

other mutagens to increase the production of L-methionine.

We thank Prof. S. C. Bhattacharya and Prof. S. L. Chakrabarty for their interest in the work.

16 April 1984; Revised 21 September 1984

1. Kinoshita, S., Udaka, M. and Shimono, M., *J. Gen. Appl. Microbiol.*, 1957b, 3, 193.
2. Rowbury, R. J., *Nature (London)*, 1965, 206, 962.
3. Childs, J. D. and Smith, D. A., *J. Bacteriol.*, 1969, 100, 377.
4. Cherest, H., Eichler, F. and Szulmajster, H. D. R., *J. Bacteriol.*, 1969, 97, 328.
5. Kase, H. and Nakayama, K., *Agric. Biol. Chem.*, 1974, 38, 2235.
6. Ozaki, H. and Shiio, I., *J. Biochem.*, 1982, 91, 1163.
7. Kase, H. and Nakayama, K., *Agric. Biol. Chem.*, 1974, 38, 2021.
8. Knight, B. C. J. G. and Proom, H., *J. Gen. Microbiol.*, 1950, 4, 508.
9. Bolinder, A. E., Lie, S. and Ericson, L. E., *Biotechnol. Bioeng.*, 1963a, 5, 131.
10. Bolinder, A. E., Lie, S. and Ericson, L. E., *Biotechnol. Bioeng.*, 1963b, 5, 147.
11. McCarthy, T. E. and Sullivan, M. X., *J. Biol. Chem.*, 1941, 141, 871.
12. Itoh, H., Morimoto, T., Kawashima, K. and Chibata, I., *Agric. Biol. Chem.*, 1974, 38, 869.

## RAPID GERMINATION OF COTTON AND OKRA POLLEN ON AN ARTIFICIAL MEDIUM

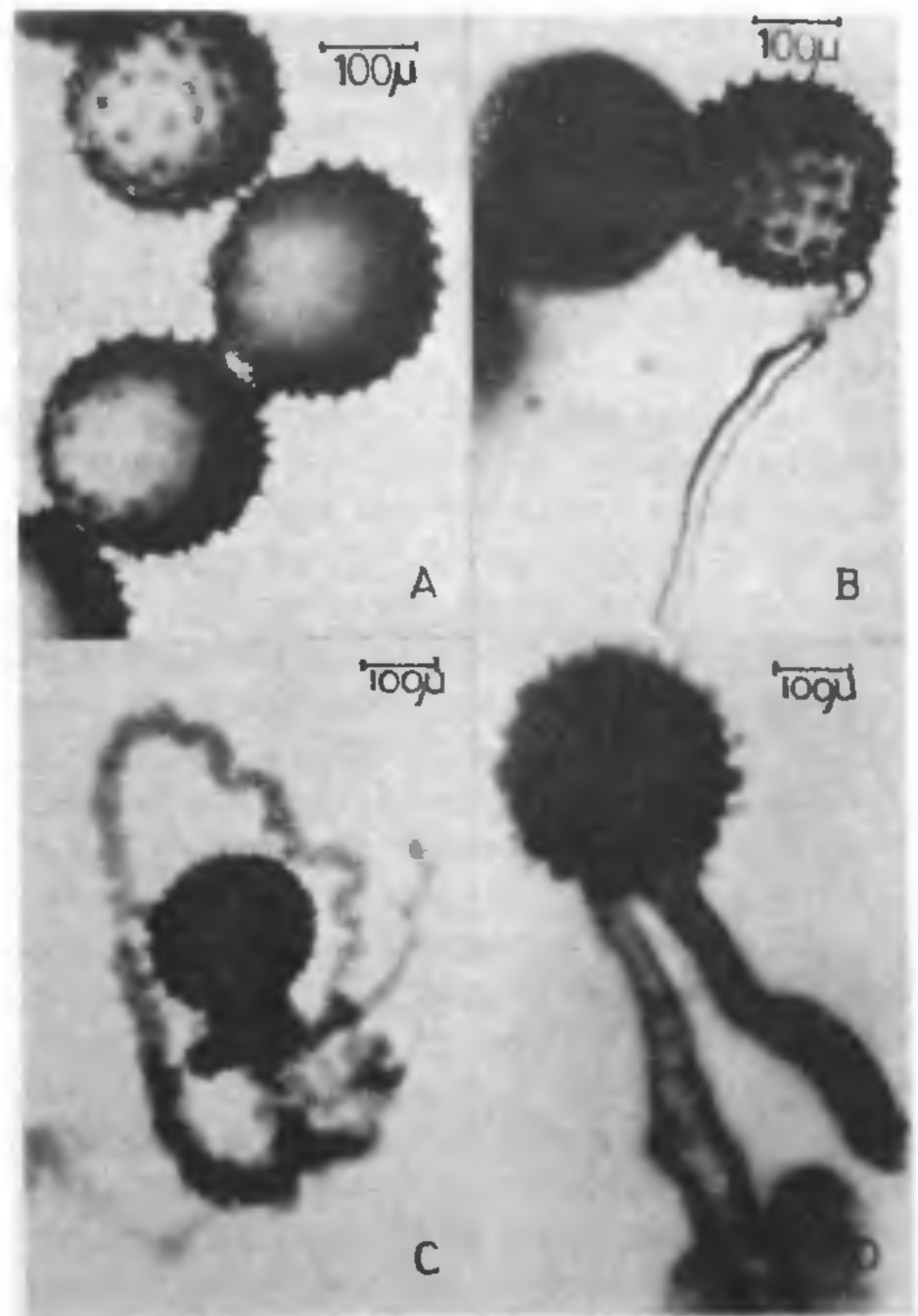
P. A. PHILOMENA and B. V. DAVID

*Fredrick Institute of Plant Protection and Toxicology,  
Padappai 601 301, India.*

THE literature on the germination of pollen (in general) is extensive<sup>1</sup>. However, a review of the literature reveals that artificial germination of cotton pollen (*Gossypium hirsutum* L.) is extremely difficult<sup>2</sup>. Banerji<sup>3</sup> tried several techniques such as cold drawn castor oil, cane sugar solution, glucose, agar-agar-sucrose combinations etc. with little success; Iyengar<sup>4</sup> failed to observe *in vitro* pollen germination and hence resorted to *in vivo* method. This paper presents a simple and rapid method, which is a modification of

Taylor's<sup>2</sup> and Barrow's<sup>5</sup> methods for *in vitro* germination of cotton pollen. The same medium was also found equally good for okra pollen germination (figure 1 A-D).

Pollen grains of *G. hirsutum* L. and *Abelmoschus esculentus* Moench. (L.) were seeded on two types of germination medium. The first one (solid medium) comprises of 3% bacto-agar, 0.04% each of boric acid and calcium nitrate, 0.07% of manganous sulphate and 25% sucrose. The second one (liquid medium) consists of all the other ingredients except bacto-agar. The agar based medium was sterilized in an autoclave at 1.5 kg/cm<sup>2</sup> pressure for 15 min and poured into



**Figure 1.** *In vitro* pollen germination in cotton and okra on an artificial culture medium. A. Ungerminated cotton pollen ( $\times 400$ ). B. Germinated cotton pollen 2 min after contact with agar-based germination medium ( $\times 400$ ). C. Germinated cotton pollen 15 min after contact with liquid culture medium ( $\times 320$ ). D. Germinated okra pollen 10 min after contact with solid (agar-based) culture medium exhibiting twin pollen tubes ( $\times 320$ ).



sterilized petridishes to a depth of 3 to 5 mm or on to glass slides to about 2 to 4 mm thickness making sure to spread evenly. Inoculation of pollen was done by dusting the dehisced anthers onto the surface of the medium with the help of a camel hair brush. Precautions were taken to avoid clumping of the pollens. Inoculated plates and slides were placed in a germination chamber at room temperature ( $28 \pm 2^\circ\text{C}$ ) and relative humidity above 70%. Germination percentages were calculated by making counts of all germinated and ungerminated grains in each of 3 to 4 random microscopic fields. Three replications were maintained each one consisting of at least 100 pollen grains per petri plate or slide. Cotton blue (Loba-chemie, India) 1% was added which helped in the staining of pollen tubes. Diameter of the pollen grains and length of the pollen tubes were measured with the help of ocular and stage micrometers.

After investigating many combinations of ingredients consistent germination of cotton as well as okra pollen grains (*in vitro*) was observed (figure 1A-D) in a medium comprising of 25% sucrose, 0.07%  $\text{MnSO}_4$  and 0.04% each of  $\text{Ca}(\text{NO}_3)_2$  and  $\text{H}_3\text{BO}_3$  in liquid medium with the addition of 3% agar in solid medium. Germination began within minutes after contact with the medium and was completed within an hour of inoculation. An average of 90% germination was noticed in cotton as well as okra pollen while Taylor<sup>2</sup> reported an average of only 30% germination in *G. hirsutum*. However, Barrow<sup>5</sup> succeeded in obtaining 98% germination employing hanging droplet method. The main differences between Taylor's and Barrow's methods and the present one are (1) instead of 3.5 g of agar/100 ml water, 3% agar was found better; (2) freshly plated agar medium was better than aged ones (24 to 48 hours) for rapid and better germination, no sinking or bursting of pollen was noticed; (3) unlike the hanging droplet method on Rodac plate, we used plain glass slides as well as petriplates, where the spread of the pollen grains were more uniform making it less cumbersome for measurements. Thus the method described here is not only much easier, but also brings about rapid and consistent germination of pollen *in vitro*. Among the two methods tried, germination in agar based medium brought about straight pollen tubes while in the liquid medium the pollen tubes were much longer but highly coiled (figure 1B, C).

9 August 1984; Revised 8 October 1984

2. Taylor, R. M., *Crop Sci.*, 1972, **12**, 243.
3. Banerji, I., *Agric. J. India*, 1932, **24**, 332.
4. Iyengar, N. K., *J. Genet.*, 1938, **37**, 69.
5. Barrow, J. R., *Crop Sci.*, 1981, **21**, 441.

### **ICERYA AEGYPTIACA (DOUGLAS) A NEW PEST OF MULBERRY (*MORUS ALBA* LINN.) IN INDIA AND ITS CONTROL**

C. SIDDAPPAJI, T. B. PUTTARAJU and S. VENKATAGIRIYAPPA

World Bank Project-A. Sericulture, Department of Entomology, University of Agricultural Sciences, GKVK, Bangalore 560065, India

PRODUCTION of high quality mulberry leaves is the primary consideration in rearing silkworms (*Bombyx mori* Linn.) and production of cocoons of high standards. Mulberry being a perennial crop is infested by a variety of pests. Among sucking insect pests, the common mealy bug *viz* *Maconellicoccus hirsutus* has been incriminated with spread of 'Tukra disease' of mulberry in West-Bengal<sup>3</sup>. Feeding on sap of mulberry by *Icerya purchasi* has been reported from Burma<sup>1</sup>. During survey in October 1983 and 1984, it was observed that mulberry (M-5) leaves were infested by another mealy bug *viz*, *Icerya aegyptiaca* (Douglas) (Homoptera; Margarodidae) causing severe damage to mulberry gardens in Bangalore north area and at G.K.V.K. Campus and *I. purchasi* Maskell on shoots of mulberry at Hebbal Campus of University of Agricultural Sciences, Bangalore. However, *I. aegyptiaca* was also reported on Areca inflorescence, Jack and different varieties of croton<sup>2</sup> from Karnataka and on *Morus alba* in the Philippines and Palestine from the other parts of the world<sup>4</sup>. On mulberry *I. aegyptiaca* was found congregating along mid-rib and veins underneath the mulberry leaves (figure 1). As a result of draining up of the sap continuously the vigour as well as quality of leaves from the infested plants was reduced and growth stunted. The infested leaves became green and put forth slight upward curling. The mealy bugs were attended by a species of black ant persistently.

Occasionally *I. purchasi* was also found to infest mulberry shoots solitarily in May 1983 and it colonized well by November 1983. The largest colony on the shoot was 70 cm length with a thick encrustation with mostly sedentary forms around mulberry branch.

1. Martin, N., *Bot. Gaz.*, 1913, **56**, 117.