Preslia, Praha, 48 : 347—353, 1976

Studies in rose pollen III. Pollen viability and germinability in eight Czechoslovak *Rosa* species

Pyl růží III. Životaschopnost a klíčivost pylu osmi československých druhů růží

Dagmar Jičínská, Marie Naděžda Končalová and Olga Sýkorová

JIČÍNSKÁ D., M. N. KONČALOVÁ et O. SÝKOROVÁ (1976): Studies in rose pollen III. Pollen viability and germinability in eight Czechoslovak *Rosa* species. — Preslia, Praha, 48 : 347—353.

A study of the pollen viability (e.g. positive TTC-reaction) and germinability in *R. pimpinellifolia* L., *R. gallica* L., *R. jundzillii* BESS., *R. canina* L., *R. majalis* HERRM., *R. pendulina* L., *R. arvensis* HUDS. and *R.* × *reversa* WALDST. et KIT. was undertaken. The percentage of morphologically good pollen grains is evaluated and compared with the viability and actual in vitro germinability. The results are discussed with regard to the taxonomy and ecology of the species examined.

Botanical Institute, Czechoslovak Academy of Sciences, 252 43 Průhonice, Czechoslovakia.

INTRODUCTION

Problems of compatibility in the genus *Rosa* as well as morphological and some physiological features of the pollen of wild roses have been dealt with elsewhere (see JIČÍNSKÁ 1975, 1976). The difference between morphologically normal and abortive pollen grains was first appreciated by the French rhodologist CRÉPIN as early as 1889. COLE (1917) published the percentage ratio of morphologically normal to abortive pollen. HARRISON (1921, 1926) concluded from microscopical studies that, in view of the high proportion of abortive pollen, the majority of wild roses were most likely of hybrid origin. HARRISON et BLACKBURN (1927) and ERLANSON (1929, 1931) published the percentages of abortive pollen in some species. CHARBONNEL (1928) supplemented his numerous herbarium labels of published exiccates of the new *Rosa* species from Auvergne with drawings of the microscopic pollen structure. Unfortunately, he failed to draw any conclusions from his observations. FLORY (1950) summarized the available data on this problem and compared them with his own results.

Few authors have studied physiological features of rose pollen. TÄCKHOLM (1922) was the first to mention pollen germinability in his paper on balanced heterogamy. Studies by MAMELI CALVINO (1951), WOHLERS et al. (1962), PEIMBERT et al. (1963) and WOHLERS et MOREY (1963) were mostly focused on cultivated roses. These authors studied the pollen germinability under different conditions in vitro using the lactophenol-cotton-blue test for the pollen viability, and the influence of storage duration on pollination. The individuals and varieties studied were often designated by the number of

the cultivar (WOHLERS et MOREY 1963); therefore no general conclusions on the taxonomy and ecology of the genus can be drawn.

Results of the studies on in vitro germinability of two wild *Rosa* species have been published (KONČALOVÁ 1975a, b). These studies also provided a methodological introduction to the present work dealing with this problem. A comparison of different methods, especially of the TTC-test and direct assessment of germinability in rose pollen, is given elsewhere (SÝKOROVÁ, JIČÍNSKÁ et KONČALOVÁ 1976). In the present paper, the percentage of morphologically normal pollen grains is evaluated and compared with the viability and acutal in vitro germinability of the pollen from 18 shrubs of wild Czechoslovak roses. The results are discussed with regard to the taxonomy and ecology of the eight species studied.

MATERIAL AND METHODS

The plants under study are grown in the rhodological collection of the Botanical Institute, Czechoslovak Academy of Sciences, Průhonice. Following is a list of species examined.

1. Sect. *Pimpinellifoliae* DC.
   R. pimpinellifolia L. (*R. spinosissima* L.)
   R 317, R 5
2. Sect. *Gallicanae* DC.
   R. gallica L. (*R. austriaca* CRANTZ, *R. pumila* JACQ.)
   R 822, R 965
   R. gallica var. *semiplena*
   R 651, R 663
3. Sect. *Jundzilliae* CRÉP.
   R. jundzillii BESS. (*R. trachyphylla* RAU, *R. marginata* auct. non WALLR.)
   R 227, R 392, R 394
4. Sect. *Caninae* DC.
   R. canina L. (incl. var. *lutetiana* (LÉM.) BAK., var. *dumalis* BAK. non BECHSTEIN, *R. fallens* DÉS., cv. *inermis* and others)
   R 37b, R 83
5. Sect. *Cinnamomeae* DC.
   R. majalis HERRM. (*R. cinnamomea* L.)
   R 38, R 161
   R. pendulina L. (*R. alpina* L.)
   R 511, R 528
6. Sect. *Synstylae* DC.
   R. arvensis HUDS. (*R. repens* SCOP., *R. silvestris* HERRM.)
   R 116, R 117

Hybrids: *R.* × *reversa* WALDST. et KIT. (*R. pimpinellifolia* L. × *R. pendulina* L.)
R 258, R 259

These species are native in Czechoslovakia and the plants have been either transplanted from the field or grown from seed. All material was identified by I. Klášterský, an expert in rhodology.

The pollen for both the viability (the term viability is used in the sense of positive reaction of cells on TTC-test) determination and germination experiments was collected and treated as in the previous study (see KONČALOVÁ 1975). The pollen was allowed to germinate at different sucrose concentrations (increasing from 5 to 40% by 5% intervals) in 1.5% agar with 0.01% boric acid at 28° C for 24 hours. For fixation and staining, ethanol-acetic acid (3 : 1, V/V) and Belling acetocarmine, respectively, were used.

The tetrazolium (TTC) test was used for the determination of the pollen viability: 1% solution of 2,3,5-triphenyltetrazolium chloride in Sörensen phosphate buffer pH = 6.8 at 22 to 25° C. The time of incubation varied from 60 minutes to 5 hours in different species.

The viability and germination of the pollen from nine flowers per individual was compared directly during one season. From each flower, 100 pollen grains were taken into account in each experimental variant.

Tab. 1. — Percentage of morphologically normal pollen (a) and of viable pollen (b) according to the TTC-test

Species		1973(a)	1973(b)	1974(a)	1974(b)
R. pimpinellifolia	R 5	91.6	60.6	76.2	51.1
	R 317	91.9	74.8	86.1	71.5
R. gallica	R 651	—	—	69.7	63.0[1])
	R 822	93.6	67.2	81.8	79.1
	R 965	95.3	57.8	77.9	76.9
R. jundzillii	R 227b	—	—	48.4	26.5[1])
	R 392	79.6	20.2	35.4	32.2
	R 394	81.0	22.3	31.0	26.5[1])
R. canina	R 37b	66.1	14.8	29.6	28.2[1])
	R 83	71.2	14.8	42.8	32.9
R. pendulina	R 511	87.4	79.8	92.5	77.9
	R 528	93.8	79.5	88.4	78.2
R. majalis	R 38	—	—	90.3	80.3
	R 161	—	—	97.6	97.3
R. arvensis	R 116	93.5	69.2	95.4	80.1
	R 117	90.8	68.3	93.8	84.2
R. × *reversa*	R 258	90.2	65.2	74.2	62.2
	R 259	94.5	68.3	77.1	53.5

[1]) TTC-test after 5 hours

RESULTS AND DISCUSSION

Morphological evaluation (Tab. 1, 2, Fig. 1, Plate XV). — The smallest percentage of morphologically normal pollen has been found in *R. canina* and *R. jundzillii*, which differ from the other species by their type of meiosis (balanced heterogamy, see TÄCKHOLM 1922). Species with normal type of meiosis produce a larger amount of morphologically good pollen. In 1973, *R. canina* and *R. jundzillii* produced 20 to 30% of morphologically normal

Tab. 2. — Percentage of normal pollen in *Rosa* species, as reported by other authors

Species	CRÉPIN (1889)	COLE (1917)	FLORY (1950)	MAMELI (1951)	KONČALOVÁ (1975)
R. pimpinellifolia	**	50—90	74.2—96.4	—	—
R. gallica	—	25	39.0—77.3	—	—
R. jundzillii	25	—	85.3—91.3	—	25—32
R. canina	33—66	—	9.3—9.8	15—16	—
R. pendulina	—	—	78.5—89.7	—	—

** reported as good

pollen, while in other species the proportion was about 90%. In 1974, the percentages of normal pollen were somewhat lower than in 1973 in all species except *R. pendulina* and *R. arvensis*. This fact may be explained by different climatic conditions during the spring periods. In 1974, when the first flowers of *R. pendulina* had appeared, frosts of $-7°$ C set in. This seems to have influenced the course of meiosis in PMC in all the species except *R. pendulina* and *R. arvensis*. The latter blooms much later, and thus the cold period probably did not affect the development of the pollen.

Pollen viability (Tab.1, Fig. 1, Plate XV). — Like the morphological evaluation of pollen, the results of the TTC-test indicate that *R. canina* and *R. jundzillii* produced the smallest amount of viable pollen, i.e. 13 to 40% of all pollen grains. The percentage in the other species was as follows: *R. pendulina* about 80%, *R. pimpinellifolia*, *R. gallica* and *R.* × *reversa* from 50 to 70%, *R. arvensis* from 70 to 90% and *R. majalis* from 80 to 100%. The results for the two years are very well comparable except *R. canina* and *R. arvensis* which produced higher percentages of viable pollen in 1974.

Pollen germinability in vitro (Fig. 1). — The pollen germinability was in good correlation with the TTC-test (SÝKOROVÁ et al. 1976). Besides the percentage of germinated pollen grains the length of pollen tubes was used as an auxiliary criterion to determine optimal concentrations of sucrose in the cultivation media. The results demonstrate that the optimal concentration of sucrose in agar was rather high in all cases (about 20%).

The demand of sucrose concentration has been apparent from the first studies dealing with the rose pollen. TÄCKHOLM (1922) used 2.5, 5 and 20% sucrose solution, the latest giving the best results. He compared the germinability of several species, but only one exact figure, i.e. 16% for *R. rubiginosa* of the *Caninae* section, was published. This approximately corresponds to our results for *R. canina*.

MAMELI CALVINO (1951) applied 5, 10, 20, 25, 30 and 35% concentrations of sucrose. She found the 20% concentration to be optimal for most roses under investigation. Nevertheless, her table shows that concentrations above 20% have not been applied to many species even though the germinability apparently increased with increasing sucrose concentration. For example, in the botanical species *R. laevigata* and *R. odorata*, the germinability was 15 and 50% in 5% sucrose, 15 and 75% in 10% sucrose and 32 and 82% in 20% sucrose, respectively. Higher concentrations were not applied. The differences between species are presented in Fig. 1, where the pollen germinability is expressed in terms of the percentage of pollen germination and of pollen tube length. In *R. pimpinellifolia*, demands of sucrose concentration varied greatly, from 10 to 25%. Similarly, for *R. pendulina* the optimum range was between 10 and 20%. For their fixed hybrid, *R.* × *reversa*, it was somewhat higher, from 15 to 25%. The pollen of *R. majalis*, belonging to the same section as *R. pendulina*, showed optimum germination in 25 to 35% sucrose. The difference between the optimums for the two species is probably due to different ecological conditions in their native habitats. While *R. pendulina* grows on cold and shady slopes, *R. majalis* prefers warm and moist climate of "cloudy forests" or of river banks. *Rosa pimpinellifolia* mostly prefers a milder climate than *R. pendulina* (compare the distribution in Flora Europaea, 1968). The pollen of *R. arvensis* and *R. gallica*, which, in the climate of Central Europe, bloom in late spring and early summer, germinates well

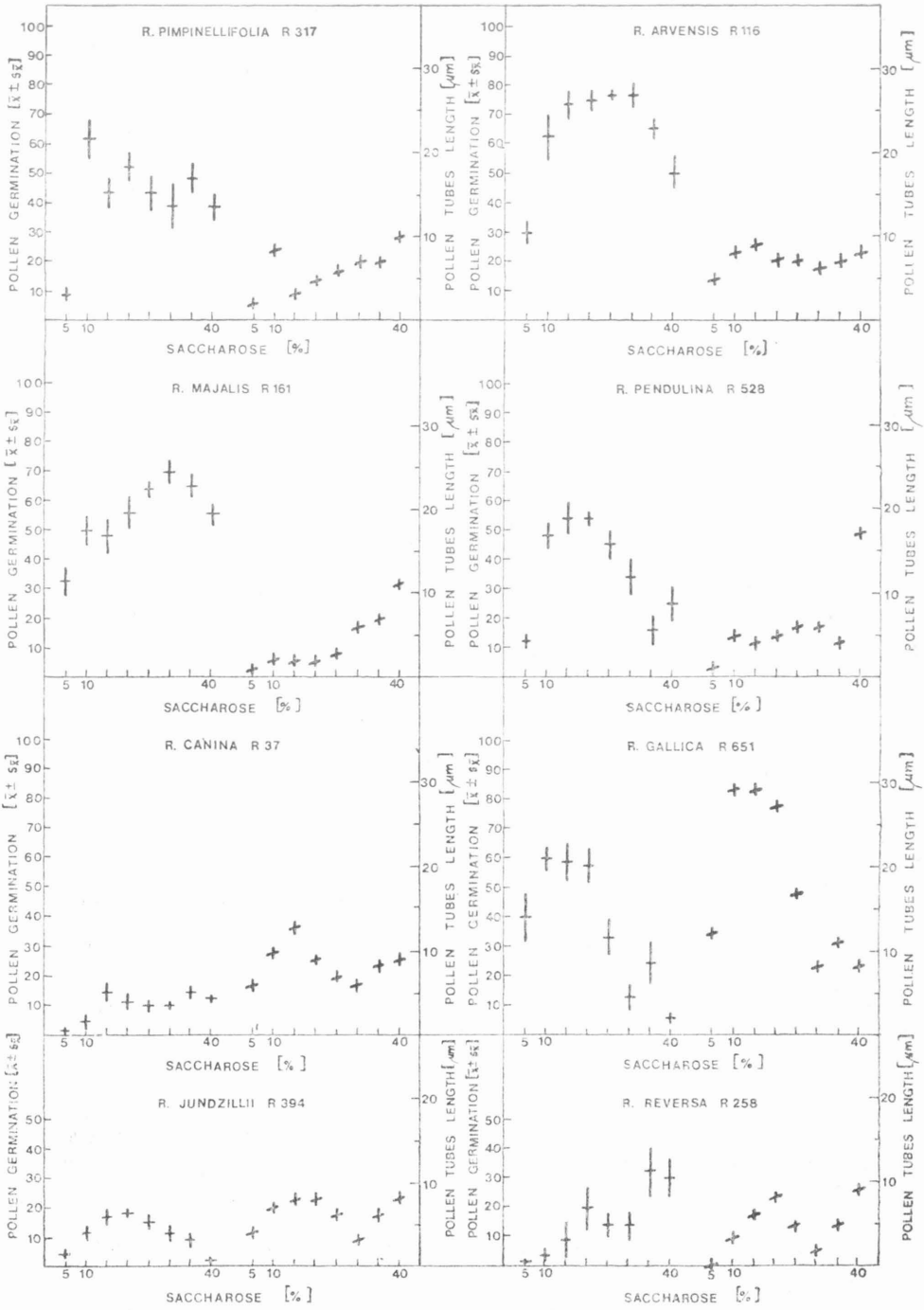

Fig. 1 — Pollen germination in percentage and pollen tubes length.

in a wide range of sucrose concentrations. The pollen of *R. canina* and *jundzillii* germinated quite well in 10 to 25% sucrose. The optimum concentrations seemed to be rather low here: 15% for *R. canina* and 20% for *R. jundzillii*. It is probably the hybrid nature of these two species and their balanced heterogamy that are responsible for the lower plasticity of their pollen demands of germination conditions.

Reviewing the present studies in the light of the results of experiments with calcium (Končalová et al. 1976), the following points may be made: In the presence of Ca in the germination media, the rose pollen germinated in lower sucrose concentrations but the optimum ranges of sucrose concentration were in both cases very similar with regard to the percentage of germination and pollen tubes length (Sýkorová et al. 1975).

SOUHRN

Ze zkoumání morfologie, vitality a klíčivosti pylových zrn u 8 druhů rodu *Rosa* vyplývá, že s výjimkou druhů ze sekce *Caninae* a *Jundzilliae* lze pyl u ostatních druhů považovat za dobrý, vitální a dobře klíčivý. Horší kvalita pylu druhů obou uvedených sekcí je způsobena odlišným průběhem meiose (balancovaná hetrogamie), která je pro obě sekce typická. Druhy sekce *Caninae* jsou determinovány jako ustálené taxonomické jednotky hybridního původu.

Optimální koncentrace sacharózy v agarovém živném médiu jsou poměrně vysoké, pohybují se kolem 20 % u *R. canina*, *R. jundzillii*, *R. pimpinellifolia*, *R. pendulina*, *R.* × *reversa*, cca 30 % u *R. majalis*. Klíčivost pylu *R. arvensis* a *R. gallica* byla vysoká v širokém rozmezí koncentrací (10—30 %) — optimum je mezi 20—25 %. Délka pylových láček po 24 hodinách růstu byla největší na těch koncentracích sacharózy, ve kterých bylo dosaženo nejvyššího procenta naklíčených pylových zrn. Hodnoty nad 35 % koncentrací jsou pravěpodobně nefyziologické, čemuž také odpovídá i veliká variabilita v délce láček.

REFERENCES

Charbonnel J. B. (1928): Roses de France. — Roffiac (Cantal).

Cole R. D. (1917): Imperfection of pollen and mutability in the genus Rosa. — Bot. Gaz,. London, 63 : 110—123.

Crépin F. (1889): Recherches sur l'etat du developpement de pollen dans diverses espèces du genre Rosa. — Bull. Soc. Bot. Belg., Gand, 28 : 114—125.

Erlanson E. W. (1929): Cytological conditions and evidences for hybridity in North American Roses. — Bot. Gaz., London, 57 : 443—506.

— (1931): Sterility in wild roses and in some species hybrids. — Genetics, Princeton, New Jersey, 16 : 75—96.

Flory W. S. (1950): Pollen conditions in some species and hybrids of Rosa, with consideration of associated phylogenetic factors. — Virginia Journ. Sci., Charlottesville, 1 : 11—59.

Harrison J. W. H. (1921): The genus Rosa, its hybridology and genetical problems. — Trans. Nat. Hist. Soc. Northumberland, Durham and Newcastle upon Tyne, 5 : 244—296.

— (1926): New British roses from Northumberland. — Proc. Univ. Durham Philos. Soc., Durham, 8 : 161—167.

Harrison J. W. H. et K. B. Blackburn (1927): The course of pollen formation in certain roses, with some deductions therefrom. — Mem. Hort. Soc., New York, 3 : 23—32.

Jičínská D. (1975): Diversity of pollination in some Rosa species. — Preslia, Praha, 47:267—274.

— (1976): Autogamy in various species of the genus Rosa. — Preslia, Praha, 48: 225—229.

Končalová M. N. (1975a): Studies in rose pollen I. In vitro germination of pollen grains of Rosa hugonis. — Preslia, Praha, 47 : 22—25.

— (1975b): Studies in rose pollen II. Branched pollen tubes. — Preslia, Praha, 47 : 111—116.

Končalová M. N., D. Jičínská et O. Sýkorová (1976): Effect of calcium and of sucrose concentration on pollen germination in vitro of six Rosa Species. — Biol. Plant., Praha, 18(1):26—30.

Mameli Calvino E. M. (1951): Ricerche sul polline del generi rosa. — Ann. Sper. Agr., Roma, 2 : 377—407.

Peimbert R., J. Krewinkle et D. Morey (1963): Factors influencing seed set in roses: II. A preliminary bio-assay of stored and fresh pollen. — Am. Rose Ann., Harrisburg, Pennsylvania, 48: 194—203.

Sýkorová O., D. Jičínská et M. N. Končalová (1976): Comparison of variable pollen germinability in vitro with tetrazolium test for determination of pollen vitality in some species of the genus Rosa. — Genetika a Šlechtění, Praha, (in press).
Täckholm G. (1922): Zytologische Studien über die Gattung Rosa. — Act. Hort. Berg., Stockholm, 7 : 97—381.
Wohlers M. A., R. Peimbert et D. Morey (1962): Factor influencing seed set in Roses. I. Pollen color changes as related to deterioration during storage. — Am. Rose Ann., Harrisburg, Pennsylvania, 47 : 103—109.
Wohlers M. A. et D. Morey (1963): Pactors influencing seed set in roses. III. Determining the actual germinability of rose pollen. — Am. Rose Ann., Harrisburg, Pennsylvania, 48: 199—204.

Received September 2, 1975
Reviewed by J. Tupý

See also Plate XV. in the Appendix.

ERRATA

In the paper "Studies in rose pollen I" by M. N. Končalová (Preslia, Praha, 47 : 22—25), the abbreviation s.e. in the figure caption on p. 23 should read 3 s.e. In Plate IX, 88° C is a misprint for 28° C (M. N. Končalová: Studies in rose pollen II. — Preslia, Praha, 47-111—116.)

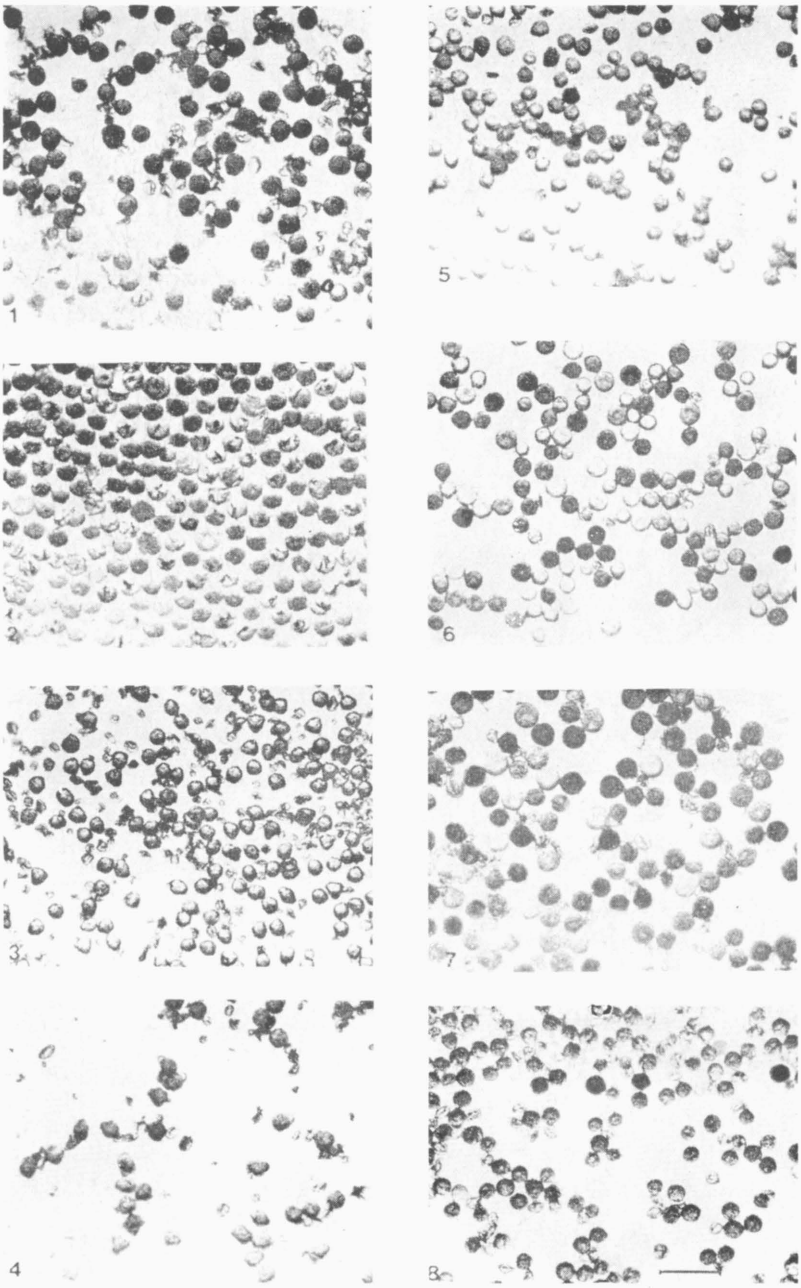

Plate XV. — TTC-test for the rose pollen. 1, *R. pimpinellifolia*. 2, *R. majalis*. 3, *R. canina*. 4, *R. jundzillii*. 5, *R. arvensis*. 6, *R. pendulina*. 7, *R. gallica* 8, *R. reversa*. Scale 10μm

D. JIČÍNSKÁ, M. N. KONČALOVÁ and O. SÝKOROVÁ: Studies in rose pollen III. Pollen viability and germinability in eight Czechoslovak *Rosa* species