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Germination of Cotton (*Gossypium hirsutum* L.) Pollen on an Artificial Medium[1]

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ABSTRACT

***Gossypium hirsutum* L. pollen was germinated by dusting it on a 3.5% agar medium containing sucrose, manganous sulfate, calcium nitrate, and boric acid. The medium was autoclaved for 5 min at 15 pounds per square inch and then poured into petri dishes to a depth of 5 to 7 mm. After cooling, the agar plates were stored in a refrigerator for 24 hours before use. Pollen was dusted onto the medium and the plates were placed in a germination chamber at 30 C and a relative humidity approaching 100%. To date, germinations have been low, ranging from 10 to 64% with an average of 30%. Advantages of this technique over the Bronckers' method are: (i) more rapid germination (2 to 3 hours); (ii) more normal-appearing tube cytoplasm, and (iii) much longer tubes.**

Additional index words: **Pollen tubes, Pollen growth.**

A REVIEW of the literature indicates that the artificial germination of cotton pollen (*Gossypium hirsutum* L.) is very difficult. There have been reports of success and counter-claims of failure scattered throughout the literature. As early as 1923 Kearney (6) was resigned to the failure of *in vitro* methods and reported a technique to determine cotton pollen viability as the percentage of pollen grains that burst when immersed in weak aqueous sugar solutions or in pure water. Baneji (2) was unsuccessful with several methods, including the agar-agar and sucrose methods that had been successful with other crops (1, 7, 9). Iyengar (5) described a method of germination and dissection of cotton pollen tubes *in situ* because of failures in attempts to germinate cotton pollen *in vitro*. In 1961 Bronkers (3) described the first technique that subsequent workers have found reliable (8). However, Miravalle (8) reports that with the Bronkers' method pollen tubes are short, the cytoplasm is cloudy and granular, and the process requires 24 hours or longer. This report summarizes the results of experiments designed to develop an improved technique for the successful germination of cotton pollen *in vitro*.

MATERIALS AND METHODS

Most of the reports of successful pollen germination on agar media state that the media used contained varying amounts of bacto-agar, sugars (generally sucrose), boric acid, and calcium nitrate dissolved in distilled water. A great number of different media were tested in this study, using different combinations of the above ingredients. Other ingredients tried were kinetins, carrot juice, coconut milk, Indole acetic acid, Gibberellin, yeast extracts, potato dextrose-agar, manganous sulfate, copper sulfate, Hoagland's complete nutrient solution No. 1 (4), and each of the ingredients of this nutrient solution in combination with the above ingredients. All ingredients were mixed together, autoclaved at 15 pounds' pressure for 5 min, then poured into petri dishes to a depth of 4 to 7 mm. The plates were immediately covered and as the agar cooled, condensate was blotted from the inside of the covers as often as required to keep it from dripping onto the agar surface. Plates not used immediately were stored under refrigeration for periods up to 14 days. Stored plates were allowed to reach room temperature before inoculation with pollen. Inoculation was accomplished either by gently shaking a cotton blossom with dehiscing anthers over the plate or by collecting the pollen from such a blossom with a camel hair brush and gently brushing it onto the agar surface. Inoculated plates were then covered and placed in a germination chamber at 30 C and at a relative humidity approaching 100%. Germination percentages were calculated by making counts of all germinated and ungerminated grains in each of three random microscope fields. These values were then averaged and the result was reported as percent germination.

RESULTS AND DISCUSSION

After investigating many combinations of ingredients it was found that *G. hirsutum* pollen could be consistently germinated on a medium consisting of 100 ml of distilled water, 3.5 g bacto-agar, 25 g sucrose, 70 mg manganous sulfate , 40 mg calcium nitrate, and 40 mg boric acid. At the levels tested kinetin, carrot juice, coconut milk, indole acetic acid, gibberellin, yeast extract, potato dextrose-agar, copper sulfate, and the components of Hoagland's solution all either completely, or severely curtailed pollen germination.

It was found necessary to age the agar plates for at least 24 and preferably 48 hours before use. If this was not done the pollen grains tended to either sink into the surface of the agar where they would not germinate, or to take up excessive moisture and to rupture. Little or no sinking or rupturing was observed on plates aged for from 48 hours to 14 days. Plates were aged under refrigeration.

Inoculated plates were checked for germination at 15-min intervals from inoculation until maximum germination had taken place. Tubes were first noted on a few plates after 30 min and germination seemed complete within 3 hours. In fact, germination was completed in 2½ hours in most cases.

Pollen tubes were much longer than those reported for the Bronkers' technique. The pollen tube length averaged 15 diameters of a pollen grain (Fig. 1), with a few tubes reaching a length of 30 diameters. After most tubes grew to their maximum length, the ends ruptured, and tube contents were exuded (Fig. 2). The cytoplasm appeared normal, although at the magnifications used no nuclei could be observed. Germination counts were rather low, ranging from 10 to 64% and averaging about 30%. Table 1 shows a typical set of data collected on five consecutive days for two varieties of cotton. Fresh pollen was used each day. It is not known whether those pollen grains that did not germinate were incapable of doing so or whether they might germinate if cultured on a different medium or under different conditions. It is hoped that future refinements of media and technique or both will enable researchers to obtain higher germination percentages. It was observed that if the pollen

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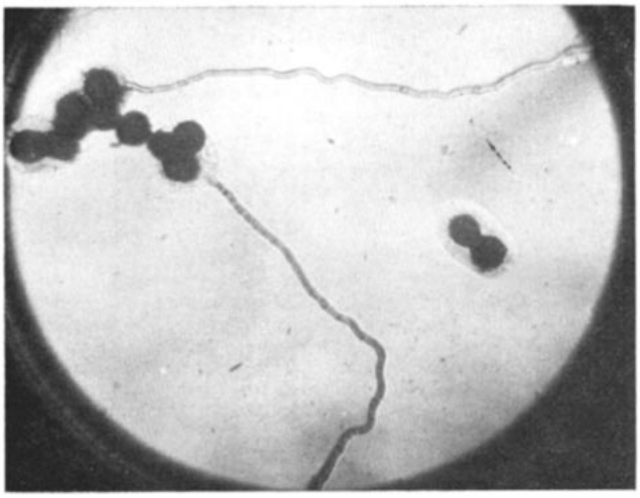

Fig. 1. Cotton pollen tube germinated on an artificial medium.

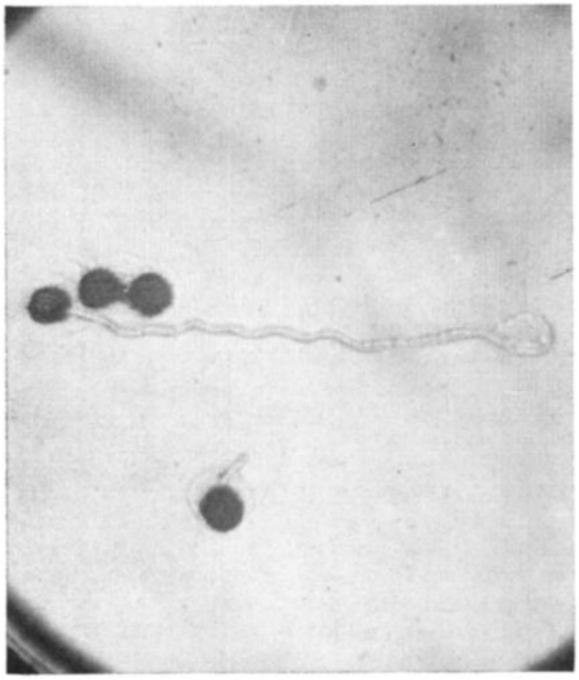

Fig. 2. Cotton pollen tube germinated on an artificial medium showing tube rupturing and exudation of cell contents.

tube grew into the agar rather than along the surface, rupturing and exudation of cell contents occurred almost immediately. This caused a "puddle" to form around the pollen grain. These puddles did not have the "milky" appearance characteristic of ruptured pollen grains. By carefully rotating such a pollen grain the short tube was usually visible; therefore, these grains were counted as germinated.

Table 1. Germination percentages of two varieties of short staple cotton pollen tested on 5 consecutive days using fresh pollen each day.

	Percent germination	
Day	Acala 1517D	Stoneville 7A
1	17	29
2	33	31
3	35	38
4	44	23
5	43	28
Average	34.4	29.8

Table 2. Germination percentages of short staple cotton pollen from the same blossom comparing dusting vs brushing as the means of inoculation (two varieties and four replications).

		Percent germination	
Cotton varieties	Rep	Dusted	Brushed
Stoneville 7A	1	13	8
	2	31	24
	3	40	31
	4	18	16
Average		25.5	19.7
Acala 1517D	1	18	17
	2	16	16
	3	39	18
	4	26	10
Average		24.7	15.2

Table 2 indicates a slight advantage of dusting over brushing as the mode of inoculation. One disadvantage of dusting is that so many grains tend to stick together in clumps that accurate counts are difficult to obtain.

Pollen from several varieties of *G. hirsutum* has been tested and successfully germinated. Among those tested were a Delta cotton ('Stoneville 7A'), a Plains cotton ('Lockett 4789'), and an Acala cotton ('1517D'). Attempts to germinate American Pima cotton (*Gossypium barbadense* L.) pollen have been frustrating. Although one or two grains per plate will germinate, the vast majority will not do so on this medium. It is hoped that future work will yield a medium more suited to the germination of Pima pollen.

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