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Studies in rose pollen II. Branched pollen tubes

Pyl růží II. Větvené pylové láčky

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Branched pollen tubes were observed in the germinating pollen of some individuals of wild *Rosa* species. The possibility of a connection between their occurrence and the recent hybrid origin of the plants is discussed.

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INTRODUCTION

The branching of pollen tubes has been reported for various systematic groups (see JOHNSTON 1959, HESEMANN 1973, and others). As far as roses are concerned, MAMELI CALVINO (1951) observed forked pollen tubes in some cultivars. Because information on germination of rose pollen is scanty (for references, see KONČALOVÁ 1975), the question was raised, whether the branching of pollen tubes is common and genetically fixed or whether it is dependent upon special environmental conditions.

MATERIAL AND METHODS

The following 24 individuals of 11 species of wild roses belonging to six sections were examined:

Section	Species	Designation of individuals	2n
Pimpinellifoliae DC.	*Rosa pimpinellifolia* L.	R 5	28
		R 317	28*
Gallicanae DC.	*R. gallica* L.	R 965	28*
		R 822	28
	R. gallica L. var. *officinalis* THORY	R 651	28
Jundzilliiae CRÉP.	*R. jundzillii* BESS. var. *trachyphylla* (RAU.) CRÉP.	R 392	42*
		R 394	42*
		R 442	42*
		NK 196/73	42
	var. *heteracantha* (CHRIST) R. KELL.	R 227b	49*
Caninae DC.	*R. canina* L. var. *lutetiana* (LÉM.) BAKER	R 37b	35*
		R 83	35*
	R. rubiginosa L. var. *umbellata* (LEERS) DU MORT	R 428	35*
		R 459	35*
	R. elliptica TAUSCH	R 475	35*
	R. schulzei (R. KELL.) KLÁŠT.	R 686	42*
Cinnamomae DC.	*R. majalis* HERRM.	R 161	14
	var. *elliptica* (MAYER) KLÁŠT.	R 38	28
	R. pendulina L.	R 511	28*
		R 528	28*
Synstylae DC.	*R. arvensis* HUDS.	R 116	14*
		R 117	14*
Hybrids	*R.* × *reversa* WALDST. et KIT.	R 258	28*
	(= *R. pimpinellifolia* × *R. pendulina*)	R 259	28*

R indicates samples from the rose collection of the Botanical Institute, Průhonice. All material was identified by I. Klášterský, a leading expert in the taxonomy of roses. The individual NK 196/73 was collected in the wild (hill Husova kazatelna near Sedlčany, Central Bohemia) and identified by the taxonomist V. Větvička.

The pollen was collected and treated in the same way as described in my previous paper (Končalová 1975). The cultivation medium for pollen germination was 5 to 50% sucrose (in 5% intervals) in: (1) distilled water; (2) solution with calcium ions, according to Kwack 1965 [200 mg/l $MgSO_4 . 7 H_2O$, 100 mg/l KNO_3, 300 mg/l $Ca(NO_3)_2 . 4 H_2O$]; (3) 1.5% agar in distilled water.

To each medium 100 mg/l boric acid was added. For comparison, an observation of the pollen of *R. jundzillii* R 227b in 20% sucrose in agar without any admixture of boric acid was also made. The pollen was incubated at 28°C in germinating boxes for 24 hours, fixed in ethand: acetic acid (3 : 1) and stained with acetocarmine (Němec 1962 : 361). In series (1) and (2) a pollen mixture from 10 flowers was cultivated in Petri dishes, and 500 pollen grains per dish were evaluated. In series (3) 100 pollen grains per flower were evaluated in each concentration (Končalová 1975) to establish the variation range for at least 10 flowers from one shrub.

Chromosome counts marked by an asterisk were made by Klášterská (1969, 1974, unpublished observations), the other have been ascertained by the present author in squashes of apical shoots fixed in acetic acid : ethanol : chloroform (1 : 2 : 1), macerated in ethanol : chloric acid (1 : 1) and stained in lactopropionic orcein.

RESULTS AND DISCUSSION

The rose pollen viability and in vitro germinability under different conditions will be discussed elsewhere.

An examination of 8000 to 18000 pollen grains per individual reveals that in 21 individual the branching of pollen tubes occurs in less than 0.14% (Plate IX, fig. 1, 2, Plate XII, fig. 15—18). In these cases we were unable to find any correlation between the occurrence of branched pollen tubes and the percentage of germination or the environmental conditions. This very low proportion of forked tubes occurs also in hybrids *R. reversa* (R 258, R 259), which arose by spontaneous hybridization of *R. pimpinellifolia* and *R. pendulina*.

Branched pollen tubes were more frequent only in *R. pimpinellifolia* R 317 (Plate XII, fig. 13, 14) and *R. jundzillii* R 227b and NK 196/73 (Plate IX, fig. 3, 4, Plate X, fig. 5—8).

The occurrence of forked tubes was especially striking in some sucrose concentrations in *R. jundzillii* (R 227b). Therefore branched pollen tubes among germinating pollen of this individual vere counted in all experimental variants.

The percentage of morphologically good pollen in R 227b is relatively low (25% in 1973, 37% in 1974), but is comparable with values obtained for other individuals of this species. *Rosa jundzillii* is supposed to be an ancient fixed hybrid of *R. canina* and *R. gallica* (Christ 1884). Because one of the parents, *R. canina*, is also supposed to be a fixed hybrid of unknown origin, the number of aborted pollen grains in *R. jundzillii* is not surprising and the results obtained are in agreement with those of other authors. A review is given by Flory (1950). The shape of a normal pollen grain of *R. jundzillii* is very similar to that of *R. gallica*, i.e. it is oval in equatorial view, usually tri-colporate, but some pollen grains with more germ pores have also been observed (Plate XI., fig. 11, 12). For instance, Mameli Calvino (1951) reports 1% of tetra-colporate pollen grains for *R. canina* and in garden hybrid tea roses they are reported to be very rare ("Briarcliff") to very frequent ("Golden Emblem" or "Texas Centennial"). She does not report any for the other wild species examined, as for instance *R. foetida* Herrm. or *R. indica* Red. et Thor.

If only the percentage of germinated pollen is taken as a criterion to determine optimal conditions for pollen germination, it would appear that 25 to 40% sucrose solution in 1.5% agar and 30—35% sucrose solution with calcium is most suitable for *R. jundzillii* R 227b (Fig. 1). In other individuals

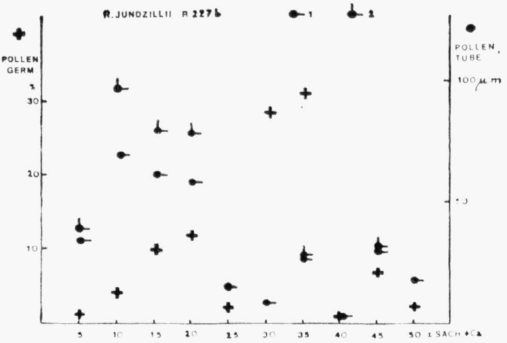

Fig. 1. — *Rosa jundzillii* Bess. R 227b. Pollen germination (% from 500 grains marked +) and pollen tube growth (average from 30 tubes; 1 without, 2 with the second branch) in 5—50% saccharose in Ca-medium after 24 hours' incubation at 28°C.

under investigation pollen tubes with maximal average length occur generally in the same conditions under which the highest percentage of germinating pollen was observed. This is not the case in R 227b, however, where the longest pollen tubes were obtained in 5—10% sucrose solution with calcium (Fig. 1). The highest number of branched pollen tubes also occurred in these concentrations. The values obtained were as follows:

percentage of branched pollen tubes	sucrose solution with calcium
13.3	5%
36.6	10%
30.0	15%
35.3	20%

In other concentrations of this medium the occurrence of branched pollen tubes was as low as in other species. Similar results were obtained in the agar-sucrose medium. In sucrose solution in distilled water the pollen germinated very poorly; the maximal value observed was 7% in 30% sucrose, the mean value being 1.8%; an evaluation of forked tubes was therefore not possible in this medium.

In the genus *Rosa*, branched pollen tubes have been only reported by Mameli Calvino (1951). She mentions this phenomenon in genetically complex cultivars of hybrid tea roses, as for instance "Texas Centennial", "Maddalena", "Golden Emblem" and suggests that the occurrence of forked pollen tubes might be due to the presence of boron in germination media or to the influence of radioactive water. Both these factors strongly increased the germination of pollen. Boron is known to be a non-specific growth stimulant. Not only does it reduce the bursting of pollen in vitro, but it also increases the percentage of germination and the length of pollen tubes (Steffen 1963). For example, Mameli Calvino reports the pollen germinability of "Texas Centennial" rose to be 0.1% in 30% sucrose and 90% in 30% sucrose with 0.001% boric acid, i.e. 900 times higher in the presence of boron. This indicates that the samples studied should be much larger, to discover less frequent abnormalities, such as forked pollen tubes in media without boron.

We tried to germinate the pollen of *R. jundzillii* R 227b and NK 196/73 in 20% sucrose in 1.5% agar without boron. Pollen from 18 flowers was investigated and 200 pollen grains per flower were evaluated. Branched tubes occurred in all but two flowers in which the germinability was lower then 7%. Average pollen germinability under these conditions was 22% of good pollen, and 18% of them had branched pollen tubes. On the contrary, using boron in all other experiments, the frequency of branched pollen tubes was very low in the majority of other wild species, except individuals mentioned above.

When compared with hexaploid plants of *R. jundzillii*, the heptaploid shrub R 227b shows many interesting abnormalities during meiosis in PMC, such as long difuse stage, irregular contraction of chromosomes in late prophase, prometaphase and metaphase as well as multivalent like configurations (see also KLÁŠTERSKÁ 1974, KLÁŠTERSKÁ et NATARAJAN 1974). During the first meiotic division the formation of 14 to 21 bivalents was observed. In spite of difficulties in observation, caused by the small size and large number of chromosomes in second meiotic division, the further course of meiosis seems to be more regular than that in the hexaploid plant. As a result of a more normal anaphase II, the pollen "tetrads" of the heptaploid contain a lower number of pollen grains. It may be concluded therefore that the nuclei of some microspores arose from 14 or more chromosomes, while seven is common for normal *R. jundzillii*.

EIGSTI (1940a, b) noticed that branched pollen tubes are especially frequent after colchicine treatment causing similar abnormalities during cell division. This supports the assumption that forked pollen tubes of the rose examined might be in connection with the abnormalities described above.

The origin of this plant is uncertain. Despite its morphological identity with typical representatives of *R. jundzillii*, the possibility cannot be excluded that it arose from hybridization not before long. In 1935, seeds of *R. jundzillii* were collected on a basalt slope below the top of the hill Soudničný vrch (formerly Richterstein) in the České středohoří Mts., N. Bohemia. Only few seedlings germinated. One of them was grown in the collection under no. R 84 until 1960 when it died. Seeds from free pollination were sown out again in 1941 and one selected seedling has been grown in the collection under no. R 227b. Herbarium material from the parent shrub, from shrub R 84 and from individual R 227b is available. These herbarium specimens conform with each other and belong beyond any doubt to *R. jundzillii* BESS. (KLÁŠTERSKÝ, personal communication.) The only difference is the height of plants. On Richterstein there occur low shrubs, whereas in the collection, R 227b is about two meter high (in 1974). Environmental conditions in both habitats are not comparable, though. Another heptaploid plant was recently found in the above locality, where this individual could possibly arose by crossing *R. jundzillii* (egg cell 35 chromosomes) with *R. gallica* (pollen 14 chromosomes). Both these species are growing there in the proximity of *R. canina* and their flowering times largely coincide. Possibly not only the heptaploid, but also some hexaploid shrubs of *R. jundzillii* in that locality are of recent hybrid origin (unpublished cytological observations).

The occurrence of forked pollen tubes in *R. jundzillii* NK 196/73 is difficult to explain, because nothing is known of the origin of this individual. This was the only shrub from natural habitat, used as a source of pollen for germination experiments. Owing to the 14 days' storage at 25°C, the physiological properties of this pollen might not have been entirely identical with those of the other samples from our rose collection. The values obtained are therefore not quite comparable and are listed here only for the sake of completeness. A recent hybrid origin of this individual cannot be excluded. It also has the typical appearance of the variety, as for example R 442. In the native habitat it is accompanied by several species of the section *Caninae*, but no *Rosa gallica* has been found in the surroundings (VĚTVIČKA, personal communication). Therefore a cytological observation was made and the somatic chromosome number was found to be 2n=42. Meiotic division has not yet been studied, because of the late date of collection. Should NK 196/73 prove to be a hybrid between *R. jundzillii* and a member of the section *Caninae*, the chromosome number would be the same as in *R. jundzillii:* 2n=42, i.e. 35 from the egg cell of *R. jundzillii* and 7 from the pollen of *R. canina* or other species of that section. It is virtually impossible for this case to be recognized either morphologically or cytologically.

The third individual with relatively numerous forked pollen tubes is *R. pimpinellifolia* R 317. Its exact genetic origin is not known, either. This shrub was obtained from municipal rose nursery at Ďáblice, Prague, in 1949. The morphological and cytological features correspond to the description of the species. Hybridization experiments suggest that this individual may be of recent hybrid origin, because the F_1 generation is not uniform when using this shrub as a maternal or paternal plant (JIČÍNSKÁ, unpublished results).

STEFFEN (1963) suggests that in groups, in which branched pollen tubes do not normally develop, their occurrence is connected with the hybrid condition. An example is triploid pear trees (ZVONÍČKOVÁ-SOSNOVÁ 1949) or triploid apple trees (TUPÝ, personal communication). If the branched pollen tubes in roses are an evidence of a recent hybrid origin, they might prove useful in identifying hybrids when morphological features fail.

The results of the above observations may be sumarized as follows:

1) In wild roses, branched pollen tubes in pollen cultures in vitro are not at all frequent.

2) The occurrence of forked pollen tubes is probably due to unbalanced physiological conditions which could be in connection with a recent hybrid origin of the plants.

3) When under optimal germination conditions forked pollen tubes occur in more than 1% of germinated pollen, the plant may be supposed to be of hybrid origin.

4) The low frequency of forked pollen tubes is no evidence of a non-hybrid nature of the individual.

SOUHRN

U jedenácti druhů z šesti sekcí rodu *Rosa* bylo sledováno klíčení pylu in vitro v různých podmínkách. Z pozorování 8 000 až 18 000 pylových zrn u každého ze sledovaných 24 keřů vyplývá, že výskyt větvených pylových láček v kulturách pylu planých růží není obecným a častým jevem. Rozvětvené pylové láčky se vyskytovaly ve větší míře pouze u tří rostlin, které byly sice morfologicky shodné s jinými jedinci stejného druhu, ale jejichž některé genetické vlastnosti opravňují k předpokladu, že se jedná o recentní hybridy. Poněvadž větvení pylových láček u růží bylo dosud pozorováno pouze u kultivarů s mnohonásobně heterozygotním charakterem, je vyslovena domněnka, že větvení pylových láček by mohlo být pomocným kriteriem při potvrzení hybridního stavu zkoumaných jedinců rodu *Rosa*.

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See also plates IX.—XII. in the Appendix.

G. Jacobi (ed.):

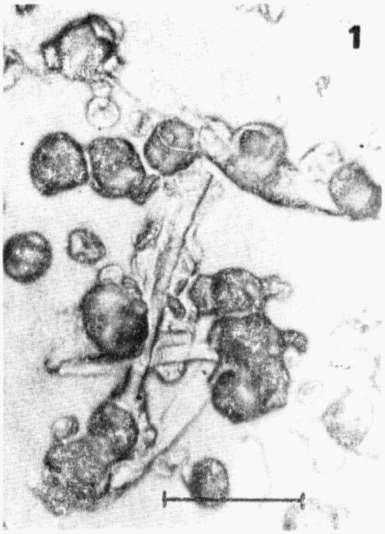
Biochemische Cytologie der Pflanzenzelle

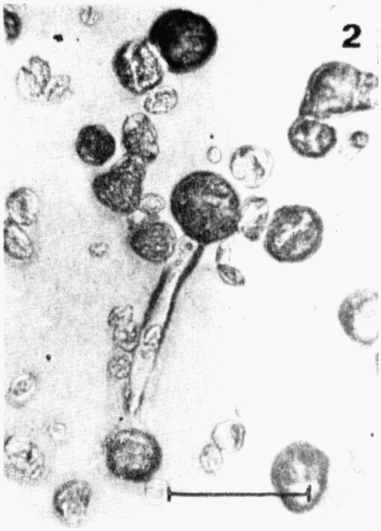
Ein Praktikum

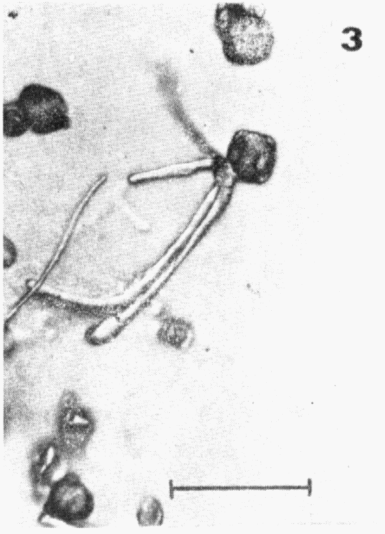
G. Thieme Verlag, Stuttgart 1974, (10) + 197 str., 46 obr., 12 tab., cena váz. 14,80 DM. (Kniha je v knihovně ČSBS.)

V biochemické cytologii se používá dvojí způsob práce: in situ a in vitro. V prvním případě se pracuje na řezech, v druhém jsou materiálem obvykle homogenáty, příp. frakce z nich izolované. Zatímco metodických příruček věnovaných prvnímu způsobu práce na rostlinných objektech existuje přece jen několik, nebyly dosud k dispozici příručky, zabývající se druhým z uvedených způsobů, zejména pokud jde o rostlinný materiál. Už tato skutečnost je dostatečným důvodem uvítat recenzovanou knihu, na níž za redakce G. Jacobiho spolupracovali H. Beevers, J. Feierabend, W. Franke, C. A. Lembi, Ph. Matile, C. Mehard, D. J. Morré, B. Parthier, R. Theimer, W. J. van der Woude a A. Wiemken. Redaktor napsal úvodní kapitolu a partii o izolaci plastidů. I ostatní kapitoly — každá z nich se zabývá jedním typem buněčných složek — napsali autoři skutečně povolaní, mající na vypracování uváděných metod velký podíl. Redaktor se pokusil o sestavení praktika. Nejedná se ovšem o nějaké primitivní školní praktikum. Svůj záměr prof. Jacobi splnil: do knihy malé rozsahem se mu podařilo seřadit hodně solidních praktických informací. Snad by stálo za úvahu rozšířit rozsah příštího vydání, aby bylo možno uvést některé další údaje a souvislosti, např. rozsáhlejší teoretický výklad k používaným operacím, problematiku identifikace izolátů a posuzování jejich stavu, korelaci nálezů in vitro a in situ atd. Také by bylo vhodné poukázat na jistou originalitu, resp. jednostrannost některých v knize zastávaných koncepcí — mám zde na mysli problematiku rostlinných lysozómů atp. Jinak však posuzovaná kniha své poslání výborně splní a specialistům v této problematice i širší veřejnosti ji lze vřele doporučit.

K. Beneš







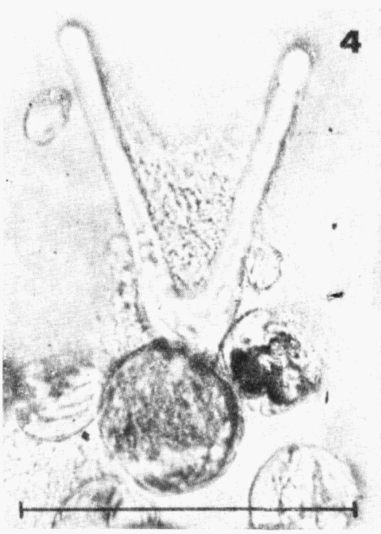


Plate IX. — Pollen of *Rosa jundzillii* germinating 24 hours at 88°C in 20% saccharose (medium without boron). Scale = 0.1 mm. — 1, 2: Individual R 442, pollen germinating with one tube only. — 3: Individual NK 196/73, pollen tube with four branches. Other three colpi also visible. — 4: Heptaploid individual R 227b, forked pollen tube.

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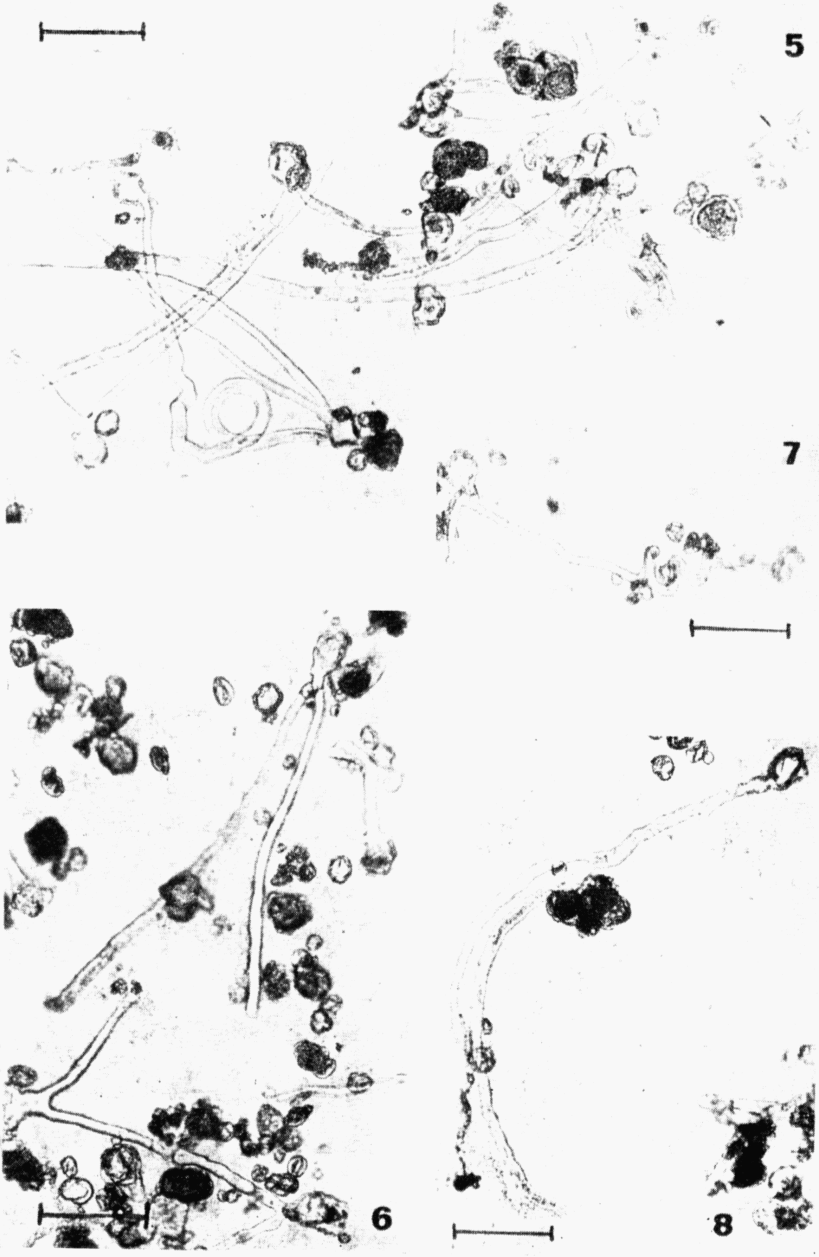

Plate X. — Pollen of *Rosa jundzillii* R 227b germinating 24 hours at 28°C (media with boron and calcium). Scale = 0.1 mm. — 5, 6: In 10% saccharose. — 7, 8: In 15% saccharose.

Plate XI. — Pollen of *Rosa jundzillii* R 227b germinating 24 hours at 28°C (medium with boron). Scale = 0.1 mm. — 9: In 45% saccharose with calcium. — 10: In 50% saccharose with calcium. — 11: In 10% saccharose. Six germ pores visible in two levels. — 12: In 10% saccharose. Tetracolporate pollen grain.

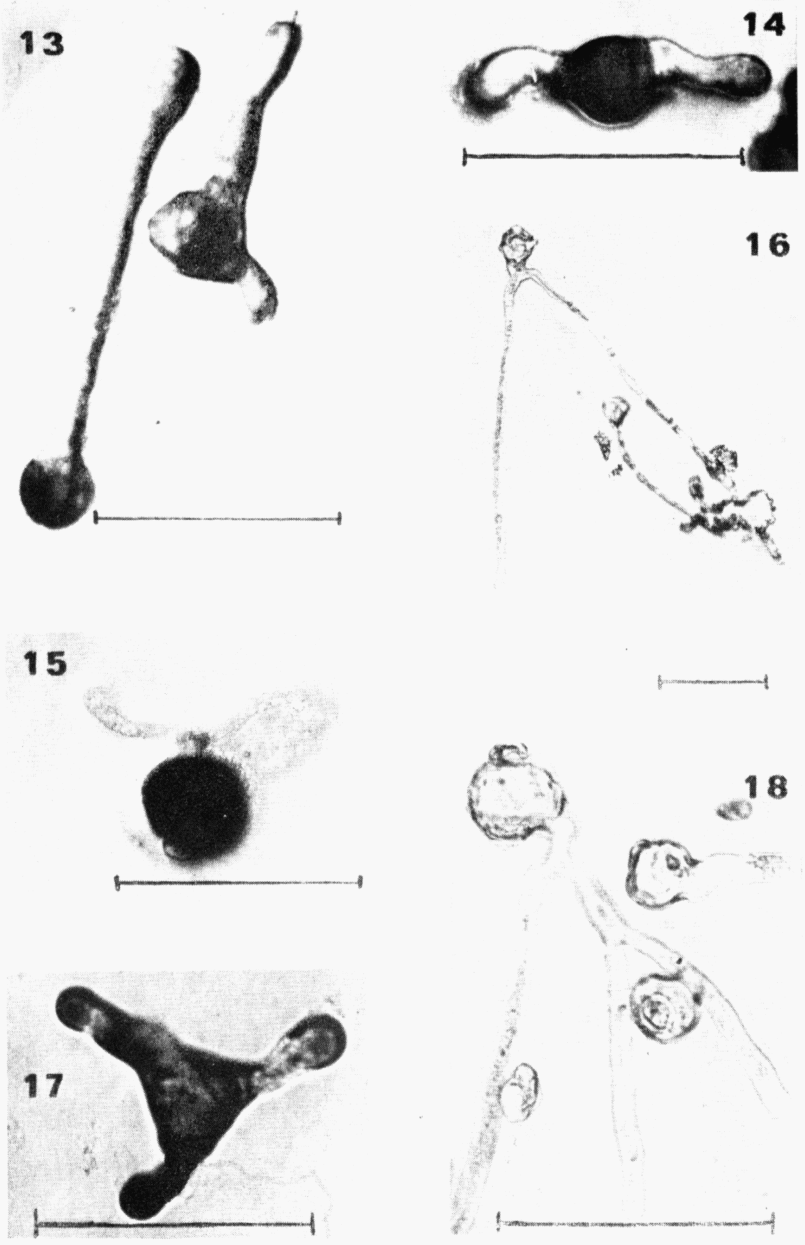

Plate XII. — Abnormal pollen germination in some wild *Rosa* species. Incubation 24 hours at 28°C. Media with boron. Scale = 0.1 mm. — 13, 14: *Rosa pimpinellifolia* R 317, pollen tubes growing from two different pores. (13: 40% saccharose in distilled water, 14: 35% saccharose in 1.5% agar). — 15, 16, 18: Branched pollen tubes: *Rosa schulzei* R 686 (16: In 20% saccharose with calcium, 18: In 30% saccharose with calcium) and *Rosa arvensis* R 116 (15: In 15% saccharose in 1.5% agar). — 17: *Rosa reversa* R 258, 35% saccharose in 1.5% agar. Beginning of germination from all three pores.