WEBBIA Journal of Plant Taxonomy and Geography

Firenze University Press
www.fupress.com/webbia

OPEN ACCESS

Citation: Lechowicz K., Bocianowski J., Wrońska-Pilarek D. (2021) Pollen morphology and variability of species from the genus *Rubus* L. (Rosaceae) alien and invasive in Poland. *Webbia. Journal of Plant Taxonomy and Geography* 76(1): 109-121. doi: 10.36253/jopt-10355

Received: January 19, 2021

Accepted: March 18, 2021

Published: April 27, 2021

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Editor: Richard Hebda

ORCID
KL: https://orcid.org/0000-0002-7362-0409
JB: https://orcid.org/0000-0002-0102-0084
DW-P: https://orcid.org/0000-0003-2431-6192

Pollen morphology and variability of species from the genus *Rubus* L. (Rosaceae) alien and invasive in Poland

Kacper Lechowicz[1], Jan Bocianowski[2], Dorota Wrońska-Pilarek[1,*]

[1] *Department of Forest Botany, Faculty of Forestry and Wood Technology, Poznań University of Life Sciences, Wojska Polskiego 71 d, 60-625 Poznań, Poland*
[2] *Department of Mathematical and Statistical Methods, Faculty of Agronomy and Bioengineering, Poznań University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, Poland*
*Corresponding author. E-mail: dorota.wronska-pilarek@up.poznan.pl

Abstract: *Rubus* L. is one of the most species-rich and most taxonomically challenging genera in the family Rosaceae. The aim of this study was to investigate pollen morphology and the ranges of inter- and intraspecific variability of the studied *Rubus* alien species, as well as verify the taxonomic usefulness of these traits in distinguishing studied taxa from this genus. We analysed six quantitative pollen characteristics and the following qualitative ones: exine ornamentation, pollen outline and shape. The study was conducted on 24 samples of six alien *Rubus* species found in Poland. 720 pollen grains were measured in total. The most important pollen features included exine ornamentation and length of the polar axis (P). In the conducted studies there were no features of pollen indicating "invasiveness". The results of our research fill the gap in knowledge on pollen structure in Rubus species alien to Poland and Europe. They may constitute a foundation for further research on the reproduction of these species (e.g. pollen viability and fertility), thus facilitating identification of features determining their expansive character.

Keywords: pollen morphology, interspecific variability, intraspecific variability, *Rubus*, alien and invasive brambles.

INTRODUCTION

Many bramble species are found outside their natural distribution area as a result of their use in horticulture, while some have been introduced accidentally (Király 2018). Alien brambles can become established very far from their original range and some of them became invasive (Evans and Weber 2003; Henderson 2007; Bennett et al. 2011; Clark et al. 2013). The identification of the taxa in the secondary range is difficult, especially in the case of apomictic species of Eurasian origin, with misidentifications, even using molecular methods, being relatively frequent (Alice et al. 2014; Bruckart et al. 2017). Király (2018) expressed an opinion that alien species from the genus *Rubus* generate significant ecological and nature conservational threats worldwide; however, the European flora and vegetation are not seriously affected by their impacts.

Lambdon et al. (2008) reported that in Europe 35 *Rubus* species are 'aliens in Europe', 10 species are 'aliens of European origin' and 21 species – 'aliens to Europe'. According to DAISIE (2009), in Europe 34 alien *Rubus* taxa are found, including all the studied species. Kurtto et al. (2010) reported in Europe 10 "non-native" *Rubus* species, and about 20 native species with secondary occurrences outside the original range. In turn, Tokarska-Guzik et al. (2012) showed for Poland the occurrence of six alien bramble species.

Rubus is one of the most taxonomically challenging genera in the family Rosaceae. Difficulties arise from the large number of species with similar morphological characteristics and often small, local ranges of natural occurrence, as well as polyploid hybridisation and apparently frequent facultative apomixis (Weber 1996; Alice and Campbell 1999). Therefore, among brambles, pollen of only 48 European species had been characterised until 2019 (Erdtman et al. 1961; Reitsma 1966; Teppner 1966; Eide 1981a, 1981b; Gonzalez Romano and Candau 1989; Monasterio-Huelin and Pardo 1995; Tomlik-Wyremblewska 1995; Wrońska-Pilarek et al. 2012, 2016; Kosiński et al. 2018). These data have been supplemented by the latest comprehensive palynological study by Lechowicz et al. (2020), who described pollen grains of 58 Polish and European *Rubus* species, and by Xiong et al. (2019), who examined 155 species and 13 varieties representing all the 12 subgenera of this genus.

The most important features of bramble pollen grains include exine ornamentation (ornamentation type, width and orientation of striae and grooves), length of colpori, type of the bridge, costae colpi and the number and size of perforations (Reitsma 1966; Naruhashi and Takano 1980; Eide 1981a, 1981b; Kosenko et al. 1982; Gonzalez Romano and Candau 1989; Hebda and Chinnappa 1990, 1994; Ueda 1992; Monasterio-Huelin and Pardo 1995; Tomlik-Wyremblewska 1995, 2000; Li et al. 2001; Tomlik-Wyremblewska et al. 2004; Wrońska-Pilarek et al. 2006, 2012; Wang et al. 2007; Kasalkheh et al. 2017; Gupta and Dash 2018; Xiong et al. 2019; Lechowicz et al. 2020). In the opinion of Tomlik-Wyremblewska (1995, 2000), pollen size and shape prove to be poor criteria in species identification.

The aim of this study was to describe and analysed pollen morphology and the inter- and intraspecific variability of all six alien *Rubus* species (on the basis of 24 pollen samples) found in Poland, based on all pollen features. All of the studied bramble species, are distributed throughout Europe as aliens. We also investigated if pollen grains of the invasive and expansive bramble species differ in structure from pollen of other species, and whether any differences may affect their invasive properties.

MATERIALS AND METHODS

Pollen morphology

The plant material was collected in the herbarium of the Institute of Dendrology, Polish Academy of Sciences in Kórnik (KOR) and stored in the herbarium of the Department of Forest Botany, the Poznań University of Life Sciences (PZNF), which did not require any permits to conduct research.

The study was conducted on 24 samples of six alien *Rubus* species (*R. allegheniensis* Porter, *R. armeniacus* Focke, *R. canadensis* L., *R. laciniatus* Willd., *R. odoratus* L. and *R. xanthocarpus* Bureau & Franch) found in Poland, the Czech Republic, Ukraine, Germany and France. A list of the species analysed with their affiliation to series is shown in Table 1.

In this paper the taxonomic classification of the studied taxa from the genus *Rubus* was adopted after Zieliński (2004), with further modifications (Kosiński et al. 2018). The verification of the taxa was made by Prof. Jerzy Zieliński (Institute of Dendrology, Polish Academy of Sciences in Kórnik), a batologist - taxonomist specialising in the genus *Rubus*.

Several, randomly selected inflorescences (flowers) were collected from 24 localities representing invasive Rubus species in five European countries (Table 2). Pollen grains were acetolysed according to the method of Erdtman (1960). The pollen grain samples were then mixed with 96% alcohol and centrifuged 4 times, with processed grains subsequently divided into two groups. One half of the processed sample was immersed in an alcohol-based solution of glycerin for LM, while the other was placed in 96% ethyl alcohol in preparation for scanning electron microscopy (SEM). The SEM observations were carried out using a Zeiss Evo 40 microscope,

Table 1. The taxonomic classification of the *Rubus* species studied.

No	Species	Subgenus	Section	Subsection	Series
1	*R. xanthocarpus*	*Chamaerubus*	-	-	*Xanthocarpi*
2	*R. odoratus*	*Anoplobatus*	-	-	-
3	*R. canadensis*	*Rubus*	*Rubus*	*Rubus*	*Canadenses*
4	*R. allegheniensis*	*Rubus*	*Rubus*	*Rubus*	*Alleghenieses*
5	*R. armeniacus*	*Rubus*	*Rubus*	*Hiemales*	*Discolores*
6	*R. laciniatus*	*Rubus*	*Rubus*	*Hiemales*	*Rhamnifolii*

while LM measurements of acetolysed pollen grains were conducted at a 400× magnification using a Levenhuk D870T microscope equipped with a camera and software enabling accurate grain measurement. The exine sculptural elements were measured when taking SEM photos on an area of 25 μm^2 according to the methods of Ueda and Tomita (1989). SEM pictures were taken from samples numbered 1–5 (see: Table 2). Measurements taken from 30 mature, randomly selected, properly developed pollen grains were made using the light microscopy (LM), with 720 pollen grains measured in total.

The pollen grains were analysed for six quantitative characters: length of the polar axis (P) and equatorial diameter (E), length of the ectoaperture (Le), distance between apices of two ectocolpi (d), and the P/E, and Le/P ratios (Figure 1). The pollen shape classes (P/E ratio) were adopted according to the classification proposed by Erdtman (1952): oblate-spheroidal (0.89–0.99), spheroidal (1.00), prolate-spheroidal (1.01–1.14), subprolate (1.15–1.33) and prolate (1.34–2.00). In addition, the following qualitative characters were also determined: outline, shape, operculum structure and exine ornamentation.

Exine ornamentation types were identified based on the classification proposed by Ueda (1992). The types and subtypes of the striate exine ornamentation were characterised by the height and width of grooves, width of striae, and the number and diameter of perforations.

The descriptive palynological terminology followed Punt et al. (2007) and Halbritter et al. (2018).

Statistical analysis

The normality of distributions for the studied traits (P, Le, d, E, P/E and Le/P) was tested using Shapiro-Wilk's normality test (Shapiro and Wilk 1965). Multivariate analysis of variance (MANOVA) was performed on the basis of the following model using a MANOVA procedure in the GenStat 18th edition: $\mathbf{Y}=\mathbf{XT}+\mathbf{E}$, where: $\mathbf{Y}$ is the $(n \times p)$-dimensional matrix of observations, n is the number of all observations, p is the number of traits (in this study p=6), $\mathbf{X}$ is the $(n \times k)$-dimensional matrix of design, k is the number of species (in this study k=24), $\mathbf{T}$ is the $(k \times p)$-dimensional matrix of unknown effects, and $\mathbf{E}$ – is the $(n \times p)$-dimensional matrix of residuals. Next, one-way analysis of variance (one-way ANOVA)

Table 2. List of localities of the *Rubus* species studied.

No	Species	Localities	Geographical coordinates	Collector, herbarium
1	*R. allegheniensis* 1	Czech Republic, Fryšták	49°16'04,6"N, 17°40'01,5"E	Zieliński; KOR
2	*R. allegheniensis* 2	Czech Republic, Lukov	49°17'24,4"N, 17°43'45,8"E	Zieliński; KOR
3	*R. allegheniensis* 3	Poland, Janowo	54°02'18,2"N, 14°58'09,3"E	Zieliński; KOR
4	*R. allegheniensis* 4	Poland, Łukęcin	54°02'34,9"N, 14°52'23,8"E	Zieliński; KOR
5	*R. armeniacus* 1	Poland, Resko	53°46'24,2"N, 15°24'20,2"E	Boratyńska, Dolatowska, Zieliński; KOR
6	*R. armeniacus* 2	Poland, Golędzinów	51°16'07,6"N, 16°55'25,5"E	Zieliński; KOR
7	*R. armeniacus* 3	Poland, Dziwnów	54°01'30,3"N, 14°45'09,5"E	Boratyńska, Dolatowska, Zieliński; KOR
8	*R. armeniacus* 4	Poland, Trzebiatów	54°03'45,7"N, 15°15'56,6"E	Tomlik; KOR
9	*R. armeniacus* 5	Poland, Cichowo	51°59'39,2"N, 16°58'45,9"E	Boratyńska; KOR
10	*R. canadensis* 1	Poland, Międzygórze	50°13'40,6"N, 16°46'00,1"E	Kosiński; KOR
11	*R. canadensis* 2	Poland, Stronie Śląskie	50°17'29,9"N, 16°52'25,3"E	Kosiński, Zieliński; KOR
12	*R. canadensis* 3	Germany, Pulsnitz	51°10'57,3"N, 14°00'50,3"E	Hans-Werner; KOR
13	*R. canadensis* 4	Poland, Nowa Morawa	50°14'11,9"N, 16°53'28,1"E	Illegible name; KOR
14	*R. laciniatus* 1	Poland, Rzeszów	50°02'14,8"N, 22°00'16,9"E	Wróblewski; KOR
15	*R. laciniatus* 2	Poland, Glinna	53°17'12,6"N, 14°43'11,9"E	Celiński; KOR
16	*R. laciniatus* 3	Poland, Poznań	52°27'14,0"N, 16°50'54,9"E	Krysztofiak; KOR
17	*R. laciniatus* 4	Poland, Małolesie	53°17'50,9"N, 14°42'33,5"E	Wróblewski; KOR
18	*R. laciniatus* 5	France, Paris	48°50'35,6"N, 02°21'34,2"E	Wróblewski; KOR
19	*R. odoratus* 1	Poland, Mościenica	52°15'01,5"N, 17°03'47,4"E	Bugała; KOR
20	*R. odoratus* 2	Poland, Niedrzwica Duża	51°06'51,3"N, 22°23'16,2"E	Illegible name; KOR
21	*R. odoratus* 3	Poland, Kórnik	52°14'28,4"N, 17°05'34,5"E	Zieliński; KOR
22	*R. odoratus* 4	Ukraine, Rudki	49°38'30,2"N, 23°29'27,5"E	Wróblewski; KOR
23	*R. odoratus* 5	Poland, Rzeszów	50°02'14,8"N, 22°00'16,9"E	Wróblewski; KOR
24	*R. xanthocarpus* 1	Poland, Miedzianka	50°50'22,5"N, 20°22'03,3"E	Maciejczak, Bróż, Zieliński; KOR

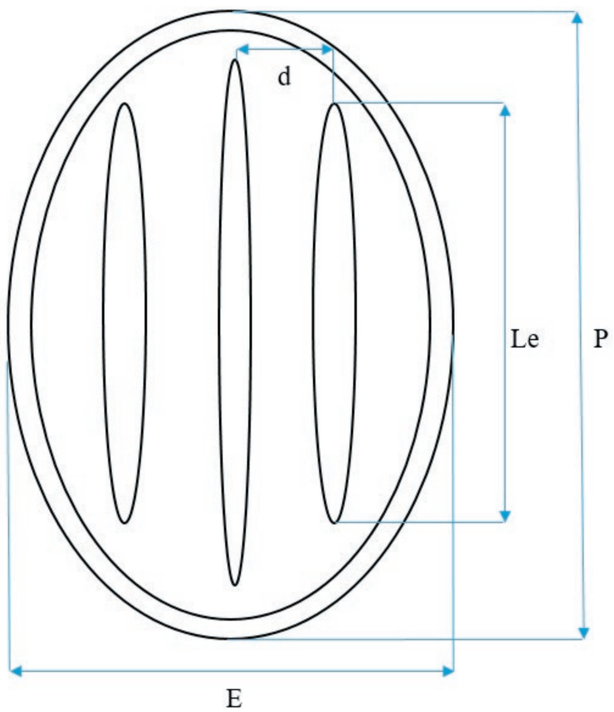

Figure 1. Scheme of the investigated quantitative characters (P, E, Le and d).

was carried out to determine the effects of *Rubus* samples on variability of P, Le, d, E, P/E and Le/P. The minimal, maximal and mean values as well as standard deviations of traits were calculated. Fisher's least significant differences (LSDs) were calculated for individual traits and on this basis homogeneous groups were established. The relationships between observed traits were assessed on the basis of Pearson's correlation. Relationships of six observed traits were presented in a heatmap. Results were also analysed using multivariate methods. The canonical variate analysis was applied in order to present multitrait assessment of similarity of tested samples in a lower number of dimensions with the least possible loss of information (Rencher 1992). This makes it possible to illustrate variation in species in terms of all observed traits in the graphic form. The Mahalanobis distance (Mahalanobis 1936) was suggested as a measure of "polytrait" species similarity (Seidler-Łożykowska and Bocianowski 2012), which significance was verified by means of critical value D_{α} called "the least significant distance" (Camussi et al. 1985). Mahalanobis distances were calculated for all species samples. The differences between the analysed species were verified by cluster analysis using the nearest neighbour method and Euclidean distances. All the analyses were conducted using the GenStat 18th edition statistical software package.

RESULTS

General morphological description of pollen

A description of pollen grain morphology of the *Rubus* species studied is given below and illustrated in SEM photographs (Figures 2 and 3). Morphological observations for the other quantitative characters of pollen grains are shown in Tables 3 and 4.

Pollen grains of the *Rubus* species studied were tricolporate, isopolar monads (Figure 2). According to the pollen size classification by Erdtman (1952) based on the length of the polar axis, analysed pollen grains were small (10–25 μm; 54.5%) or medium-sized (25.1–50 μm; 45.5%). Pollen grains had a small range of average values for trait P, ranging from 20.57 to 27.82 μm.

The average length of the polar axis (P) was 24.75 (15.16–33.65) μm (Table 3). The smallest mean P was found for pollen of *R. xanthocarpus* (20.57 μm), while the largest – for *R. armeniacus* (27.82 μm) (Table 3). In the *R. xanthocarpus* sample all measured pollen grains were small at a narrow range of polar axis length (18–24

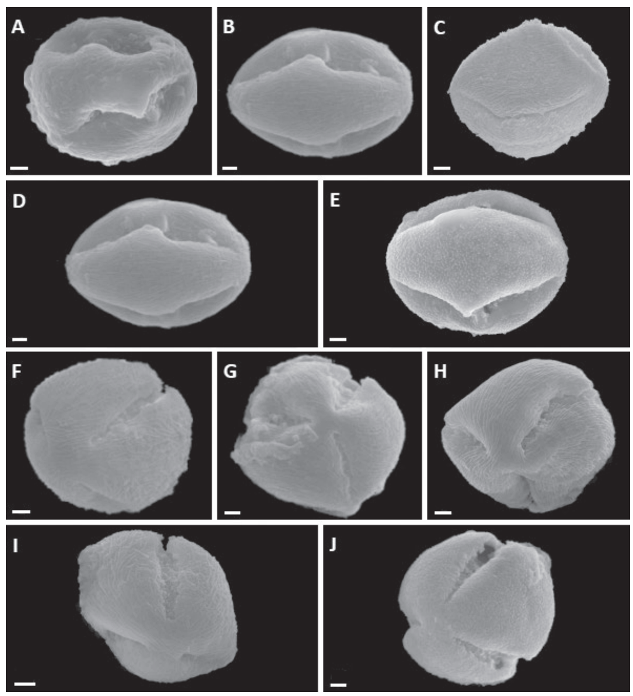

Figure 2. Equatorial and polar views of pollen grains under a scanning electron microscope (SEM) (A-E). (A) *R. allegheniensis,* (B) *R. armeniacus,* (C) *R. canadensis,* (D) *R. laciniatus,* (E) *R. odoratus* – pollen grains in equatorial view, two colpori and exine ornamentation visible. (F-J) (F) *R. allegheniensis,* (G) *R. armeniacus,* (H) *R. canadensis,* (I) *R. laciniatus,* (J) *R. odoratus* - pollen in polar view, three colpori and exine ornamentation visible. Scale bars = 2 μm.

Table 3. Minimal, maximal and mean values as well as standard deviations (s.d.) for P, Le and d.

Trait *Rubus*	P					Le					d				
	Mean		Min	Max	s.d.	Mean		Min	Max	s.d.	Mean		Min	Max	s.d.
R. allegheniensis 1	27.01	ab#	23.00	33.65	2.35	21.81	abcde	16.63	29.27	2.82	6.40	a	3.46	9.23	1.53
R. allegheniensis 2	26.55	abc	22.05	30.17	2.44	20.89	cdefg	16.68	23.9	2.04	5.83	a	3.15	9.17	1.68
R. allegheniensis 3	25.24	cdef	22.52	30.26	1.90	20.86	cdefg	17.01	24.88	1.71	4.79	b	3.21	6.16	0.81
R. allegheniensis 4	24.47	efgh	22.00	28.00	1.72	20.80	cdefg	16.00	24.00	1.63	4.27	bcdef	2.00	8.00	1.36
R. armeniacus 1	27.02	ab	22.74	31.45	2.11	22.25	abc	19.56	26.06	1.78	3.96	bcdefg	2.60	5.59	0.76
R. armeniacus 2	27.41	a	23.13	29.62	1.65	22.31	abc	17.15	26.73	2.12	4.45	bc	2.96	6.15	0.75
R. armeniacus 3	27.01	ab	23.44	30.18	1.79	22.85	ab	17.81	26.74	2.16	3.95	bcdefg	2.00	5.16	0.90
R. armeniacus 4	27.13	ab	22.19	29.81	1.47	21.86	abcde	16.79	25.38	1.63	3.92	cdefg	2.32	6.00	0.86
R. armeniacus 5	27.82	a	22.98	30.80	2.00	23.16	a	18.27	26.21	2.05	4.06	bcdefg	2.42	6.45	0.89
R. canadensis 1	21.98	jkl	19.88	24.80	1.19	18.79	ijk	15.75	21.6	1.30	2.31	k	1.67	4.38	0.59
R. canadensis 2	23.03	hijk	20.27	27.80	1.93	19.64	ghij	17.06	24.32	1.80	2.58	jk	1.62	6.23	0.88
R. canadensis 3	25.71	bcde	20.37	29.02	1.98	21.20	cdef	16.16	24.78	1.98	4.33	bcd	2.00	7.28	1.32
R. canadensis 4	21.27	l	20.00	24.00	1.23	18.13	jk	14.00	20.00	1.57	2.60	ijk	2.00	4.00	0.86
R. laciniatus 1	26.46	abc	23.22	29.31	1.37	21.91	abcd	18.81	25.13	1.64	3.53	defgh	2.00	6.17	1.01
R. laciniatus 2	24.84	defg	21.29	30.16	2.68	20.34	efgh	17.11	26.28	2.38	3.45	fghi	1.93	4.88	0.79
R. laciniatus 3	26.36	abcd	22.63	28.82	1.63	21.48	bcde	16.23	25.05	1.75	3.27	ghij	1.76	5.32	1.01
R. laciniatus 4	24.61	efg	15.16	27.48	2.53	20.66	defg	16.00	23.76	1.70	2.96	hijk	1.15	6.37	1.06
R. laciniatus 5	24.44	efgh	21.96	26.52	1.08	19.89	fghi	16.61	22.74	1.50	4.43	bc	3.04	5.82	0.83
R. odoratus 1	23.85	fghi	21.49	26.71	1.31	19.92	fghi	16.14	22.44	1.64	3.69	cdefgh	2.36	5.23	0.72
R. odoratus 2	22.86	ijk	20.62	25.70	1.35	18.71	ijk	15.02	21.84	1.71	3.86	cdefg	2.12	5.32	0.84
R. odoratus 3	23.38	ghij	20.81	25.78	1.32	18.95	hijk	15.80	22.60	1.65	4.30	bcde	2.51	6.28	0.90
R. odoratus 4	21.66	kl	18.71	23.95	1.35	17.64	kl	14.67	20.34	1.40	3.45	efgh	2.67	5.12	0.72
R. odoratus 5	21.78	kl	19.37	23.43	0.94	17.90	k	15.60	19.70	1.14	3.51	defgh	2.00	5.46	0.90
R. xanthocarpus 1	20.57	l	18.00	24.00	1.43	16.23	l	14.00	18.00	1.31	3.87	cdefg	2.00	6.00	1.07
$LSD_{0.001}$	1.5					1.54					0.85				
ANOVA *F*			47.35***					29.89***					26.10***		

a, b, ... – values with different letters in columns are significantly different (P<0.001).

μm). On the other hand, the longest pollen grains were found in *R. armeniacus* (22.19–31.45 μm).

The mean equatorial diameter (E) was 17.49 (14–28.23) μm. The shortest mean equatorial diameter was recorded in pollen of *R. xanthocarpus* (17.60 μm), and the longest was found in *R. allegheniensis* (24.19 μm; Table 4).

The outline in the polar view was mostly circular with obtuse apices, less often elliptic, whereas in the equatorial view the outline was mostly elliptic, rarely circular (Figure 2).

The mean P/E ratio was 1.16, ranging from 0.66 in *R. laciniatus* to 2.00 in *R. armeniacus* (Table 4). On average the P/E ratio values were always above 1.00, ranging from 1.10 in *R. odoratus* to 1.24 in *R. laciniatus*. Pollen grains of the examined species were most frequently subprolate (48.8% – 351 pollen grains) or prolate-spheroidal (42.9% – 309), rarely prolate (5% – 36) and very rarely oblate-spheroidal (1.9% – 14).

The polar area index (PAI) or the apocolpium index, in other words the d/E ratio, averaged 0.18 (0.05–0.41). The lowest mean value of this ratio (0.05) was recorded in *R. laciniatus*, while the highest (0.41) – in *R. allegheniensis*.

Rubus pollen grains usually have three apertures colpori. The colpori were arranged meridionally, regularly, more or less evenly spaced and were usually long, with a mean length of 20.34 (14.00–29.27) μm (Table 3). On average, the shortest colpori were found in *R. xanthocarpus* and *R. canadensis* (14.00 μm), while the longest was recorded in *R. armeniacus* (19.56 μm). On average, the length of the colporus (Le) constituted 82% of the polar axis length (P) and ranged from 67 to 97%. The colpori were narrow, linear or fusiform in outline. Their width varied and was usually greatest in the equatorial region. Sculpturing of ectocolpus membranes approached rugulate, rarely partly psilate (Figure 3). Colpus margins frequently had small undulations (Figure 3). In all the studied species a bridge was observed,

Table 4. Minimal, maximal and mean values as well as standard deviations (s.d.) for E, P/E and Le/P.

Trait *Rubus*	E					P/E					Le/P				
	Mean		Min	Max	s.d.	Mean		Min	Max	s.d.	Mean		Min	Max	s.d.
R. allegheniensis 1	24.19	a[#]	19.07	27.23	1.93	1.12	cde	0.96	1.37	0.10	0.81	def	0.68	0.90	0.07
R. allegheniensis 2	23.33	abc	16.87	27.06	2.63	1.14	cde	1.01	1.37	0,09	0.79	f	0.67	0,91	0.05
R. allegheniensis 3	22.09	cdef	20.06	24.50	0.99	1.14	cde	1.02	1.35	0,08	0.83	abcde	0.68	0,94	0.06
R. allegheniensis 4	21.20	efgh	18.00	24.00	1.45	1.16	abcde	1.00	1.40	0,10	0.85	ab	0.73	0,92	0.06
R. armeniacus 1	22.79	abcd	19.86	25.04	1.33	1.19	abc	1.08	1.45	0,08	0.83	abcdef	0.69	0,94	0.05
R. armeniacus 2	23.30	abc	20.20	25.82	1.46	1.18	abcde	1.08	1.30	0,06	0.81	bcdef	0.68	0,90	0.05
R. armeniacus 3	23.54	abc	20.42	27.76	2.02	1.15	bcde	1.02	1.29	0,08	0.85	abcd	0.75	0,94	0.04
R. armeniacus 4	24.09	a	20.93	28.23	1.51	1.13	cde	0.99	1.28	0,06	0.81	def	0.74	0,87	0.03
R. armeniacus 5	23.82	ab	15.14	27.27	2.45	1.18	abcde	0.94	2.00	0,18	0.83	abcd	0.76	0,90	0.04
R. canadensis 1	19.07	hi	16.26	22.48	1.42	1.16	bcde	1.01	1.28	0,07	0.85	a	0.72	0,93	0.04
R. canadensis 2	19.75	hgh	16.76	23.58	1.66	1.17	abcde	0.96	1.40	0,08	0.85	ab	0.75	0,90	0.03
R. canadensis 3	23.40	abc	17.83	27.24	2.42	1.10	de	0.99	1.36	0,09	0.83	abcdef	0.71	0,92	0.05
R. canadensis 4	18.47	hi	14.00	20.00	1.46	1.16	bcde	1.00	1.43	0,10	0.85	ab	0.70	0,91	0.05
R. laciniatus 1	22.47	bcde	18.83	26.55	1.84	1.18	abcd	1.04	1.38	0,09	0.83	abcde	0.75	0,91	0.04
R. laciniatus 2	21.30	defg	17.31	26.78	2.45	1.17	abcde	0.97	1.37	0,10	0.82	abcdef	0.74	0,89	0.03
R. laciniatus 3	22.52	bcde	16.47	27.84	2.33	1.18	abcde	0.99	1.60	0,14	0.81	bcdef	0.72	0,88	0.04
R. laciniatus 4	21.46	def	16.88	26.12	2.20	1.16	bcde	0.66	1.52	0,14	0.85	abc	0.76	1,45	0.12
R. laciniatus 5	19.78	ghgh	17.88	21.76	0.95	1.24	a	1.10	1.38	0,08	0.81	bcdef	0.70	0,88	0.04
R. odoratus 1	19.51	gh	15.60	22.60	1.63	1.23	ab	1.06	1.38	0,08	0.84	abcd	0.72	0,91	0.05
R. odoratus 2	19.44	gh	16.25	21.82	1.54	1.18	abcde	1.06	1.46	0,08	0.82	abcdef	0.71	0,89	0.04
R. odoratus 3	20.73	fghg	17.96	23.37	1.35	1.13	cde	1.01	1.35	0,08	0.81	cdef	0.71	0,89	0.04
R. odoratus 4	19.76	hgh	16.43	22.41	1.76	1.10	e	0.94	1.34	0,10	0.81	bcdef	0.74	0,88	0.03
R. odoratus 5	18.93	hi	14.79	21.82	1.98	1.16	abcde	1.02	1.49	0,12	0.82	abcdef	0.71	0,91	0.05
R. xanthocarpus 1	17.60	i	16.00	22.00	1.55	1.18	abcde	1.00	1.50	0.11	0.79	ef	0.64	0.90	0.06
$LSD_{0.001}$	1.55					0.08					0.04				
ANOVA *F*			36.27***					3.30***					4.06***		

\# a, b, ... – values with different letters in columns are significantly different (P<0.001).

crossing the colpus at the equator and dividing it into two parts, formed by two bulges of the ectexine that meet in the middle (Figure 3). These equatorial extensions were of the same or unequal length.

Exine ornamentation in all species was striate-perforate, with the exception of *R. odoratus*, which had a verrucate ornamentation with small perforations (Figure 3). Exine ornamentation elements were highly variable (Figure 3). Striae and grooves usually ran parallel to colpori and the polar axis, but frequently they also formed fingerprint-like twists. Striae were straight or forked and of varying length, width and height.

The investigated pollen of individual *Rubus* species was classified according to the striate exine ornamentation classification proposed by Ueda (1992). The cited author distinguished six types (I-VI) and six subtypes (I-III, each A and B). In our study only subtypes IIA and IIIA were found (Figure 3). Subtype IIA had prominent perforations between ridges and with short intervals of ridges. Subtype IIIA had more distinct ridges due to their steep slope; the ridges had small diameter (0.15~0.30 μm). Four species (*R. allegheniensis, R. armeniacus, R. xanthocarpus, R. laciniatus*) belonged to the IIA subtype, which was characterised by distinct striae, narrow grooves and frequently by prominent, numerous perforations. Subtype IIIA was represented by only one species (*R. canadensis*) with higher (i.e. more conspicuous than in subtype IIA), fairly narrow striae in comparison with subtype IIA. Striate-verrucate exine ornamentation with flattened and wavy muri and small, few perforations was found only in *R. odoratus*.

Interspecific variability of pollen grains

Results of MANOVA indicated that the all the *Rubus* samples were significantly different when investigated in terms of all the six quantitative traits jointly

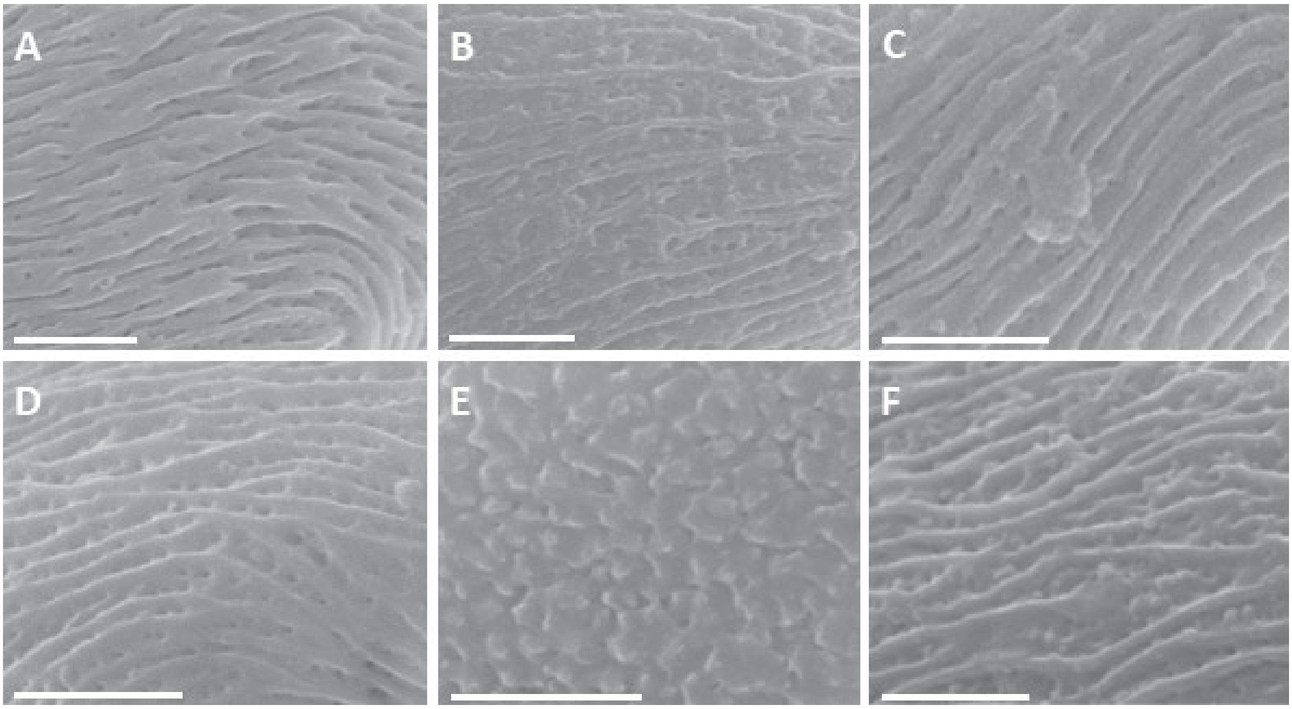

Figure 3. Types and subtypes of striate exine ornamentation. (A) *R. allegheniensis* (subtype-IIA), (B) *R. armeniacus* (IIA), (C) *R. canadensis* (IIIA), (D) *R. laciniatus* (IIA), (E) *R. odoratus* (verrucate ornamentation), (F) *R. xanthocarpus* (IIA). Scale bars = 2 μm.

(Wilk's l=0.1495; $F_{23;696}$=11.27; P<0.0001). The analysis of variance for the six biometric traits [P ($F_{23;696}$=47.25), Le ($F_{23;696}$=29.89), d ($F_{23;696}$=26.10), E ($F_{23;696}$=36.27), P/E ($F_{23;696}$=3.30) and Le/P ($F_{23;696}$=4.06)] confirmed variability of the tested species at a significance level α=0.001 (Tables 3 and 4). The mean values and standard deviations for the observed traits indicated a high variability among the tested samples, for which significant differences were found in terms of all the analysed morphological traits (Tables 3 and 4). The density plots of P and P/E by *Rubus* species samples are presented in Figures 4 and 5, respectively. Variability of the observed traits across all *Rubus* species on the basis of *F*-statistics ranked as follows: P>E>Le>d>Le/P>P/E. The ranking of variability in the *Rubus* species calculated as a sum of coefficients of variations for the six observed traits is: *R. allegheniensis* (sample 1), > *R. laciniatus* (4) > *R. allegheniensis* (2) > *R. canadensis* (3) > *R. laciniatus* (3) > *R. canadensis* (2) > *R. canadensis* (4) > *R. laciniatus* (2) > *R. allegheniensis* (4) > *R. armeniacus* (5) > *R. xanthocarpus* > *R. odoratus* (5) > *R. laciniatus* (1) > *R. armeniacus* (3) > *R. odoratus* (2) > *R. odoratus* (4) > *R. canadensis* (1) > *R. odoratus* (3) > *R. armeniacus* (1) > *R. odoratus* (1) > *R. allegheniensis* (3) > *R. armeniacus* (4) > *R. armeniacus* (2) > *R. laciniatus* (5).

The performed correlation analysis indicated statistically significant correlation coefficients for six out of 15 coefficients (Figure 6). Trait P was significantly correlated with Le (r=0.969), d (0.508) and E (0.955). Additionally, E was correlated with Le (0.915) and d (0.551). A negative significant correlation was observed between d and Le/P (-0.615).

In the presented dendrogram, as a result of the nearest neighbour grouping using the Euclidean distances method, the *Rubus* samples were divided into two groups (Figure 7). The first group comprised 14 samples, while the other was composed of the other 10 samples. In the dendrogram (Figure 7) a trend towards clustering of samples of individual species is observed, indicating similar traits of their pollen grains. The first group comprised almost all samples of *R. laciniatus* (samples 1-4), *R. armeniacus* (samples 1-5), and *R. allegheniensis* (samples 1-4), while the other group was composed of samples of *R. canadensis* and *R. odoratus* (Figure 7).

Individual traits vary in terms of their importance and share in the joint multivariate variation. A study on the multivariate variation for *Rubus* species samples includes also identification of the most important traits in the multivariate variation of species. Analysis of canonical variables is a statistical tool making it possible to solve the problem of multivariate relationships (Seidler-Łożykowska et al. 2013, Lahuta et al. 2018, Wrońska-Pilarek et al. 2018, Bocianowski and Majchrzak 2019). Figure 8 shows vari-

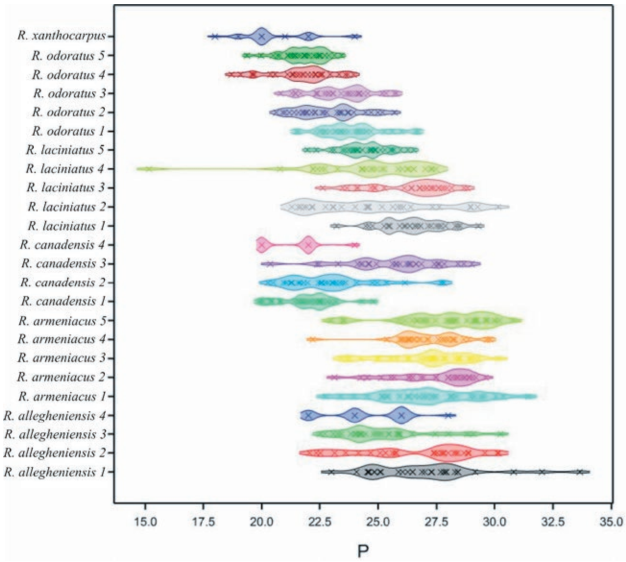

Figure 4. The density plot of P for individual *Rubus* species. The point "x" denotes trait observation for particular species. The points are plotted along a line, with a kernel density smooth on either side to indicate the density of points along the line.

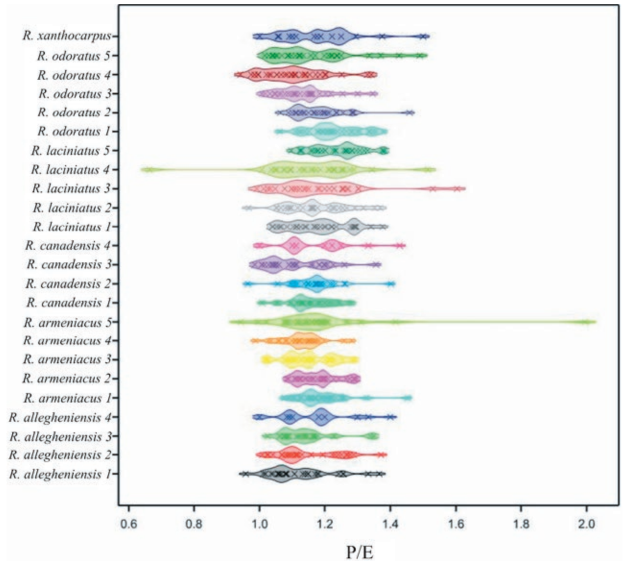

Figure 5. The density plot of P/E for individual *Rubus* species. The point "x" denotes trait observation for particular species. The points are plotted along a line, with a kernel density smooth on either side to indicate the density of points along the line.

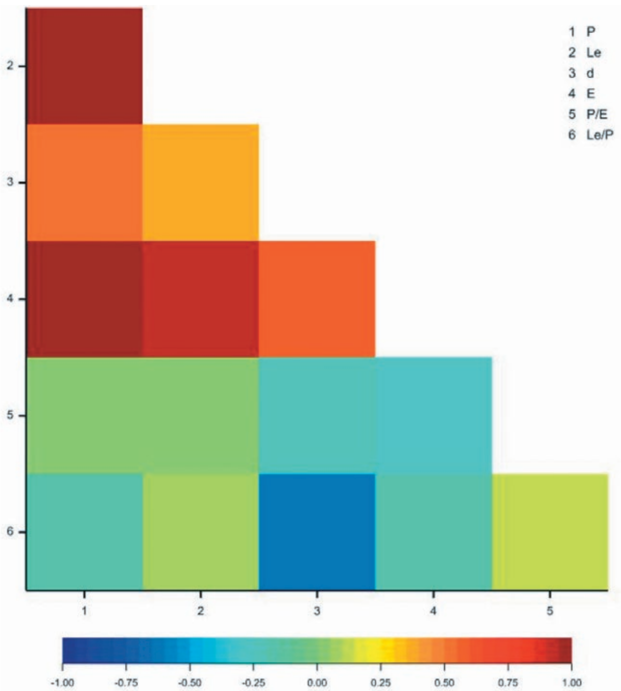

Figure 6. Heatmap for linear Pearson' correlation coefficients between observed traits (r_{cr}=0.406). The heatmap provides a graphical representation of a correlation matrix between pairs of the observed traits. Each element of the correlation matrix is represented by a shaded rectangle indicating the value at that location, using a different colour or shading density.

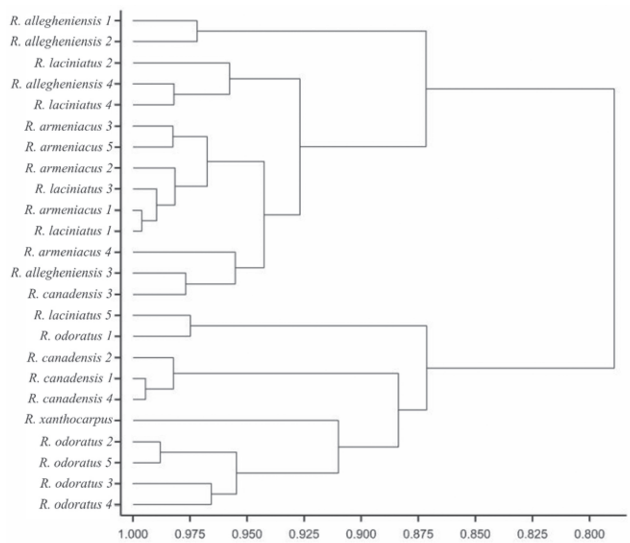

Figure 7. Dendrogram of the nearest neighbour cluster grouping of *Rubus* species on the basis of six traits. The length of the lines denotes similarity/distance between two species or between two groups of species and determined hierarchical clusters.

ability of the six pollen grain traits in 24 studied *Rubus* samples in terms of the first two canonical variables. In the graph the coordinates of the point for particular species are the values for the first and second canonical variables, respectively. The first two canonical variables

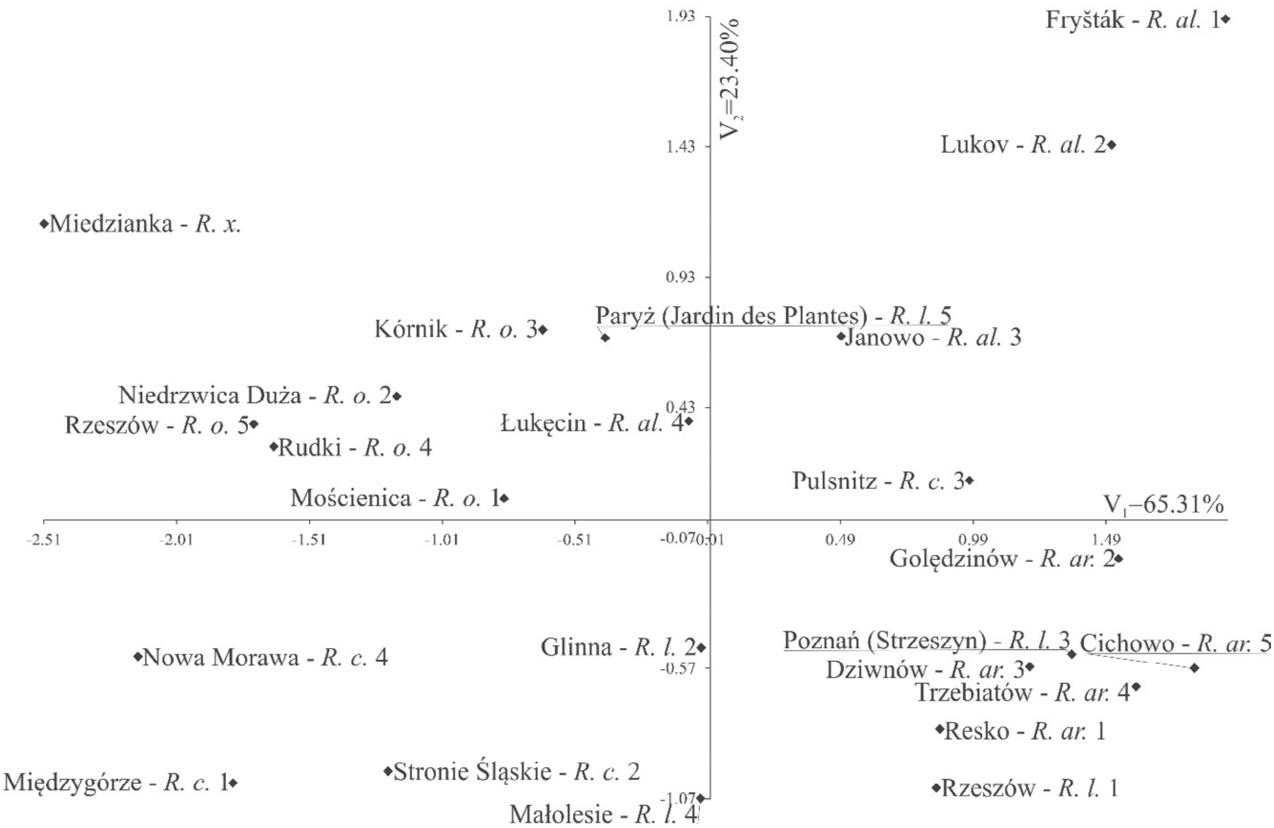

Figure 8. Distribution of 24 *Rubus* species in the space of the first two canonical variables.

accounted for 88.71% of the total multivariate variability among the individual species samples (Table 5, Figure 8). The most significant, positive, linear relationship between the first canonical variables was found for P, Le, d and E (Table 5). The second canonical variable was significantly positively correlated with d and negatively correlated with Le/P (Table 5). The greatest variation in terms of all the traits jointly (measured Mahalanobis distances) was found for *R. allegheniensis* (sample 2) and *R. canadensis* (sample 4), with the Mahalanobis distance between them amounting to 4.78 (Table 6). The greatest similarity (0.56) was found for *R. armeniacus* (sample 1) and *R. armeniacus* (sample 4). The Mahalanobis distance is unitless and scale-invariant, and takes into account the correlations of the data set. On the basis of Mahalanobis distances we identified samples showing greatest similarity for all observed traits jointly.

Table 5. Correlation coefficients between the first two canonical variables and original traits.

Trait	First canonical variable	Second canonical variable
P	0.989***	-0.102
Le	0.942***	-0.256
d	0.595**	0.802***
E	0.982***	-0.035
P/E	-0.116	-0.195
Le/P	-0.216	-0.623**
Percentage variation	65.31%	23.40%

** P<0.01; *** P<0.001

DISCUSSION

In the lists of alien species of Europe (Lambdon et al. 2008; DAISIE 2009; Kurtto et al. 2010), the studied *Rubus* species have the status of 'aliens in Europe' and naturalised within the continent (*R. allegheniensis, R. armeniacus, R. canadensis, R odoratus, R. xanthocarpus*) or 'aliens of European origin' - naturalised and neophytes within the continent (*R. laciniatus*). We studied all the six bramble species considered by Tokarska-Guzik et al. (2012) to be alien for Poland. All these species are domesticated kenophytes in Poland, occur-

Table 6. Mahalanobis' distances between studied *Rubus* species.

Rubus species		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
R. allegheniensis 2	2	1.03																						
R. allegheniensis 3	3	1.96	1.52																					
R. allegheniensis 4	4	2.61	2.25	0.78																				
R. armeniacus 1	5	2.76	2.20	1.56	1.81																			
R. armeniacus 2	6	2.29	1.78	1.47	1.92	0.56																		
R. armeniacus 3	7	2.59	2.37	1.67	1.81	1.09	1.09																	
R. armeniacus 4	8	2.72	2.17	2.02	2.38	1.19	1.10	1.25																
R. armeniacus 5	9	2.57	2.29	2.04	2.30	1.25	1.12	0.79	1.02															
R. canadensis 1	10	4.76	4.17	2.88	2.28	3.11	3.51	3.29	3.51	3.73														
R. canadensis 2	11	4.29	3.72	2.40	1.82	2.53	2.93	2.69	2.98	3.13	0.63													
R. canadensis 3	12	2.11	1.77	1.31	1.60	1.66	1.52	1.23	1.28	1.38	3.13	2.63												
R. canadensis 4	13	4.78	4.21	2.94	2.33	3.45	3.79	3.61	3.85	4.05	0.62	1.08	3.31											
R. laciniatus 1	14	3.00	2.46	1.65	1.70	0.69	1.03	0.92	1.12	1.14	2.72	2.12	1.42	3.09										
R. laciniatus 2	15	3.18	2.54	1.45	1.28	1.38	1.68	1.66	1.74	1.98	1.91	1.36	1.54	2.20	0.99									
R. laciniatus 3	16	3.25	2.63	2.04	2.10	1.16	1.41	1.34	0.99	1.28	2.77	2.22	1.50	3.16	0.63	1.12								
R. laciniatus 4	17	3.64	2.98	2.03	1.79	1.71	2.10	1.99	1.89	2.18	1.87	1.41	1.85	2.25	1.32	0.99	1.23							
R. laciniatus 5	18	2.88	2.31	1.32	1.14	2.15	2.23	2.47	2.78	2.73	2.44	2.06	2.20	2.38	2.08	1.51	2.36	2.17						
R. odoratus 1	19	3.40	2.86	1.65	1.11	2.24	2.46	2.42	2.81	2.76	1.73	1.34	2.22	1.72	1.96	1.24	2.18	1.75	0.82					
R. odoratus 2	20	3.49	2.95	1.78	1.34	2.66	2.83	2.83	3.11	3.23	1.68	1.48	2.41	1.50	2.41	1.52	2.59	2.11	1.07	0.80				
R. odoratus 3	21	2.88	2.38	1.25	1.08	2.37	2.42	2.47	2.68	2.89	2.14	1.85	1.89	2.05	2.19	1.41	2.40	2.08	1.17	1.23	0.78			
R. odoratus 4	22	4.01	3.50	2.36	1.96	3.19	3.38	3.25	3.41	3.71	1.50	1.56	2.71	1.26	2.87	1.93	2.95	2.34	2.04	1.67	1.02	1.19		
R. odoratus 5	23	4.00	3.47	2.36	1.88	3.22	3.41	3.31	3.53	3.69	1.47	1.53	2.78	1.11	2.88	1.94	2.96	2.31	1.71	1.28	0.72	1.27	0.75	
R. xanthocarpus 1	24	4.60	4.11	3.19	2.82	4.19	4.32	4.33	4.52	4.71	2.41	2.60	3.75	1.92	3.92	2.99	4.01	3.48	2.38	2.23	1.57	2.02	1.46	1.23

$D_{0.05}$=2.73

ring naturally in North America and Asia; except for *R. laciniatus* – native to Eurasia, which in Poland is an invasive anthropophyte (www.iop.krakow.pl). In turn, *R. xanthocarpus* colonise anthropogenic plant communities, *R. armeniacus* – anthropogenic and seminatural ones, while the other four species are found in anthropogenic, seminatural as well as natural communities (Zieliński 1991; Bróż and Zieliński 1993; Weber 1993; Tokarska-Guzik et al. 2012).

All palynologists agree that the most important pollen features for the species in the genus *Rubus* are the width, number and course of grooves (muri) and the width of the striae, as well as the number and diameter of perforations (Ueda and Tomita 1989; Ueda and Okada 1994; Tomlik-Wyremblewska 1995, 2000; Li et al. 2001; Wrońska-Pilarek et al. 2006, 2012, Xiong et al. 2019; Lechowicz et al. 2020). Some authors considered pollen size and shape as potentially important features in the diagnosis of the analysed *Rubus* species (Candau and Romanos 1987; Monasterio-Huelin and Pardo 1995; Wrońska-Pilarek et al. 2012; Ghosh and Saha 2017), while others claimed that they have no diagnostic significance (e.g. Li et al. 2001; Tomlik-Wyremblewska et al. 2004). Based on Candau and Romanos (1987); Monasterio-Huelin and Pardo (1995); Wrońska-Pilarek et al. (2012) or Ghosh and Saha (2017) and our results, we partially agree with they former opinion, because the length of the polar axis (P) has been an important feature.

According to current palynological studies, European native bramble species are less variable in terms of exine ornamentation than Asian ones. Comprehensive palynological studies by Li et al. (2001), Xiong et al. (2019) and Lechowicz et al. (2020) confirmed this thesis. Li *et al.* (2001) examined 103 *Rubus* species from China, which belonged to four types and 11 subtypes, and Xiong et al. (2019) divided pollen grains of 168 taxa into six types and three subtypes of exine ornamentation. However, in the study of Lechowicz et al. (2020) in the examined 58 European *Rubus* species only two types of exine ornamentation were found (striate and striate-verrucate with microgranules). Other European palynologists distinguished in brambles mainly striate or striate-perforate exine ornamentation (Reitsma 1966; Eide 1981a, 1981b; Monasterio-Huelin and Pardo 1995; Tomlik-Wyremblewska 1995, 2000; Wrońska-Pilarek et al. 2006, 2012; Polyakova and Gataulina 2008; Ghosh and Saha 2017; Kasalkheh et al. 2017). Ueda and Tomita (1989) and Ueda (1992) distinguished six types and six subtypes of striate exine ornamentation in species from the genus *Rosa* and the family Rosaceae, including the genus *Rubus*. Hebda and Chinnappa (1990) divided Canadian rosaceous pollen types into two broad categories based on sculpturing. In the current study the analysed *Rubus* pollen grains were classified into two sculpturing subtypes (II A, III A), which were most frequently represented among the European native bramble pollen (e.g. Reitsma 1966; Eide 1981a, 1981b; Wrońska-Pilarek et al. 2012; Kasalkheh et al. 2017; Lechowicz et al. 2020).

Authors of many palynological studies (e.g. Tomlik-Wyremblewska 1995, 2000; Li et al. 2001; Wrońska-Pilarek et al. 2012) reported that the bridges are located in most of the studied *Rubus* species. In a study by Lechowicz et al. (2020) and in our study, bridges were observed in all the analysed species. This structure was not used as a basis for the identification of species, because they were very similar in all species.

In the opinion of Li et al. (2001), pollen shape in *Rubus* varied from spheroidal, subspheroidal, prolate and perpolate, to occasionally rhomboid and hexagonal in the equatorial view. In turn, Monasterio-Huelin and Pardo (1995) stated that they were only prolate or spheroidal, while other authors distinguished several pollen shape types - subprolate, prolate spheroidal, prolate or perprolate (Tomlik-Wyremblewska 1995, 2000; Wrońska-Pilarek et al. 2006, 2012; Ghosh and Saha 2017; Kasalkheh et al. 2017). Xiong et al. (2019) showed that pollen shape varies from suboblate, spheroidal, subprolate and prolate to perprolate, while Lechowicz et al. (2020) distinguished the greatest number, i.e. six types of pollen shape (subprolate**,** prolate-spheroidal, prolate, spheroidal, oblate-spheroidal and perprolate). We agree with the opinions by Tomlik-Wyremblewska (1995, 2000) and Lechowicz et al. (2020) that pollen shape turned out to be a poor criterion in identifying bramble species, because most pollen grains (ca. 90%) are similar in shape – either subprolate or prolate-spheroidal.

So far, morphological features of pollen grains have not been analysed in terms of their impact on the invasive character of the alien bramble species. Our results showed no differences in the pollen structure of the invasive *R. laciniatus* compared to the other five studied alien bramble species (Figures 7 and 8), but also with dozens of *Rubus* species native to Poland and Europe described by our team in another paper (Lechowicz et al. 2020). It may be stated that pollen morphology does not determine expansion of the studied species. Pollen viability and germination may have a greater impact on the expansive properties of such bramble species as *R. laciniatus*. The arrangement of the investigated samples on the dendrogram (Figure 7) showed that samples of the same species (e.g. *R. allegheniensis, R. armeniacus, R. odoratus*) often form clusters, except for *R. laciniatus*. This means that *R. laciniatus* pollen grains were more similar to those of other species than to one another.

CONCLUSIONS

- Interspecific variability of pollen features was found with but the highest and the lowest variability observed for pollen of the same bramble species (e.g. *R. allegheniensis* - samples 1 and 3, *R. laciniatus* - samples 4 and 5 or *R. armeniacus* - samples 5 and 2) (Figure7).
- In the case of the invasive anthropophyte (*R. laciniatus*), the greatest intraspecific variability was found, as individuals of this species differed the most from each other (Figure 7), while no interspecies variation with other non-invasive bramble species was found. Thus so it may be assumed that pollen morphology probably does not affect invasive traits of this species.
- Results of our studies fill the gap in knowledge on pollen structure of species from the genus *Rubus* alien for Poland and Europe. They may be used in further studies on reproduction of these species (e.g. pollen viability and fertility), facilitating identification of traits determining their expansive character.

ACKNOWLEDGEMENTS

We kindly thank Prof. Mirosław Makohonienko and Monika Panfil, M.Sc. from the Department of Quaternary Geology and Paleogeography at the Adam Mickiewicz University in Poznań for providing access to laboratory equipment and assistance with acetolysis.

We would like to thank the Reviewer for the detailed and valuable comments on the manuscript.

REFERENCES

Alice LA, Campbell CS. 1999. Phylogeny of *Rubus* (rosaceae) based on nuclear ribosomal DNA internal transcribed spacer region sequences. Am J Bot. 86: 81–97.

Alice LA, Goldman, DH, Macklin JA, Moore G. 2014. *Rubus*. In: Flora of North America Editorial Committee (ed): Flora of North America north of Mexico. New York (NY): Oxford University Press.

Bennett JR, Young EJ, Giblin DE, Dunwiddie PW, Arcese P. 2011. Avian dispersal of exotic shrubs in an archipelago. Écoscience. 18: 369–374.

Bocianowski J, Majchrzak L. 2019. Analysis of effects of cover crop and tillage method combinations on the phenotypic traits of spring wheat (*Triticum aestivum* L.) using multivariate methods. Appl Ecol Environ. Res. 17: 15267–15276.

Bróź E, Zieliński J. 1993. *Rubus xanthocarpus* (Rosaceae) - a new naturalized species in the flora of Poland. Fragm Flor Geob. 38: 153–157.

Bruckart WL, Michael JL, Sochor M, Trávníček B. 2017. Invasive blackberry species in Oregon: their identity and susceptibility to rust disease and the implications for biological control. Invasive Plant Sci Manag. 10: 143–154.

Camussi A, Ottaviano E, Caliński T, Kaczmarek Z. 1985. Genetic distances based on quantitative traits. Genetics. 111: 945–962.

Candau P, Romanos LG. 1987. “Rosaceae”. In Atlas polínico de Andalucía Occidental, Edited by: Valdés, B., Díez, M.J. and Fernández, I. Sevilla: Univ Sevilla & Diput Cádiz Inst Desarollo Reg. 43: 179–184.

Clark LV, Evans KJ, Jasieniuk M. 2013. Origins and distribution of invasive *Rubus fruticosus* L. agg. (Rosaceae) clones in the western United States. Biological Invasions. 15: 1331–1342.

DAISIE (ed.). 2009. Handbook of Alien Species in Europe, Invading Nature: Springer Series in Invasion Ecology, Springer, Dordrecht, the Netherlands.

Eide F. 1981 a. Key for Northwest European Rosaceae pollen. Grana. 20: 101–118.

Eide F. 1981 b. On the pollen morphology of *Rubus chamaemorus* L. (Rosaceae). Grana. 20: 25–27.

Erdtman G. 1952. Pollen morphology and plant taxonomy. Angiosperms. An introduction to palynology. Stockholm: Almquist and Wiksell.

Erdtman G. 1960. The acetolysis method. A revised description. Sven Bot Tidskr. 54: 561–564.

Erdtman G, Berglund B, Praglowski J. 1961. An Introduction to a Scandinavian Pollen Flora. Grana. 2: 3–86.

Evans KJ, Weber HE. 2003. *Rubus anglocandicans* (Rosaceae) is the most widespread taxon of European blackberry in Australia. Aust Syst Bot. 16: 527–537.

Ghosh A, Saha I. 2017. Pollen morphological study of some selected Indian taxa of Rosaceae. Indian J Applied & Pure Bio. 32: 121–130.

Gonzalez Romano ML, Candau PA. 1989. Contribution to palynological studies in the Rosaceae. Acta Bot Malac. 14: 105–116.

Gupta C, Dash SS. 2018. A new species of *Rubus* (Rosaceae) from Arunachal Pradesh, India. Blumea. 63: 26–30.

Halbritter H, Ulrich S, Grímsson F, Weber M, Zetter R, Hesse M, Buchner R, Svojtka M, Frosch-Radivo A. 2018. Illustrated Pollen Terminology. 2nd ed. Vienna: Springer.

Hebda RJ, Chinnappa CC.1990. Studies on pollen morphology of Rosaceae in Canada. Rev Palaeobot Palynol. 64: 103–108.

Hebda RJ, Chinnappa CC. 1994. Studies on pollen morphology of Rosaceae. Bot Lett. 141: 183–193.

Henderson L. 2007. Invasive, naturalized and casual alien plants in southern Africa: a summary based on the Southern African Plant Invaders Atlas (SAPIA). Bothalia. 37: 215–248.

IOP: *Rubus laciniatus* Willd. – Cut-leaved blackberry. 2009–2020. [accessed 2020 July 10]. www.iop.krakow.pl/ias/gatunki/149

Kasalkheh R, Jorjani E, Sabouri H, Habibi M, Sattarian A. 2017. Pollen morphology of the genus *Rubus* L. subgenus *Rubus* (Rosaceae) in Iran. Nova Bioliogica Reperta. 4: 9–18.

Király G. 2018. Alien *Rubus* species in Hungary: Distribution, habitats and threats. Dendrobiology. 80: 1-11, 2018.

Kosenko VN, Nguen TH, Jacovlev GP. 1982. Palynomorphological study of the representatives of the genus *Rubus* (Rosaceae) in the flora of Vietnam. Bot Z. 69: 497–503.

Kosiński P, Maliński T, Sliwinska E, Zieliński J. 2018. *Rubus prissanicus* (Rosaceae), a new bramble species from North West Poland. Phytotaxa. 344: 239–247.

Kurtto A, Weber HE, Lampinen R, Sennikov AN. 2010. Atlas florae Europaeae. Distribution of vascular plants in Europe, Vol. 15: Rosaceae (*Rubus*). Helsinki: Committee for Mapping the Flora of Europe and Societas Biologica Fennica Vanamo.

Lahuta LB, Ciak M, Rybiński W, Bocianowski J, Börner A. 2018. Diversity of the composition and content of soluble carbohydrates in seeds of the genus *Vicia* (Leguminosae). Genet Resour Crop Evol. 65: 541–554.

Lambdon PW, Pysek P, Basnou C, Hejda M, Arianoutsou M, Essl F, Jarosik V, Pergl J, Winter M, Anastasiu P, et al. 2008. Alien flora of Europe: species diversity, temporal trends, geographical patterns and research needs. Preslia. 80: 101–149.

Lechowicz K, Wrońska-Pilarek D, Bocianowski J, Maliński T. 2020. Systematic importance of pollen morphological features of selected species from the genus *Rubus* (Rosaceae). PLoS One. 15: e0221607.

Li WL, He SA, Gu Y, Shu P, Pu ZM. 2001. Pollen morphology of the genus *Rubus* from China. Acta Phytotax Sin. 39: 234–247.

Mahalanobis PC. 1936. On the generalized distance in statistics. Proc Nat Inst Sci India. 12: 49–55.

Monasterio-Huelin E, Pardo C. 1995. Pollen morphology and wall stratification in *Rubus* L. (Rosaceae) in the Iberian Peninsula. Grana. 34: 229–236.

Naruhashi N, Takano H. 1980. Size variation of pollen grains in some *Rubus* species. J Phytogeogr Taxon. 28: 27–32.

Polyakova TA, Gataulina GN. 2008. Morphology and variability of pollen of the genus *Spiraea* L. (Rosaceae) in Siberia and the Far East. Contemp Probl Ecol. 1: 420–424.

Punt W, Hoen PP, Blackmore S, Nilsson S, Le Thomas A. 2007. Glossary of pollen and spore terminology. Rev Palaeobot Palynol. 143: 1–81.

Reitsma T. 1966. Pollen morphology of some European Rosaceae. Acta Bot Neerl. 15: 290–307.

Rencher AC. 1992. Interpretation of canonical discriminant functions, canonical variates, and principal components. Am Stat. 46: 217–225.

Seidler-Łożykowska K, Bocianowski J. 2012. Evaluation of variability of morphological traits of selected caraway (*Carum carvi* L.) genotypes. Ind Crops Prod. 35: 140–145.

Seidler-Łożykowska K, Bocianowski J, Król D. 2013. The evaluation of the variability of morphological and chemical traits of the selected lemon balm (*Melissa officinalis* L.) genotypes. Ind Crops Prod. 49: 515–520.

Shapiro SS, Wilk MB. 1965. An analysis of variance test for normality (complete samples). Biometrika. 52: 591–611.

Teppner H. 1966. Zur Kenntnis der Gattung Waldsteinia L. - Schlüssel zum Bestimmen von Rosaceen Polleeinschliesslich ählicher Pollen - formen aus andere Familien. Phyton. 3-4: 224–238.

Tokarska-Guzik B, Dajdok Z, Zajac M, Zajac A, Urbisz A, Danielewicz W, Hołdynski C. 2012. Rosliny obcego pochodzenia w Polsce ze szczególnym uwzględnieniem gatunków inwazyjnych [Alien plants in Poland with particular reference to invasive species]. Warszawa: Generalna Dyrekcja Ochrony Środowiska.

Tomlik-Wyremblewska A. 1995. Pollen morphology of genus *Rubus* L. Part I. Introductory studies of the European representatives of the subgenus *Rubus* L. Acta Soc Bot Pol. 64: 187–203.

Tomlik-Wyremblewska A. 2000. Pollen morphology of genus *Rubus* L., Part II. Introductory studies on the Malesian species of subgenus Micranthobatus. Acta Soc Bot Pol. 69: 31–40.

Tomlik-Wyremblewska A, Van der Ham RWJM, Kosiński P. 2004. Pollen morphology of genus *Rubus* L. Part III. Studies on the Malesian species of subgenera *Chamaebatus* L. and *Idaeobatus* L. Acta Soc Bot Pol. 73: 207–227.

Ueda Y, Tomita H. 1989. Morphometric analysis of pollen patterns in Roses. J Japan Soc Hort Sci. 58: 211–220.

Ueda Y. 1992. Pollen surface morphology in the genus *Rosa*, related genera. Japan J Palynol. 38: 94–105.

Ueda Y, Okada Y. 1994. Discrimination of rose cultivar groups by pollen surface structure. J Hortic Sci. 69: 601–607.

Wang X, Tang H, Huang L, He Z, Dong X, Fu H, Deng Q. 2007. Comparative studies on pollen submicroscopic morphology of some wild species and cultivars of bramble (*Rubus* L.). Acta Hortic Sinica. 34: 1395–1404.

Weber HE. 1993. Zur entstehung, taxonomie und nomenklatur des *Rubus laciniatus* (Rosaceae). Willdenowia. 23: 75–81.

Weber HE. 1996. Former and modern taxonomic treatment of the apomictic *Rubus* complex. Folia Geobot Phytotax. 31: 373–380.

Wrońska-Pilarek D, Maliński T, Lira J. 2006. Pollen morphology of Polish species of genus *Rubus* L. - *Rubus gracilis* J. Presl & C. Presl. Dendrobiology. 56: 69–77.

Wrońska-Pilarek D, Jagodziński AM, Maliński T. 2012. Morphological studies of pollen grains of the Polish endemic species of the genus *Rubus* L. (Rosaceae). Biologia. 67: 87–96.

Wrońska-Pilarek D, Danielewicz W, Bocianowski J, Maliński T, Janyszek M. 2016. Comparative Pollen Morphological Analysis and Its Systematic Implications on Three European Oak (*Quercus* L., Fagaceae) Species and Their Spontaneous Hybrids. PLoS One. 11: e0161762.

Wrońska-Pilarek D, Szkudlarz P, Bocianowski J. 2018. Systematic importance of morphological features of pollen grains of species from *Erica* (Ericaceae) genus. PLoS One. 13: e0204557.

Xiong X, Zhou X, Li M, Xul B, Dengl H, Yul Q, Gao X. 2019. Pollen morphology in *Rubus* (Rosaceae) and its taxonomic implications. Plant Syst Evol. 305: 705–716.

Zieliński J. 1991. *Rubus laciniatus* i *R. armeniacus* (Rosaceae) we florze Polski. [*Rubus laciniatus* and *R. armeniacus* (Rosaceae) in the flora of Poland] Fragm Flor Geobot. 35: 217–224.

Zieliński J. 2004. The genus *Rubus* (Rosaceae) in Poland. Pol Bot Stud. 16: 1–300.