METHODS FOR GROWING POLLEN TUBES FOR PHYSIOLOGICAL AND CYTOLOGICAL STUDIES*

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A number of excellent methods for growing pollen tubes in artificial media have been devised by Trankowsky (1930—31), Beatty (1937), Maheshwari and Wulff (1937), and Newcomber (1938), but none of these procedures give an adequate discussion of the methods for growing pollen tubes for physiological studies. In most cases mention is made of a moist chamber, but no further reference is made concerning the construction or use of such a chamber. During the past three years the present writer and his students have been engaged in physiological and cytological studies of pollen tubes in angiosperms (Eigsti, 1939, 1940; Smith, 1939, 1940), and in these studies an especially constructed chamber was used successfully. In the opinion of this writer, the dearth of available information concerning methods of growing pollen tubes, and the satisfactory results obtained from apparatus used for this purpose in our laboratory, warrant a presentation of its construction and use.

The dimensional details of the chamber for growing pollen tubes are explained in the accompanying diagram. (Figs. 4—5). Galvanized iron (No. 28) was used for the chamber (Fig. 4). The total cost of the chamber varies from $2.50 to $5.00 depending upon local costs of material and labor. The exact size of the pan can be made to suit the particular projects under consideration. Two sizes have been found adaptable, viz., one holding 120 slides (Fig. 4) and another accommodating only 60 slides. The smaller pans can be removed to constant temperature cases when studies involving the effects of temperature are under consideration.

The general procedure involving the use of this chamber (Fig. 4), the calibrated vial (Fig. 3), and the water bath (Figs. 1—3) is described in eleven steps.

(1) Flowers with unopened anthers were brought into the laboratory prior to the dehiscence of the anther. The pollen adhering to the wall of the opened anther was used for pollen inoculations.

(2) The solution for growing the pollen tubes was prepared by adding the desired amount of sucrose to a 2% solution of agar. (The concentration of sucrose must be twice that required for the germination of each particular type of pollen for reasons which will be made clear in the following steps.)

(3) The substances tested, such as colchicine, 3-indole acetic acid, vitamin B_1, etc., were made up in an aqueous solution with distilled water. For a control, distilled water was used.

(4) The solution of sucrose in agar (2) and the solution used in the treatment (3) were mixed in equal portions in a calibrated vial (Fig. 3). This mixture of the two solutions halves the concentration of each solution used.

(5) The calibrated vials (Fig. 3) were placed in small bottles (Fig. 2) which served as water baths (Fig. 1) to keep the temperature of the

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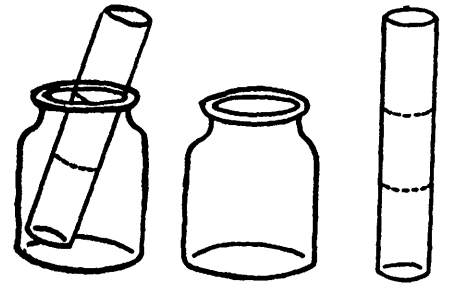
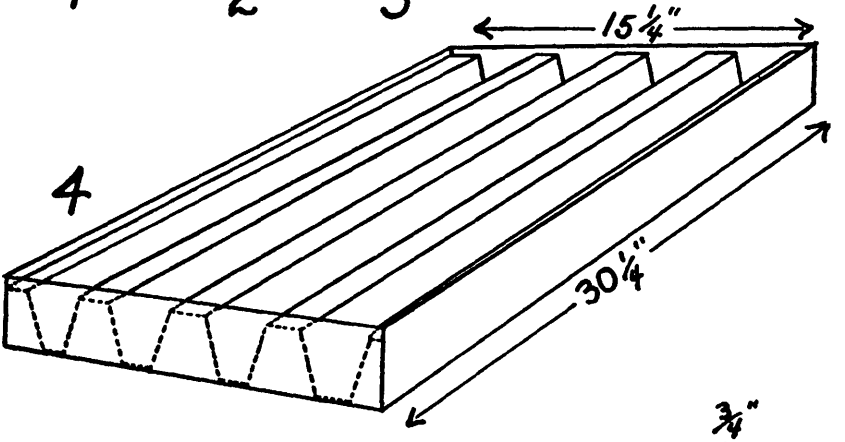


1 2 3

Dimensions of Lid

Length - 30½"
Width - 15½"
Heighth - 1"



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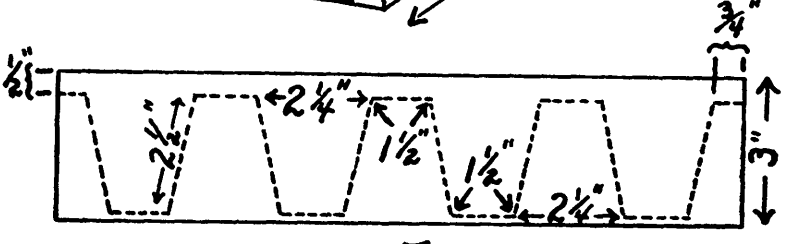

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solution between 50°—53° C. This temperature was found suitable for making an inoculating film on a glass slide.

(6) The film of cultural media (5) was spread over the slide with a camels hair brush No. 3; a separate brush was used for each solution.

(7) Immediately after the film was spread on the slide, pollen was introduced onto the medium. This was done by shaking the flower above the slide or by removing the anther and dipping it into the film.

(8) When the inoculation was made, the slide was inverted in the germinating chamber (Fig. 4) with the film facing the water in the moat. Each moat was filled with water to a depth of about one or two inches (Fig. 5).

(9) When all slides for a specific experiment were prepared and placed in the chamber (8), a small amount of hot water (80° C.) was added to each moat of the chamber (Fig. 5) prior to closing with the lid (see accompanying diagram for explanation of the dimensions of the lid). The addition of this water increased the humidity in the germinating chamber.

(10) Slides were removed from the chamber at the desired interval of time, depending upon the particular problem under consideration. In the experience of the writer, this interval of time has ranged from 15 minutes to 96 hours. However, those remaining in the chamber longer than 24 hours became contaminated with bacteria and various other fungi.

(11) The culture was removed from the chamber, observed briefly under the microscope, and if satisfactory was placed in a basket for subsequent fixation and staining.

These methods for growing pollen tubes have been used successfully in this laboratory for investigations dealing with the physiological, morphological, and cytological phases of pollen tubes.

SUMMARY

1. The construction of a chamber for growing pollen tubes is described.

2. A general procedure for growing pollen tubes is outlined.

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