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BRIEF COMMUNICATION

The Technique of Obtaining Germinating Pollen Without Microbial Contamination

EVA PETRŮ, EVA HRABĚTOVÁ, JAROSLAV TUPÝ

Department of Plant Physiology and Genetics,
Institute of Experimental Botany, Czechoslovak Academy of Sciences, Praha*

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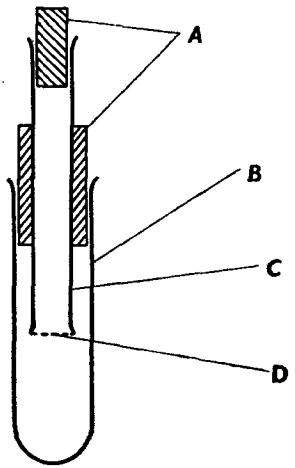
Pollen without microbial contamination but with normal germinating capacity is absolutely necessary, primarily in physiological experiments requiring long-term cultivation of pollen under artificial conditions. It is equally necessary for fertilization in vitro together with long-term cultivation of the fertilized ovum.

Our experiments with the disinfection of apple and tobacco pollen with solutions of Famosept,



A — cotton plug
B — test tube
C — small glass tube
D — net

Fig. 1. Simple apparatus for shaking out pollen from anthers under aseptic conditions

* *Address*: Na cvičišti 2. Praha-Dejvice,

Ajatin, sodium hypochlorite such as "Chloramin B", calcium hypochlorite and hydrogen peroxide were not successful. We did obtain noncontaminated pollen, but it did not germinate. HELLMERS and MACHLIS (1956) reached the same conclusion in the case of pine pollen. In further experiments we treated mature anthers from which we obtained normally germinating, noncontaminated pollen. The best procedure was found to be the following:

Anthers were shaken for 1 min. in a solution of Famosept (10 ml. sol. aq. phenylhydrargyri borici $2^0/_{00}$ + 90 ml. H_2O) or in a solution of Chloramin B (10 g. substance + 90 ml. H_2O), thoroughly washed with sterile water, dried between filter paper and transferred to a Petri dish. To obtain rapid shedding of the pollen the Petri dishes were placed over dried silicagel in a vacuum exsiccator, which was evacuated by laboratory suction pump. Soon after dehiscence the anthers were transferred aseptically into a little tube placed in a test tube (fig. 1). On shaking, the pollen fell out of the anthers into the bottom of the test tube. Before use the glass and filter paper were sterilized in hot air at 140° C for $1^1/_2$ hour. Test tubes with pollen were stored at −10° C.

We used this method of obtaining pollen without microbial contamination from the apple (*Malus* cultivars) and tobacco (*Nicotiana alata* LINK et OTTO) in 1962 and again in 1963 with good results. The germinating capacity of the pollen and the growth rate of the pollen tubes were normal and no infection occurred in the culture even after 18 days.

Reference

HELLMERS, H., MACHLIS, L.: Exogenous substrate utilization and fermentation by the pollen of *Pinus ponderosa*. — Plant Physiol. **31** : 1—6, 1956.

E. PETRŮ, E. HRABĚTOVÁ, J. TUPÝ, Ústav experimentální botaniky ČSAV, Praha: Technika získávání klíčivého, mikrobiálně nekontaminovaného pylu. — Biol. Plant. 6 : 68 — 69, 1964.

Sterilizují se dospělé prašníky roztoky Famoseptu nebo Chloraminu B. Po opláchnutí vodou se rychle vysuší ve vakuu za nízké vlhkosti vzduchu. Z otevřených prašníků se pyl vyklepává v jednoduchém zařízení (obr. 1).

Э. ПЕТРУ, Э. ГРАБЕТОВА, Я. ТУПЫ, Институт экспериментальной ботаники ЧСАН, Прага: **Техника получения микробами незараженной, способной прорастать пыльцы.** — Biol. Plant. 6 : 68 — 69, 1964.

Зрелые пыльники стерилизуют растворами Фамосепта или Хлорамина Б. После ополаскивания водой их быстро высушивают в вакууме в условиях пониженной влажности. Из открытых пыльников вытряхивают пыльцу в простом приборчике (рис. 1.).