Preslia, Praha, 47 : 22—25, 1975

Studies in rose pollen I. In vitro germination of pollen grains of *Rosa hugonis*

Pyl růží I. Klíčení pylových zrn u *Rosa hugonis* in vitro

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

Pollen germination in *Rosa hugonis* HEMSL. was studied under various temperature and in various agar-saccharose media. The best results were obtained at 28 and 35° C with 30, 35 and 40% saccharose in 1.5% agar. Under these conditions more than 90% of normal grains germinated and the pollen tubes achieved their maximum length. Ecological and genetic factors influencing the low fertility of the plant examined and general problems of the germination of rose pollen are also discussed.

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INTRODUCTION

The pollen morphology of *Rosa* has been dealt with by many authors. A review of these studies will be found in FLORY (1950). Physiological properties of rose pollen have been examined by TÄCKHOLM (1922), MAMELI CALVINO (1951), WOHLERS et al. (1962), PEIMBERT et al. (1963), and WOHLERS et MOREY (1963). The results of pollen germination studies are evident from the following statement: "The germination of rose pollen under our conditions has proven to be an extremely variable and unpredictable event... Despite all our work no basic patterns of response could be seen..." (WOHLERS and MOREY 1963 : 109 et 202).

One reason causing the difficulty may be the large number of species and cultivars compared in previous experiments. Therefore it was decided to gain an insight into the problem by examining one single species. *Rosa hugonis* HEMSL. was chosen because of its early florescence, and large number of flowers per shrub. Also, it seemed desirable to explain the absence of hips in a specimen of *Rosa hugonis* grown in the collection of the Botanical Institute, Czechoslovak Academy of Sciences, Průhonice.

MATERIAL AND METHODS

The shrub examined was collected as a seedling by C. SCHNEIDER in Central China around 1918 and has been in cultivation at Průhonice since that time. The plant was identified by I. KLÁŠTERSKÝ as a typical representative of *Rosa hugonis* HEMSL. It has been flowering every year but no fruits were collected or seen until 1973. Therefore a study was made of the mitosis in meristematic cells of apical shoots. The material was pretreated in a concentrated aqueous solution of paradichlorobenzen for two hours, fixed in ethanol — acetic acid — chloroform (1 : 2 : 1) mixture (NĚMEC 1962) for at least two hours, macerated in ethanol — HCl (99 : 1) mixture for 15 minutes, thoroughly washed in distilled water and squashed in lactopropionic orcein.

The pollen was collected from flowers about to open. These were gathered from a shrub labelled R 807 between 7 and 8 a.m., from 22nd to 29th May, 1973, when the plant was in full bloom. The flowers were placed in open Petri dishes allowing anthers to dehisce at room temperature. Next morning the pollen from one flower was blown onto 10 slides covered with 1.5% agar-

sacharose medium, each slide with different concentration of sugar (increasing from 10 to 55%). The pollen was incubated in germination boxes for 24 hours, where constant temperature (22, 28 or 35° C) and standard humidity were maintained. The same procedure was repeated with ten flowers for each of the sugar concentrations. Some observations at 55° C temperature were also made. Because of the relatively small amount of pollen per flower, it was not possible to examine the behaviour of pollen from one flower in all temperature variants. For the same reason we could not accept the sample size of 500 pollen grains as suggested by FLORY who worked with a mixture of pollen from many flowers, and sometimes also from different shrubs. In our experiments the behaviour of pollen within one flower was compared, taking into account samples of 100 morphologically well-developed pollen grains per slide.

RESULTS

The somatic chromosome number for *Rosa hugonis* was confirmed to be 2n = 14. The same number has been reported by TÄCKHOLM (1922) and HURST (1928, 1931). Of 1000 pollen grains 86.3% were morphologically normal, round, 30 μm in diameter.

At 22°C the germination of pollen varied strongly not only in individual flowers, but also with regard to different concentrations of saccharose; no correlation between the concentration of sucrose and pollen germination has been found. Also, the number of germinated grains was usually very low (less than 10%) and the pollen tubes never exceeded 100 μm so that they could be mistaken for bursting ones, especially in lower concentrations. In 10% saccharose, most of the normal pollen grains swelled and burst during the first hour of incubation. On the other hand, in 55% saccharose the pollen grains were shrinking, and no pollen tubes could be found.

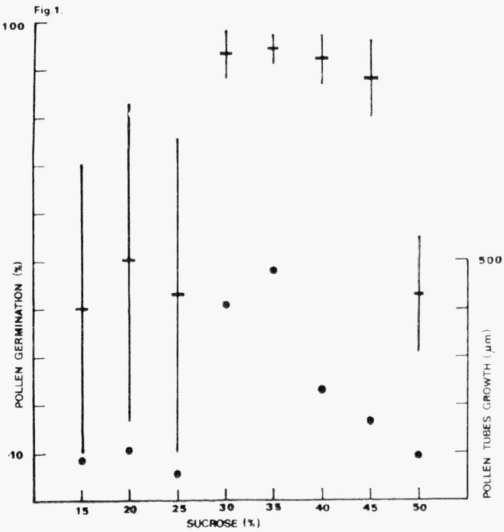

Fig. 1. — Effect of sucrose concentration on pollen germination (▬, means ± s.e. from 10 flowers) and pollen tube growth (●, means of 100 pollen tubes) after 24 hours of cultivation at 28° C.

Results obtained for 10 and 55% concentrations at 28°C temperature were similar to those described above. Germination in 15, 20 and 25% saccharose varied greatly (from 1 to 96%) and the pollen tubes never reached the length of 100 μm (Tab. VII, a, b). In 30, 35 and 40% concentrations the pollen germinated well and the pollen tubes were much longer (Tab. VII, c—f; Tab. VIII, a, b). Germination started 60 to 90 minutes after the beginning of incubation. In 35% concentration the pollen tubes reached a maximum length within 6 hours. In other concentrations the number of germinating pollen grains and the length of pollen tubes continued to increase till 24 hours (Fig. 1.)

The results obtained at 35°C were similar to those at 28°C incubation.

The pollen of *R. hugonis* was found to germinate even at 55°C, especially in 30 and 35% saccharose-agar-medium. Because this incubation was made in a paraffin stove, where a constant humidity could not be maintained, the pollen soon dried out and further interpretation was not possible.

DISCUSSION

The material examined proved to be diploid. This conforms to the results obtained by other authors. Thus, aneuploid or triploid character cannot be made responsible for the sterility of the shrubs studied.

FLORY (1950) gives for *R. hugonis* an average of 84.9 per cent of normal pollen, ranging from 75.7 to 94.1 per cent. Our result of 86. 3% is within this variation range.

It is a well known fact that the germination of rose pollen is closely dependent upon the concentration of saccharose. TÄCKHOLM (1922) used 2.5, 5 and 20% saccharose, the last concentration having been the most suitable one. MAMELI CALVINO (1951) tried concentrations from 5 to 50% for a few species and many cultivars, though not all concentrations were used for all plants under study. She considered 20% saccharose to be the best medium. Comparing our results with hers we can conclude that the pollen of some roses could probably germinate better in higher concentrations; for example, for the 5, 10 and 20% saccharose concentrations the percentage of germinating pollen grains was given as follows: 20, 20 and 32% in *R. foetida*, 15, 15 and 26% in *R. laevigata*, 50, 75 and 82% in *R. odorata*.

The results of WOHLERS and MOREY are summarized and apply to multiple horticultural hybrids. No temperature ranges were given. Likewise in MAMELI CALVINO's work, this may be the reason why the results differ so much.

Different explanations have been proposed to account for the sterility of our shrubs of *R. hugonis*. Autogamization experiments having failed (no hips were produced), it was assumed that self-sterility (known to occur in roses in different degrees, see JIČÍNSKÁ 1975a, 1975b) was involved. But in summer of 1973, lots of ripe hips with morphologically good seeds were produced. In the same year various successful hybridization experiments with *R. hugonis* as pollen donor as well as pollen acceptor were made.

PEIMBERT et al. (1962) made an interesting observation on the fresh vitality values, determined by means of the cotton-blue-lactophenol-test of the pollen in a red Hybrid Tea Rose C3-279A. The vitality values varied from 36 to 50% in different days of collecting, probably depending on temperature. The authors concluded that ecological factors influencing microsporogenesis were responsible for pollen vitality. This may also be true with *R. hugonis*. This species is known to be native in Central China, where hot springs and very dry summers prevail. Similar situation is rare in Central Europe.

Incidentally, there were several very hot days in spring of 1973, especially in early May, when the development of pollen of *R. hugonis* is likely to take place. This can be concluded from our many years' experience with collecting buds of roses for the purpose of study of meiosis in pollen mother cells. The dry, hot summer that followed was also very favourable for this *Rosa* species, causing the old shrubs in the Průhonice collection to produce hips with normal seeds only in that particular year.

The present observations suggest that pollen viability and germination in *R. hugonis* are closely dependent upon environment and are genetically fixed much more than previously assumed. The results could also explain the different pattern observed in *Rosa* species belonging to various taxonomic groups with different ecological requirements (JIČÍNSKÁ et KONČALOVÁ, in preparation) or with complicated hybrid basis, as for instance the rose cultivars (MAMELI CALVINO 1951, WOHLERS et MOREY 1963).

SOUHRN

Byly studovány podmínky klíčení pylu *Rosa hugonis* HEMSL. in vitro v souvislosti se sledováním příčin dlouholeté sterility těchto introdukovaných rostlin. Bylo zjištěno, že normálně vyvinutý pyl je zastoupen z 86 % a nejlépe klíčí při teplotě 28 až 35 °C v 30 až 40% roztoku sacharosy v 1,5% agaru. Stanovení somatického počtu chromozómů vyloučilo možnost aneuploidie nebo triploidie (2n = 14). Shodou okolností v r. 1973, kdy byly tyto pokusy prováděny, všechny keře tohoto druhu ve sbírce bohatě zaplodily z volného sprášení, i hybridizační pokusy s touto růží jako donorem či akceptorem pylu byly úspěšné. Poněvadž ten rok byly na našem území zřejmě zvlášť příhodné tepelné podmínky, zvláště v době důležité pro mikrosporogenezi *R. hugonis*, je diskutována možnost silné genetické fixace ekologických nároků růží na tvorbu pylu. To by vysvětlovalo dosud nesrovnatelné, převážně sumarizované výsledky jiných autorů s hodnocením vitality pylu u vysoce hybridních kulturních odrůd růží.

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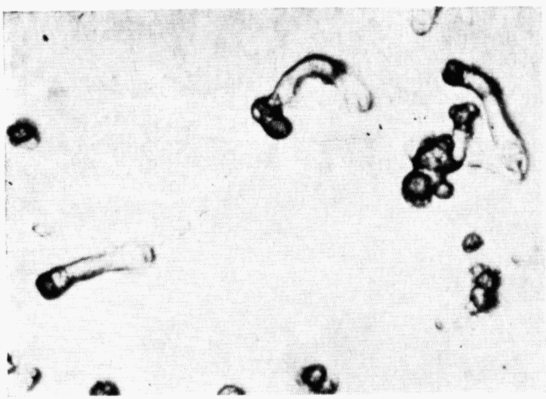
See also plates VII.—VIII. in the Appendix.



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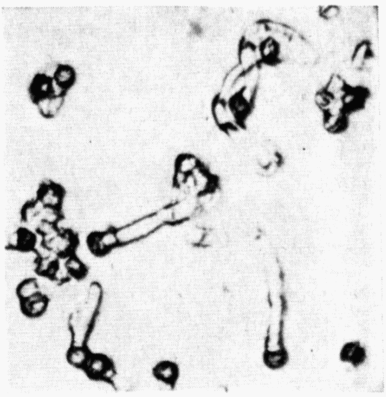

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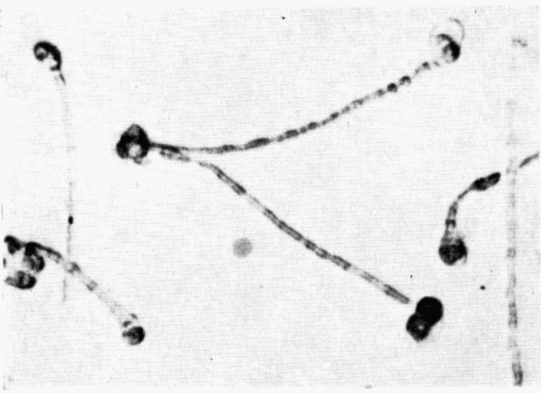

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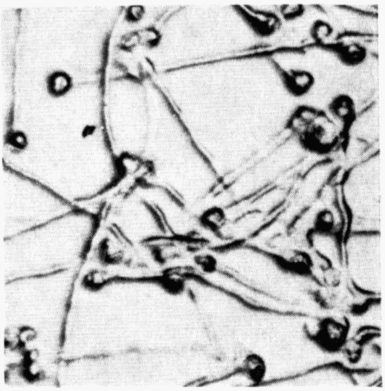

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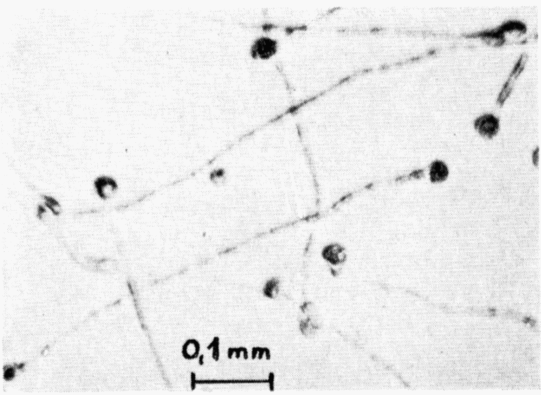

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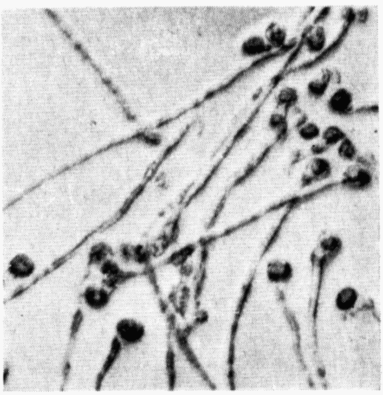
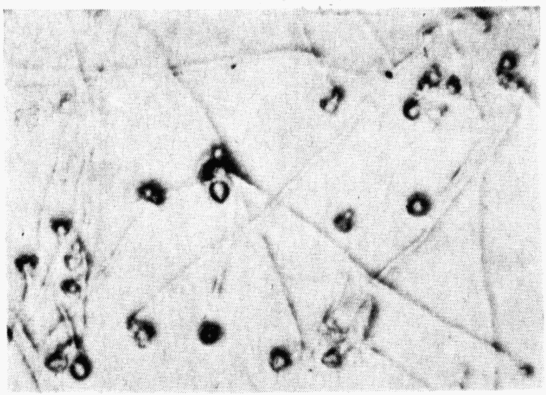


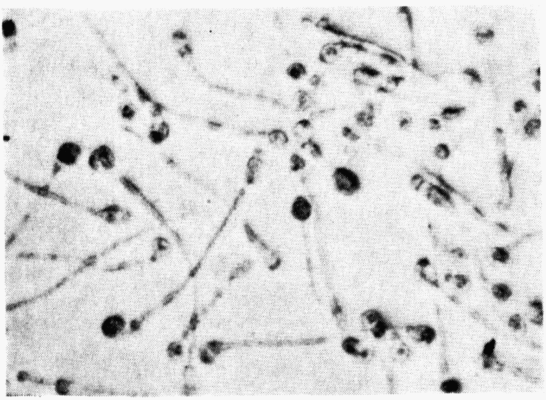
f

Tab. VII. — Germinating pollen from one flower of *Rosa hugonis*. Temperature 28°C, sucrose concentration in 1.5% agar: a, b 15%; c, d 30%; e, f 35%.



a

b



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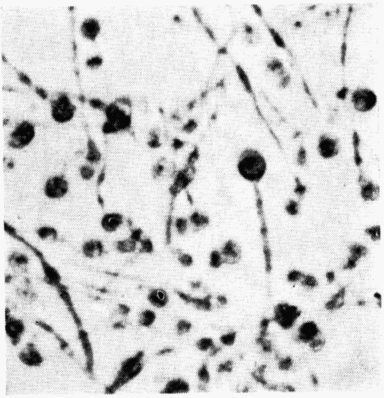

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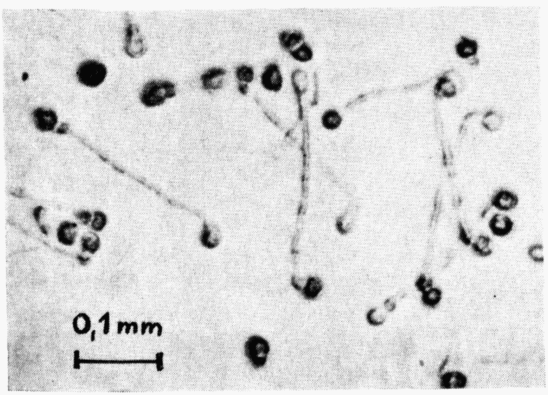

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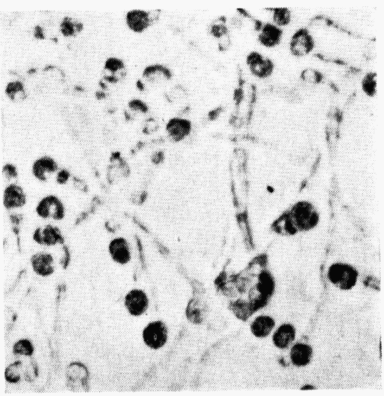

f

Tab. VIII. — Germinating pollen from one flower of *Rosa hugonis*. Temperature 28°C, sucrose concentration in 1.5% agar: a, b 40%; c, d 45%; e, f 50%.

M. N. KONČALOVÁ: Studies in rose pollen I. In vitro germination of pollen grains of *Rosa hugonis*