staining of the pollen nucleus. The method followed is described below.

Pollen grains from either freshly collected flowers or those fixed in Carnoy were placed in a drop of LPO on a slide, covered with a cover glass and kept for 24 hr for observation under the light microscope. It is seen that the protoplast of the pollen first bulged out through the pollen pore and then got completely ejected out of the exine envelope without any rupture of the membraneous intine or any apparent seriously altering damage to the inner cell contents. At the same time the nuclei were intensely stained so as to offer sufficient contrast with the background cytoplasm (figures 1 and 2). In this condition the nuclear number and morphology could be conveniently studied. Preliminary studies making use of this phenomenon were carried out with pollen from members of Oleaceae. Extended study with pollen from members of other families yielded similar results. It gave the impression that the phenomenon is universal whenever pollen is stained by LPO.

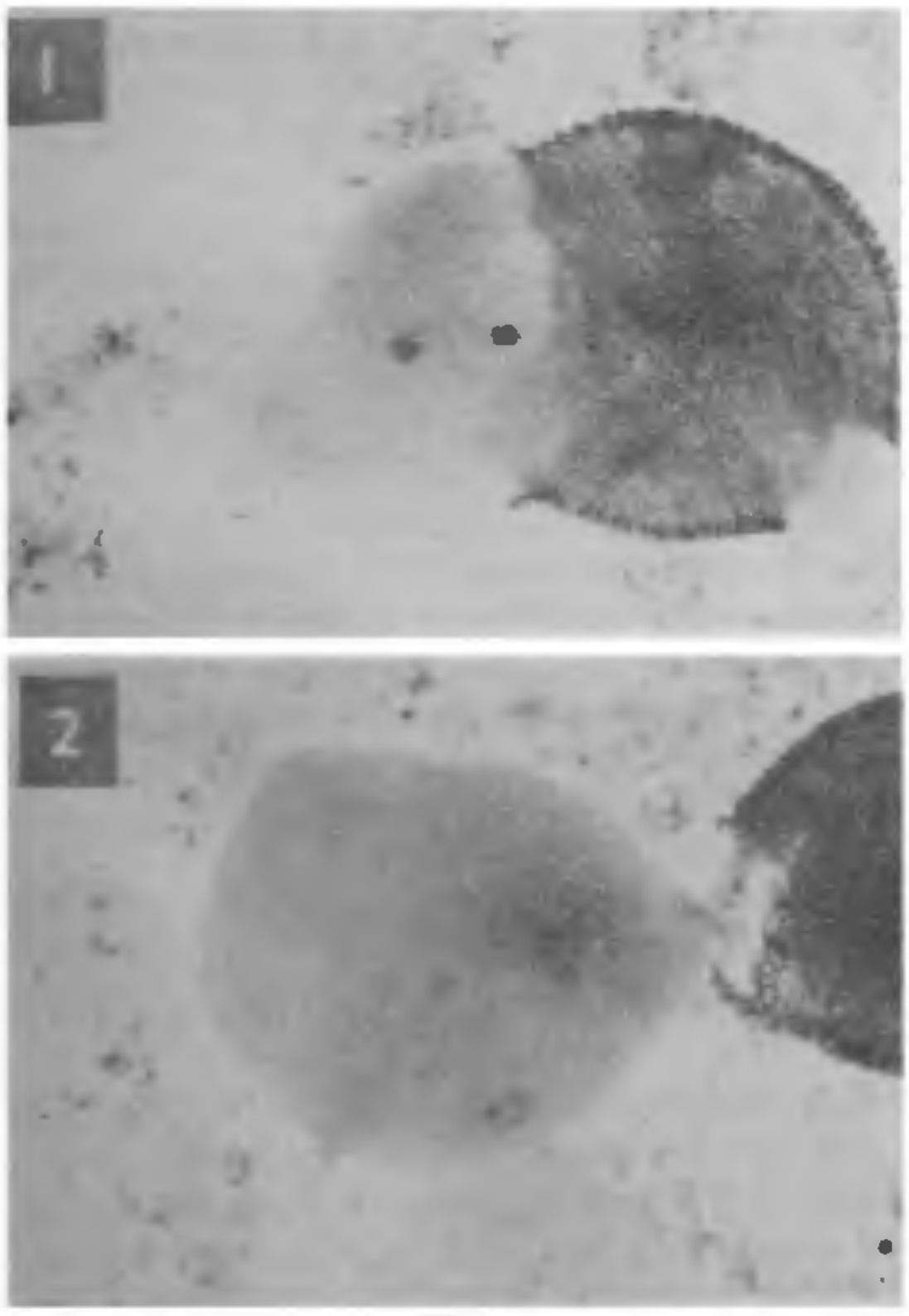

Figure 1. Pollen of *Jasminum bignoniaceum* stained with lactopropionic orcein showing ejection of protoplast having two nuclei. **2.** Protoplast completely ejected out of the exine and showing pollen nucleus in mitosis × 750

Since this phenomenon occurred only when treated with LPO and not with acetocarmine, acetic orcein, propionocarmine or propionic orcein, the factor in the LPO staining fluid which caused the ejection of protoplast was suspected to be lactic acid. This problem was investigated by keeping pollen in three separate media of 45% lactic acid, 45% propionic acid and a 1:1 mixture of 45% lactic acid and 45% propionic acid. Ejection of protoplast was noticed only in the case of pollen kept in the medium of lactic acid alone or in combination with propionic acid. Therefore, it could be inferred that when pollen is stained in LPO two events take place more or less simultaneously; 1. ejection of protoplast by the effect of lactic acid and 2. staining of nucleus by orcein. Since the ejected cell is not covered by the exine, the visibility of the stained nucleus was in no way obscured. This enables one to study the number and morphology of not only pollen nuclei but also the chromosomes in pollen mitosis. The method is particularly useful in cases of plants in which there is no natural pollen tube formation by germination. For instance, in species of *Jasminum* it is found that their mature pollen is binucleate and pollen mitosis takes place normally. Probably their nuclei are nonfunctional in tube production and fertilization.

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RECORD OF *PHOMOPSIS COCOINA* (COOKE) PUNITH. IN STEM BLEEDING AFFECTED COCONUT PALM

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STEM bleeding disease of coconut was first reported from Sri Lanka[1]. Subsequently, it was found to occur in India and other coconut growing countries[2].

The characteristic symptom of the disease is the

oozing out of a reddish brown fluid from the cracks generally at the lower portion of the trunk.

During isolations for fungi associated with the disease-affected coconut palms, it was noticed that pieces of stem tissues from the diseased palms around Kandalloor and Kayangulam (Alleppey district) yielded a fungus which produced pycnidia in abundance in the culture medium (PDA and 3% stem extract agar). The identity of the fungus as *Phomopsis cocoina* (Cooke) Punith. (Syn: *Phomopsis cocoes* Petch) was established at the Commonwealth Mycological Institute, Kew, England. The culture has been deposited at the CMI Herb: (CMI Nos. 279408 to 279410).

P. cocoes Petch was once reported to be associated with leaf spot disease of coconut[3]. Apparently there is no information on the occurrence of this organism in coconut trunk. *Phomopsis* spp. are known to cause bark diseases in plantation crops like tea and coffee[4-6].

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SEROLOGY OF RICE NECROSIS MOSAIC VIRUS

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RICE necrosis mosaic virus (RNMV), a sap and soil transmissible virus[1-3] has recently been reported from India[4]. Morphologically, the virus is rod-shaped having two modal lengths at 275 and 550 nm[5,6]. In order to ascertain the serological relationship, antigenic relationship between the present Indian isolate and the Japanese isolate of RNMV was studied.

Thirty-day-old healthy rice plants (cv. TN-1) grown in soil infected with RNMV[4] were maintained inside insect proof cages. After the appearance of distinct symptoms, leaves from infected plants were collected and utilized for serological determination. A similar number of plants grown on healthy soil under identical conditions served as controls.

Antigenic relationship was studied following slide agglutination test in sterile glass slides using antiserum to RNMV (Japanese isolate)[5] and the clarified original inoculum from leaves of TN-1 rice plant, infected with RNMV (Indian isolate), used as antigen. For this purpose, 2 g leaf tissues of rice plant (cv. TN-1), infected with RNMV were homogenized in 6 ml of 0.05 M borax and the sap was clarified by centrifugation at 3000 g for 10 min (Prof. T. Inouye, personal communication). The antiserum was diluted (1:4) with normal saline[7]. To each sterile slide 1 drop of both antigen and antiserum were added and mixed well. Sap (clarified extract) from leaves of non-infected (control) rice plants, similarly treated, served as control. The experiment was repeated twice.

Aggregation of chloroplasts and clumping of host components[8,9] occurred in slides within 10 min, of mixing (antigen and antiserum, 1:1 ratio). No such agglutination was observed when sap from control rice plants reacted with antiserum. Thus, the present investigation indicated a distinct serological relationship between Indian and Japanese isolate of RNMV and hence confirmed the occurrence of RNMV in India.

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