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Pollen morphology of Polish species of genus *Rubus* – Part I. *Rubus gracilis*

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Abstract: Pollen morphology and pollen variability of *Rubus gracilis* were studied. A total of 260 grains from 13 natural Polish localities were examined. Important characteristics include: a stretched bridge; a visible pore area and endopores; ectocolpi arranged regularly, more or less evenly spaced or, more rarely, joining one another in the apocolpium, long (79.3% length of the polar axis) and narrow; exine sculpture striate, distinct; striae and muri of equal width, rather narrow; striae usually running parallel to the polar axis, sometimes forming semicircles in the apocolpium zone; with distinct perforations. The size, outline and shape turned out to be poor criteria when identifying the species. A statistical analysis of 10 quantitative grain characteristics showed their little variability. The highest variability was found to occur in two traits connected with *d* (the distance between the apices of two ectocolpi). Statistical studies revealed no differences among the grains from the individual localities, likely the result of apomixis.

Additional key words: statistical analysis, intraspecific variability, LM, SEM

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Introduction

Poor morphological knowledge of the genus *Rubus* L. in Europe poses serious problems of a taxonomic nature owing to apomixis. Apomixis is characteristic almost exclusively of the subgenus *Rubus*, which embraces most of the European bramble species, including *R. gracilis*. Apomixis in brambles gives rise to grains that are mature and of typical structure, as well as much smaller, and not fully developed pollen. Facultative apomicts produce fewer undeveloped grains (a few per cent) than obligate ones, in which they constitute from 10 to 25% (Sudre 1917).

Pollen grain morphology in representatives of the genus *Rubus* has frequently been the subject of re-

search. The European species have been studied by: Erdtman (1952), Erdtman et al. (1961), Teppner (1965), Reitsma (1966), Stachurska et al. (1974–1975), Kuprianowa and Alyoshina (1978), Eide (1981 a, b), Fedoronchuk and Savitsky (1987), Faegri and Iversen (1989), Moore et al. (1991) and Tomlik-Wyremblewska (1995), Asiatic and north-American species have been analysed by Ikuse (1965), Huang (1972), Naruhashi and Takano (1980), Kosenko et al. (1982), Zhou et al. 1999, Tomlik-Wyremblewska (2000) and Tomlik-Wyremblewska et al. (2004).

In spite of those numerous studies, our knowledge of the morphological structure of *Rubus* grains is still only fragmentary because there are no palynological

monographs of this genus, notoriously difficult taxonomically.

The diagnostic features of pollen grains of the genus *Rubus* include: its exine sculpture (type of sculpture, width and orientation of striae and vallaes), and characteristics of apertures (length and arrangement of ectocolpi; type of bridge – contracted or stretched, number and size of granules on colpus membrane and number of perforations).

The present article is the first of a series on the morphology of pollen grains of the Polish species of the genus *Rubus*. The object of study will be 23 bramble species from all the four subgenera occurring in Poland (subg. *Chamaerubus*, subg. *Cylactis*, subg. *Idaeobatus*, subg. *Rubus*) and 21 series: *Rubus chamaemorus* L., *R. saxatilis* L. (*Saxatiles*), *R. idaeus* L., *R. nessensis* HALL (*Nessenses*), *R. plicatus* Weihe & Ness (*Rubus*), *R. grabowskii* Weihe ex Günther & al. (*Discolores*), *R. gracilis* J. Presl & C. Presl (*Rhamnifolii*), *R. macrophyllus* Weihe & Nees (*Sylvatici*), *R. sprengelii* Weihe (*Sprengeliani*), *R. pyramidalis* Kaltenb. (*Vestiti*), *R. glivicensis* (Sprib. ex Sudre) Sprib. (*Micantes*), *R. radula* Weihe (*Radulae*), *R. schnedleri* H. E. Weber (*Pallidi*), *R. koehleri* Weihe (*Hystrix*), *R. pedemontanus* Pinkw. (*Glandulosi*), *R. lamprocaulos* G. Braun (*Subrectigeni*), *R. czarnunensis* (Sprib.) Sprib. (*Sepincoli*), *R. gothicus* Frid. & Gelert ex E. H. L. Krause (*Subthysoidei*), *R. camptostachys* G. Braun (*Subsylvatici*), *R. fasciculatus* P. J. Müll. (*Subcanescentes*), *R. fabrimontanus* (Sprib.) Sprib. (*Subradulae*), *R. seebergensis* Pfuhl ex Sprib. (*Hystriopsis*) and *R. caesius* L. (*Caesii*). The research will be summed up by detailed descriptions of the pollen grains as well as a key for the identification of the domestic species of the genus *Rubus* on the basis of their pollen grain morphology.

Rubus gracilis J. Presl & C. Presl (syn. *R. villicaulis* Köhler ex Weihe & Nees) belongs to the subgenus *Rubus*, series *Rhamnifolii* (Weber 1995, Zieliński 2004). It is a species distributed throughout northern and central Europe (Weber l.c.). In Poland it can be found in the central and southern regions as well as in a few western localities of West Pomerania (Zieliński 2001). Morphologically, it is one of the most variable bramble species in Poland. Despite this variability, the species is easy to identify because of its fairly strong, permanently patent-hairy shoots, strong, dark-reddish prickles, and usually strongly armed inflorescences (Zieliński 2004).

Rubus gracilis was selected for analysis because its pollen grains have already been studied by Tomlik-Wyremblewska (1995). Her work presents their morphological characteristics with SEM and LM photographs. In accordance with the principles adopted in this type of research, she based her results on the measurements of 10 grains coming from one sample, while in the present study 260 grains from 13 samples were measured. A comparison of the data ob-

tained by the cited author and the present authors will make it possible to establish the extent to which measurements of grains from a small sample differ from those from a large one. The data will also allow later statistical comparisons of the properties of *R. gracilis* with other Polish bramble taxa selected for examination.

The aim of the study was to describe the morphology of *Rubus gracilis* pollen grains on the basis of a large sample under LM and SEM, and then to discuss the opinions of other authors concerning the importance of the diagnostic traits of the pollen grains of this species

Material and methods

Dried material came from the Herbarium of the Institute of Dendrology, Kórnik (Poland). The pollen grains were collected from 13 localities in the wild (Table 1). Twenty pollen grains were examined for each collection; 260 pollen grains in total. They were analysed for 10 quantitative features and the following qualitative ones: outline, shape, type of the bridge and exine sculpture (Table 2). Only fully developed grains were measured.

All samples were acetolysed according to Erdtman's method (1952), with insignificant modifications (Wrońska-Pilarek 1998). The terminology follows Erdtman (l.c.); Reitsma (1970); Hebda et al. (1988 a, b); Hebda, Chinnappa (1990); Punt et al. (1999) and Halbritter et al. (2005). The classes of exine thickness were determined on the basis of the thickness in selected species of the *Rosaceae* (Eide 1981 a). Relative exine thickness is the ratio of exine thickness measured along the P (Exp) axis to the length of the P axis, and along the E (Exe) axis to the length of the E axis.

The observations were carried out both with light microscope (Biolar 2308, Nikon HFX-DX) and scanning electron microscope (Hitachi S-3000N).

The empirical data from grain measurements embraced quantitative features listed in table 2. Their analysis included descriptive statistics and correlation coefficients, univariate analysis of variance and Tukey's procedure, agglomerative grouping by Ward's method – dendrogram (Caliński, Kaczmarek 1973; Karoński, Caliński 1973; Dobosz 2001).

Results

Pollen morphology of *Rubus gracilis*

J. Presl & C. Presl

The grains of *Rubus gracilis* are zonocolporate (Figs 1–6, Table 3). Pollen size is usually medium (91.5%), rarely small (8.5%). Subprolate grains predominate, prolate and prolate spheroidal shapes are much less

Table 1. Location of studied pollen samples of *Rubus gracilis* (KOR – Herbarium of the Institute of Dendrology, Kórnik)

No of sample	Location	Longitude E	Latitude N	Collector, date of collection, herbarium, number
1.	Prov. Dolnośląskie, Golejów, Pogórze Izerskie	15°35'	51°01'	Awzan L, Boratyński A., 30.07.1987, KOR 30885
2.	Prov. Podkarpackie, Łubienko	21°33'	49°39'	Oklejewicz K., 17.07.1989, KOR 29217
3.	Prov. Podkarpackie, near Wesoła	22°08'	49°45'	Oklejewicz K., 06.07.1992, KOR 29275
4.	Prov. Wielkopolskie, Gołaszyn	16°50'	52°37'	Ferstch W., 01.07.1964, KOR 11123
5.	Prov. Podkarpackie, near Wólka Sokołowska	22°11'	50°15'	Oklejewicz K., 17.07.1993, KOR 30539
6.	Prov. Lubuskie, Pieski	15°26'	52°25'	Zieliński J., 28.08.1992, KOR 29957
7.	Prov. Wielkopolskie, near Mięskowo	17°25'	52°05'	Awzan L, Zieliński J., 09.07.1986, KOR 30866
8.	Prov. Lubuskie, near Glisno	15°14'	52°28'	Zieliński J., 31.07.1975, KOR 7180
9.	Prov. Dolnośląskie, Rudawy Mountains	15°55'	50°53'	Boratyński A., Zieliński J., 09.09.1987, KOR 30875
10.	Prov. Łódzkie, near Dzierżnki	18°36'	51°06'	Zieliński J., 14.07.1993, KOR 30508
11.	Prov. Małopolskie, near Droginia	20°03'	49°51'	Zieliński J., 24.07.1991, KOR 24811
12.	Prov. Podkarpackie, Kraczkowa	22°12'	50°03'	Oklejewicz K., 08.07.1992, KOR 29268
13.	Prov. Podkarpackie, Grudna Kępska	21°18'	49°44'	Oklejewicz K., 27.07.1989, KOR 25593

frequent. Exine is two-layered, with ectexine and endexine of about the same thickness. Exine thickness is medium, with a very narrow range of thickness (1.8–2.0 mm). The value and range of the coefficients of the relative thickness of the exine measured along P (Exp) and along E (Exe) are similar, which indicates that thickness of exine measured along both axes is similar (cf. Table 3).

Exine sculpture distinct, striate. Striae and muri of similar width. Muri rather narrow, cylindrical, running \pm parallel to polar axis, straight, less frequently bifurcating, forming semicircles near apocolpium. Striae usually rather narrow. On their bottom, more or less numerous elliptic or circular perforations of similar diameters.

Apertures consist of 3 ectocolpi and 3 endopores. Ectocolpi narrow, arranged in equatorial zone, regular, more or less evenly spaced, but sometimes joining in polar area. Usually long, ending sharply. Width variable, usually greatest in equatorial region where a bridge occurs. Ectocolpus margins irregular, without costa. Bridge stretched, therefore endopores and pore area more or less visible. Sculpturing of ectocolpus

membrane granulate. The mean apocolpium index, or d/E ratio, is 0.18. Its value differs substantially and ranges over an interval of 0.08 to 0.33. Such a wide range indicates that the distance between adjacent ectocolpi is variable because colpi have different lengths and lie at different distances from the apocolpium, sometimes even touching it. Endopore indistinct, usually located in the middle of ectocolpus with fastigium (cf. Table 3).

Intraspecific variability

The empirical data concerning pollen measurements covered the following features: P, E, Exp, Exe, Le, d, P/E ratio and d/E ratio (cf. Table 2, 4).

Features P, E, P/E ratio and Le showed low variability (7.2%, 7.1%, 6.4%, 8.8%, respectively), while

Table 2. Pollen grains traits analysed

No	Traits
1.	Length of polar axis (P)
2.	Length of equatorial axis (E)
3.	Thickness of exine along polar axis (Exp)
4.	Thickness of exine along equatorial axis (Exe)
5.	Length of ectocolpi (Le)
6.	Distance between the apices of two ectocolpi (d)
7.	P/E ratio
8.	Apocolpium index (d/E ratio)
9.	Relative thickness of exine (P/Exp ratio)
10.	Relative thickness of exine (E/Exe ratio)

Fig. 1. (LM). Pollen grain of *Rubus gracilis* in equatorial view; two ectocolpi with bridge and exine visible ($\times 1000$)



Fig. 2. (LM). Pollen grain of *Rubus gracilis* in polar view; polar area with two ectocolpi and exine sculpture visible ($\times 1000$)

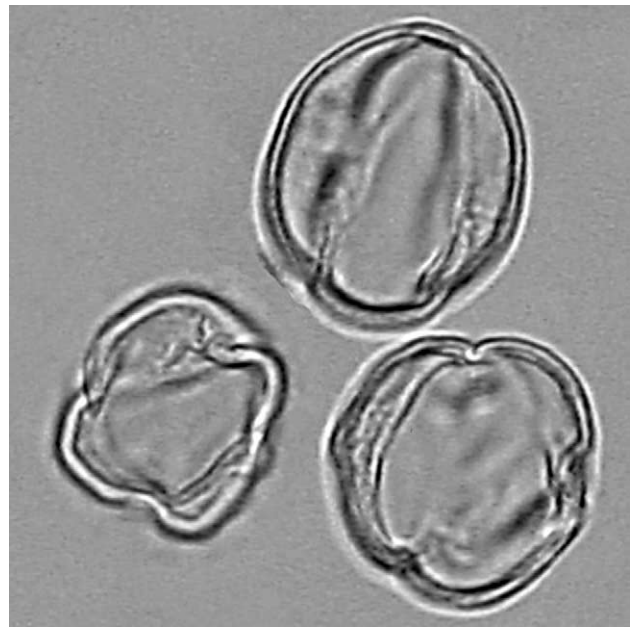


Fig. 3. (LM). Three pollen grains of *Rubus gracilis* in polar and equatorial view; ectocolpi and exine visible ($\times 1000$)



Fig. 4. (SEM). Pollen grain of *Rubus gracilis* in equatorial view; two ectocolpi with bridge and exine sculpture visible ($\times 3500$)

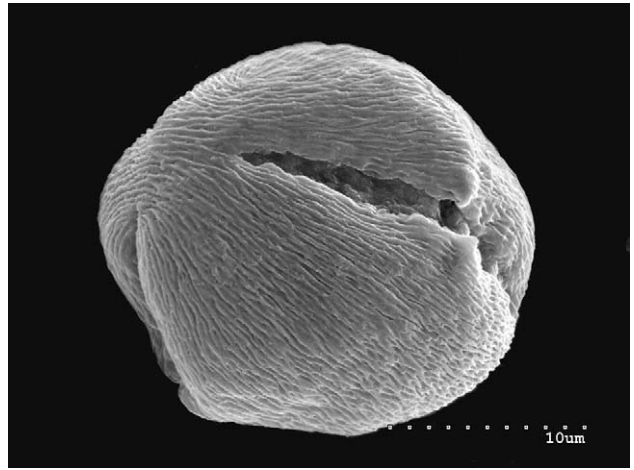


Fig. 6. (SEM). Pollen grain of *Rubus gracilis* in polar view; ectocolpus with one opened endopore visible ($\times 3500$)

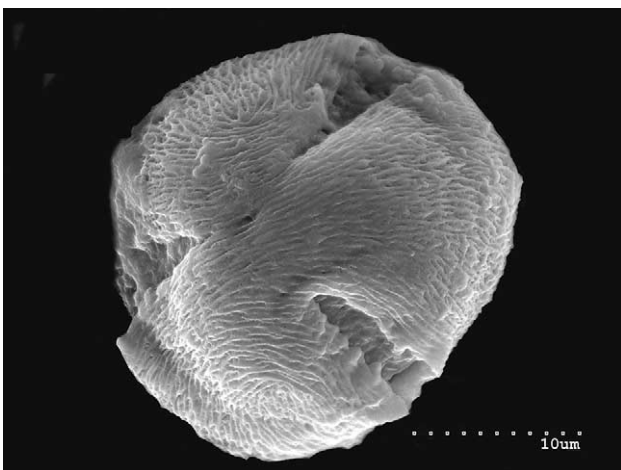


Fig. 5. (SEM). Pollen grain of *Rubus gracilis* in polar view; polar area with three ectocolpi and exine sculpture visible ($\times 3500$)

the variability of d and d/E ratio is higher and averages (22.3%, 22.0%, cf. Table 4).

In turn, Exp and Exe did not display any variability and assumed the value 99% in $2 \mu m$ measurements. That is why they were left out of further statistical analysis, and so were two derivatives of P/Exp ratio and E/Exe ratio.

On the basis of an analysis of the diagonal elements of the inverse of the R correlation matrix for the features: P , E , Le , d , P/E and d/E , those selected for further study were ones with low values of the diagonal element, which is indicative of a weak correlation between the given feature and the others. Thus, the following arrangement of features was chosen: P with the diagonal element value of 3.72, E – 1.58, Le – 2.97 and d – 1.10.

Table 3. Summary of the pollen characteristics of *Rubus gracilis* according to Tomlik-Wyremblewska (1995) and the author's of this paper

Traits	Tomlik-Wyremblewska 1995	Author's of this paper
Mean length of polar axis (P) and its range [μm]	23.7	27.86
	22–25	24–32
Mean length of equatorial axis (E) and its range [μm]	18.1	22.35
	16–20	18–26
Mean P/E ratio and its range	1.3	1.25
		1.08–1.50
Outline in polar view	circular to subtriangular	circular to triangular obtuse convex
Outline in equatorial view	elliptical	elliptical
Pollen shape [%]	–	80.0
	subprolate	3.1
	prolate spheroidal	16.9
	prolate	as beside
Bridge	present with margins constricted	as beside
Apertures	Pori and pore area Colpi	as beside
	long, narrow, 1.2 μm in the widest part; acute, colpus membrane granulate, extending 86–90% length of the polar axis	long – 22.09 (16–26) μm , extending 79.3 (67.7–86.7)% length of the polar axis, 1.2–1.4 μm in the widest part, acute, colpus membrane granulate
Ornamentation	striate; the muri and the striae about the same width, in slightly sinuous arrangement with perforations and microperforations	as beside
The orientation of the muri or striae	muri mostly parallel to the colpus, slightly sinuous on the mesocolpium; swirling on the poles; 2.5–5.5 μm long, 0.15 μm wide, with obtuse ends often merge into perforate tectum	muri running \pm parallel to polar axis, straight, less frequently bifurcating, forming semicircles near apocolpium
Anastomoses range per 10 μm	3–5	–
Exine thickness [μm]	1.5–1.6	2.0
		1.8–2.0
Exp/P	–	0.07 (0.06–0.08)
Exc/E	–	0.09 (0.08–0.11)
Exine subunits thickness [μm]	columnellae: 0.24–0.3 nexine: 0.34–0.4 tectum: 0.6–0.7	–
Apocolpium index	–	0.18
		0.08–0.33
Participation of deformed pollen grains [%]	–	30–40
Morphological variability	little variation in shape	cf. subchapter: Intraspecific variability

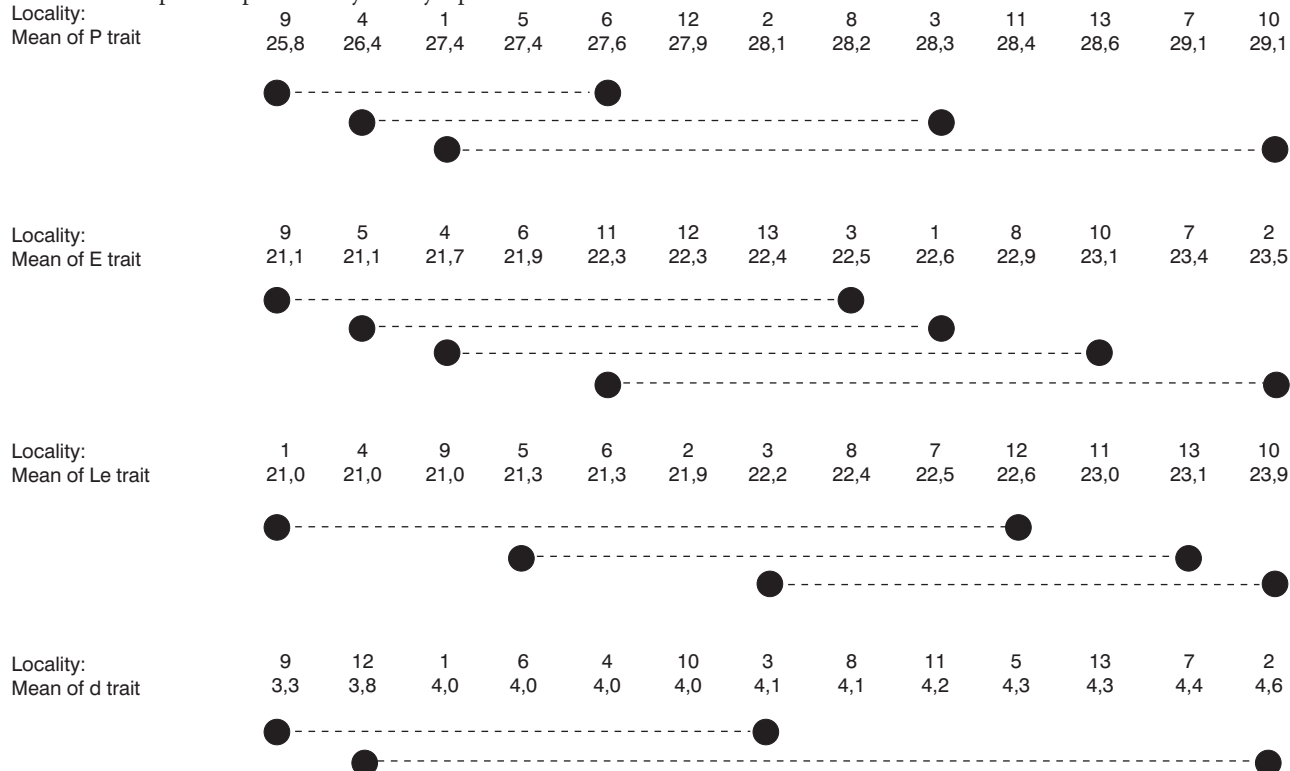
Table 4. Descriptive statistics of traits of studied pollen grains

Traits Locality	P				E				P/E				Exp				Exe			
	minimum	arithmetic mean	maximum	variance coefficient (%)	minimum	arithmetic mean	maximum	variance coefficient (%)	minimum	arithmetic mean	maximum	variance coefficient (%)	minimum	arithmetic mean	maximum	variance coefficient (%)	minimum	arithmetic mean	maximum	variance coefficient (%)
1	24	27.4	31	7.5	20	22.6	26	7.7	1.08	1.21	1.40	7.4	2.0	2.0	2.0	0.0	2.0	2.0	2.0	0.0
2	26	28.1	31	5.5	20	23.5	26	6.7	1.08	1.20	1.36	7.0	2.0	2.0	2.0	0.0	2.0	2.0	2.0	0.0
3	24	28.3	32	7.3	20	22.5	24	5.5	1.17	1.26	1.36	6.0	2.0	2.0	2.0	0.0	2.0	2.0	2.0	0.0
4	24	26.4	29	5.7	18	21.7	24	7.5	1.17	1.22	1.44	5.5	1.8	2.0	2.0	2.2	2.0	2.0	2.0	0.0
5	24	27.4	32	8.2	20	21.1	24	5.7	1.18	1.30	1.45	5.6	2.0	2.0	2.0	0.0	2.0	2.0	2.0	0.0
6	24	27.6	31	8.6	18	21.9	26	10.0	1.15	1.26	1.40	5.8	2.0	2.0	2.0	0.0	2.0	2.0	2.0	0.0
7	26	29.1	32	6.9	20	23.4	26	6.3	1.15	1.24	1.36	5.4	2.0	2.0	2.0	0.0	2.0	2.0	2.0	0.0
8	26	28.2	30	5.0	22	22.9	24	4.5	1.17	1.23	1.36	5.6	2.0	2.0	2.0	0.0	2.0	2.0	2.0	0.0
9	24	25.8	30	7.5	18	21.1	22	5.7	1.09	1.23	1.50	7.6	2.0	2.0	2.0	0.0	1.8	2.0	2.0	2.2
10	26	29.1	30	4.2	22	23.1	26	5.0	1.15	1.26	1.36	5.8	2.0	2.0	2.0	0.0	2.0	2.0	2.0	0.0
11	26	28.4	32	6.3	20	22.3	24	5.4	1.17	1.28	1.43	6.6	1.8	2.0	2.0	2.2	2.0	2.0	2.0	0.0
12	26	27.9	30	4.7	20	22.3	24	5.4	1.17	1.25	1.36	4.4	2.0	2.0	2.0	0.0	1.8	2.0	2.0	2.2
13	24	28.6	32	6.5	20	22.4	26	5.9	1.15	1.28	1.43	6.2	2.0	2.0	2.0	0.0	2.0	2.0	2.0	0.0
Traits Locality	Le				d				d/E											
	minimum	arithmetic mean	maximum	variance coefficient (%)	minimum	arithmetic mean	maximum	variance coefficient (%)	minimum	arithmetic mean	maximum	variance coefficient (%)								
1	18	21.0	24	7.2	3	4.0	6	15.3	0.13	0.18	0.25	15.6								
2	20	21.9	26	9.1	2	4.6	6	22.7	0.08	0.20	0.27	23.6								
3	20	22.2	26	8.2	3	4.1	6	16.9	0.13	0.18	0.27	17.7								
4	20	21.0	24	5.8	2	4.0	6	31.4	0.08	0.18	0.30	29.8								
5	18	21.3	26	9.8	3	4.3	6	25.2	0.14	0.20	0.27	22.0								
6	16	21.3	26	11.9	2	4.0	6	23.9	0.08	0.18	0.33	26.5								
7	20	22.5	26	8.6	3	4.4	6	21.5	0.13	0.19	0.25	19.2								
8	20	22.4	24	6.2	3	4.1	5	9.7	0.13	0.18	0.23	11.0								
9	20	21.0	26	8.5	2	3.3	4	24.3	0.09	0.16	0.20	23.2								
10	22	23.9	26	3.9	3	4.0	6	19.9	0.13	0.17	0.26	19.7								
11	20	23.0	26	7.7	2	4.2	6	22.5	0.09	0.19	0.27	22.7								
12	20	22.6	26	6.9	2	3.8	5	17.0	0.09	0.17	0.23	17.8								
13	18	23.1	26	8.2	3	4.3	6	20.0	0.14	0.19	0.30	22.7								

To verify the hypothesis about the effect of the location of the sites of the 13 specimens on the pollen grain parameters under study, a univariate analysis of variance was carried out. The assumptions verified included that of distribution normality (the Shapiro-Wilk test) and variance uniformity (the Levene test). Since the parameters showed no uniformity, a logarithmic

transformation was employed. When analysing the empirical value of F statistic and probability p , for each feature under study the differences were found to be significant at the 5% significance level (feature P – $F_{\text{calc}} = 5.73$ i $p = 0.000$, cecha E – $F_{\text{calc}} = 5.96$ i $p = 0.000$, cecha Le – $F_{\text{calc}} = 5.55$ i $p = 0.000$ oraz cecha d – $F_{\text{calc}} = 2.68$ i $p = 0.002$). x). In analysing the particu-

Table 5. Multiple comparisons by Tukey's procedure



lar specimens (1–13), a lack of significant differences for each feature was marked by two points joined with a dashed line (Table 5).

In order to distinguish the most homogeneous clusters of pollen grains collected from specimens in localities marked from 1 to 13 by the criterion of the

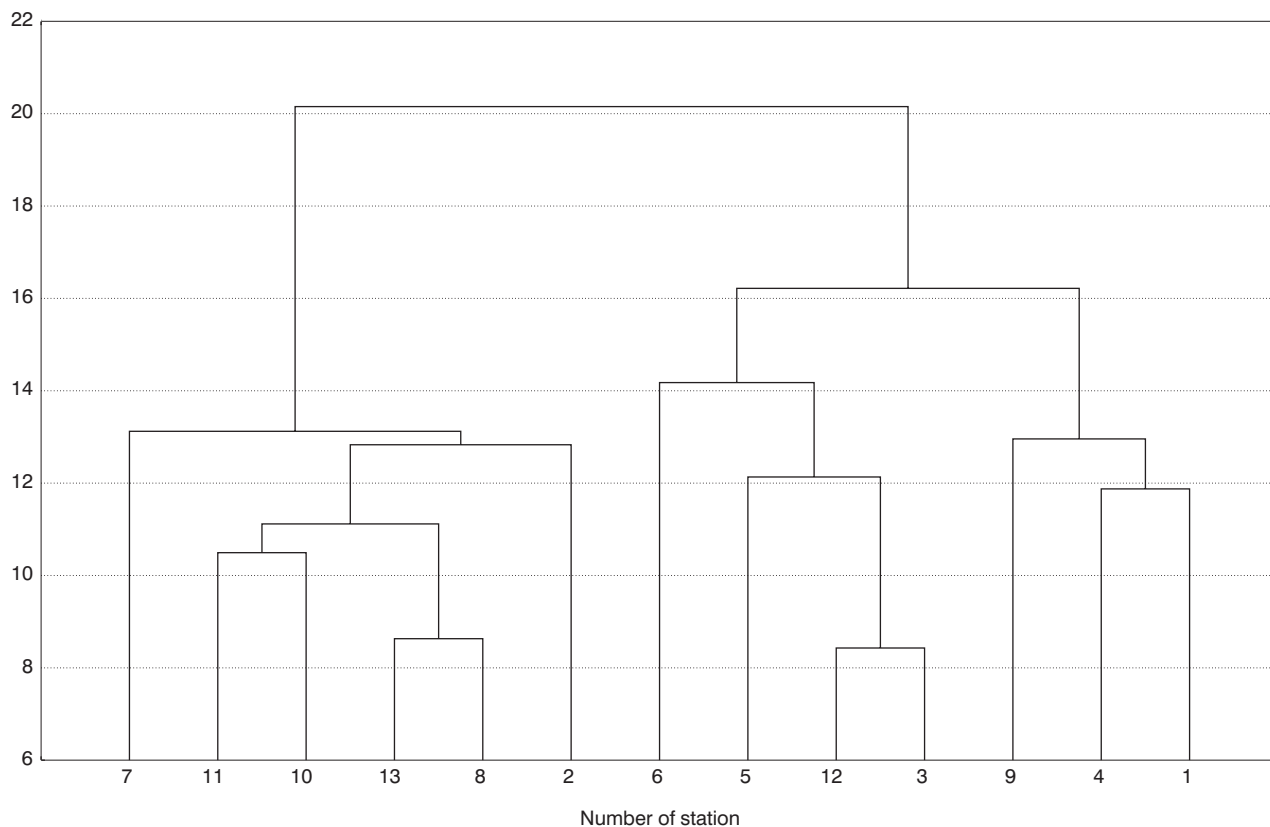


Fig. 7. Dendrogram of 13 localities for pollen grains of *Rubus gracilis* constructed on the basis of Ward's method

features under study (P, E, Lb and d), an agglomerative grouping by Ward's method was carried out to obtain a dendrogram (Fig. 7). The grouping showed the grains to fall into two groups. The first embraced localities 7, 11, 10, 13, 8 and 2, and the other 6, 5, 12, 3, 9, 4 and 1 (cf. Table 1).

Discussion

The above description of pollen grains of *Rubus gracilis* accords with the only characterisation of this species available in the palynological literature, given by Tomlik-Wyremblewska (1995).

The differences concern the length of the axes P and E as well as the exine thickness. The present authors recorded the mean length of polar axis (P) greater by about 4 μm and its range from 24 to 32 μm , while in Tomlik-Wyremblewska (1995) it amounted 22 to 25 μm . As a consequence, a much smaller proportion of small grains was recorded and a greater proportion of medium-sized ones. The same holds for the mean length of the equatorial axis (E) and its range. The results presented above differ by about 3 μm from those given by Tomlik-Wyremblewska (l.c.), and the ranges are 18–26 μm and 16–20 μm respectively. Also greater was the exine thickness, at 1.5–1.6 μm , compared to 1.8–2.0 μm in are study. The differences in the results are undoubtedly due to the fact that in the present research a substantial number of grains were measured.

It should be stated that the value of grain size as a diagnostic feature raises doubts because of the considerable grain variability in *Rosaceae* (Naruhashi, Takano 1980; Moore et al. 1991) and the way in which acetolysis was performed (Reitsma 1969).

Conclusions

- In the light of the literature cited above, pollen grains of *Rubus gracilis* have no distinct differentiating features in comparison with the bramble species examined so far. Therefore, their pollen grain morphology can only serve as one of auxiliary characteristics when identifying this species. Their most important features include: the type of bridge (stretched, therefore the pore area and endopores visible though indistinct); ectocolpi (long, averaging 79.3% of the length of the polar axis, narrow, arranged regularly, more or less evenly spaced or, more rarely, joining one another in the polar area); exine sculpture (striate, fairly distinct, striae and muri of equal width, rather narrow, striae running \pm parallel to the polar axis, straight, less frequently bifurcating, forming semicircles in the apocolpium, distinct perforations). The size, outline and shape turned out to be poor criteria when identifying the species.
- Statistical analysis showed that the pollen grains of *Rubus gracilis* are relatively uniform. The highest variability occurs in two parameters, d – 22.3% and d/E ratio (apocolpium index) – 22.0%, while those (P, E, P/E ratio, Le) displaying low variability included – 6.4 – 8.8%. Four exine features – Exp and Exe (thickness of exine along polar and equatorial axis) and relative thickness of exine (P/Exp ratio and E/Ex ratio), showed no variability.
- No statistical relations were found to occur between the morphological character of grains collected from individual plants growing in different localities. There are similarities among grains collected in regions distant from one another, while those coming from neighbouring parts of the country can be different. This result fully corroborates the current opinions about the effect of apomixis on the characteristics of representatives of the genus *Rubus* L. Apomixis leads to the development of plants with the same pollen grain characteristics as the maternal plants, irrespective of the geographical region.

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