In the third experiment, all the eight plants treated showed complete recovery while the untreated ones showed decline in growth, and absence of development of any new flush of foliage in spring, as was the case in the treated plants. The two untreated plants appear to be on the verge of collapse and death.

Full recovery of six out of eight treated trees originally having mild to moderately severe greening infection indicates high effectiveness of the chemical. This is also reflected in the overall healthy appearance of the treated trees and increase in fruit yield. However, severely affected trees did not show marked recovery or improvement in vigour. During the same period, deterioration of the control trees was more pronounced. The glasshouse experiment shows that spraying of the chemical is less effective and phytotoxicity at higher doses was an additional disadvantage in this mode of application. Reappearance of symptoms in one of the recovered plants indicates that the plant was not completely cured by spray application.

The method of direct insertion of the chemical was found to be the most suitable one as the treated plants recovered fully and reappearance of greening symptoms did not occur. This was further confirmed by indexing.

The effectiveness of the chemical CPAN No. MJN 1891 in controlling greening disease of citrus has been demonstrated. Since the pathogen is restricted to the phloem region, it is essential that chemical application be so designed that the chemical reaches the phloem region or the conducting tissues directly and in the shortest possible time. These studies will be helpful in developing greening-free nucleus planting material and if this material is grown in the vector-free areas already identified[7], it will form a crucial component of the certification programme.

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NATURAL AUTOFLUORESCENCE IN OOSPORES OF *PERONOSCLEROSPORA SORGHI* AND THEIR VIABILITY

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PERONOSCLEROSPORA SORGHI (Weston & Uppal) C. G. Shaw is a major problem for the production of sorghum (*Sorghum bicolor* (L.) Moench) and maize (*Zea mays* L.) in many parts of the world[1,2]. The pathogen produces two types of spores, viz. conidia and oospores. The oospores are mainly responsible for perpetuation of the pathogen through unfavourable seasons[3]. There are several reports on oospore germination in *P. sorghi*[2,4].

According to Williams[2] the subjects of the longevity, viability and germination of oospores of the graminaceous downy mildews are characterized by confusion and contradiction. Time and again the need was emphasized for an improved method for determining oospore viability in graminaceous downy mildews to meet the inadequacies in the previous methods[2,5]. An inverse relationship between natural autofluorescence in fungal spores and their viability was reported by Wu and Warren[6]. The present study was undertaken to determine viability of the oospores of *P. sorghi* using fluorescence microscopy.

Oospores of *P. sorghi* were collected from infected and dried leaves of sorghum by the method of French and Schmitt[7] and stored at room temperature (25 ± 5°C). One-year-old oospores were plated for germination on water agar in petri dishes[4,7]. After 3–4 days, a portion of water agar along with oospores was removed using a cork borer, mounted on a microscope slide and covered with a coverglass, and observed for the presence/absence of autofluorescence. The experiment was repeated thrice and 400 oospores were observed each time.

A Leitz Orthoplan epifluorescence system with a

Pleomopak fluorescence vertical illuminator was used to observe the oospores for autofluorescence. The light source was a 200 W high-pressure mercury lamp (HBO 200). A Leitz Wetzlar Fluota lens was used to enhance the brightness of the fluorescence at wavelengths of 450–490 μm.

Photomicrographs were taken with a normal 35 mm Leitz Vario-Orthomat camera system using ASA 400 fast film (ORWO NP 27) and 1–2 min exposure time. To show the relationship between fluorescence and viability, comparable pictures of bright field and fluorescence were taken of the same field.

Examination of the material plated for germination revealed that 56% of the oospores emitted fluorescence (figure 1). The percentage of oospore germination was 8. The germinated oospores did not show fluorescence in the protoplasm (figure 2).

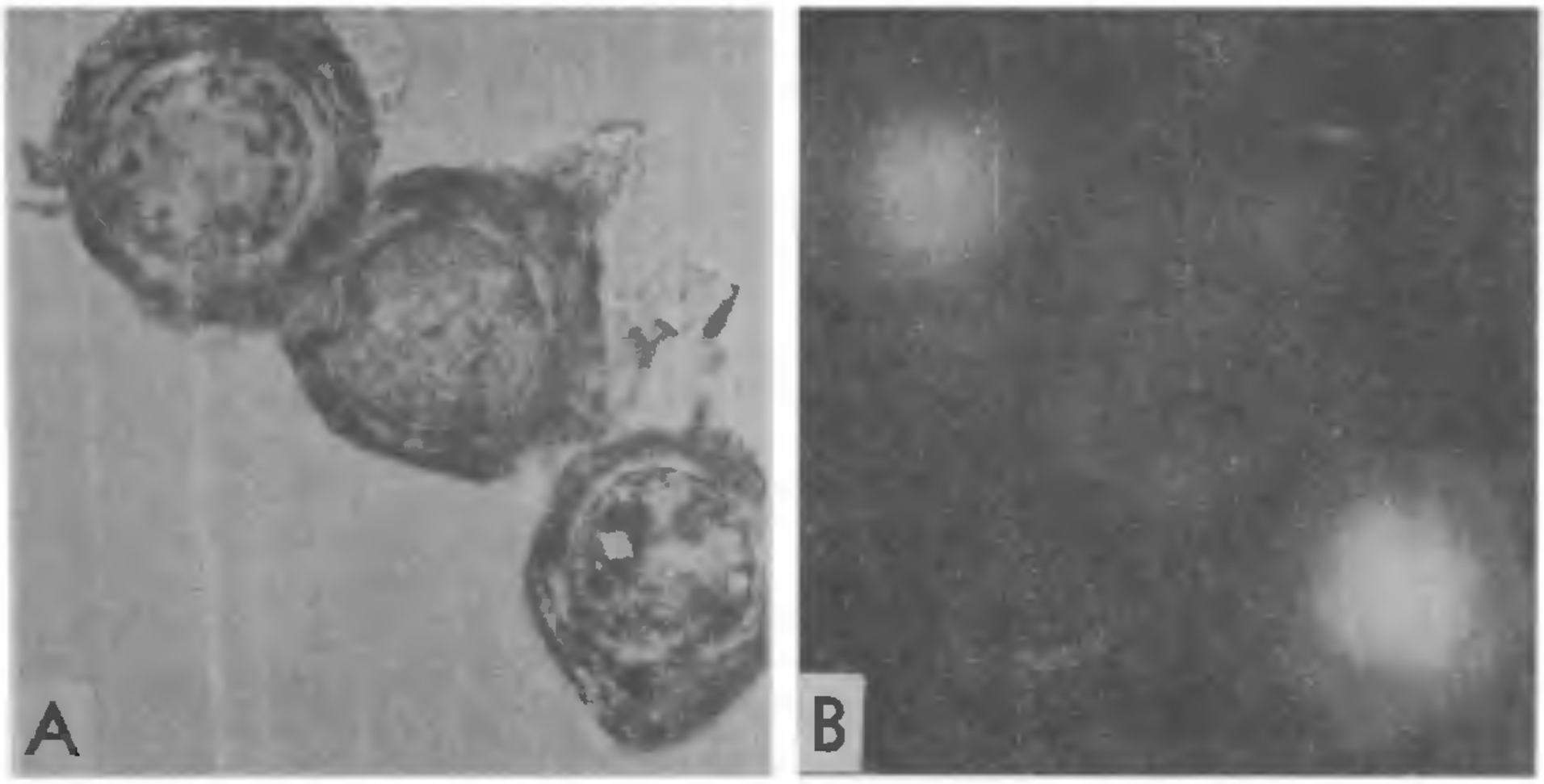

Figure 1. Oospores of *Peronosclerospora sorghi* mounted in water (×750) under (A) bright-field and (B) fluorescence microscopy.

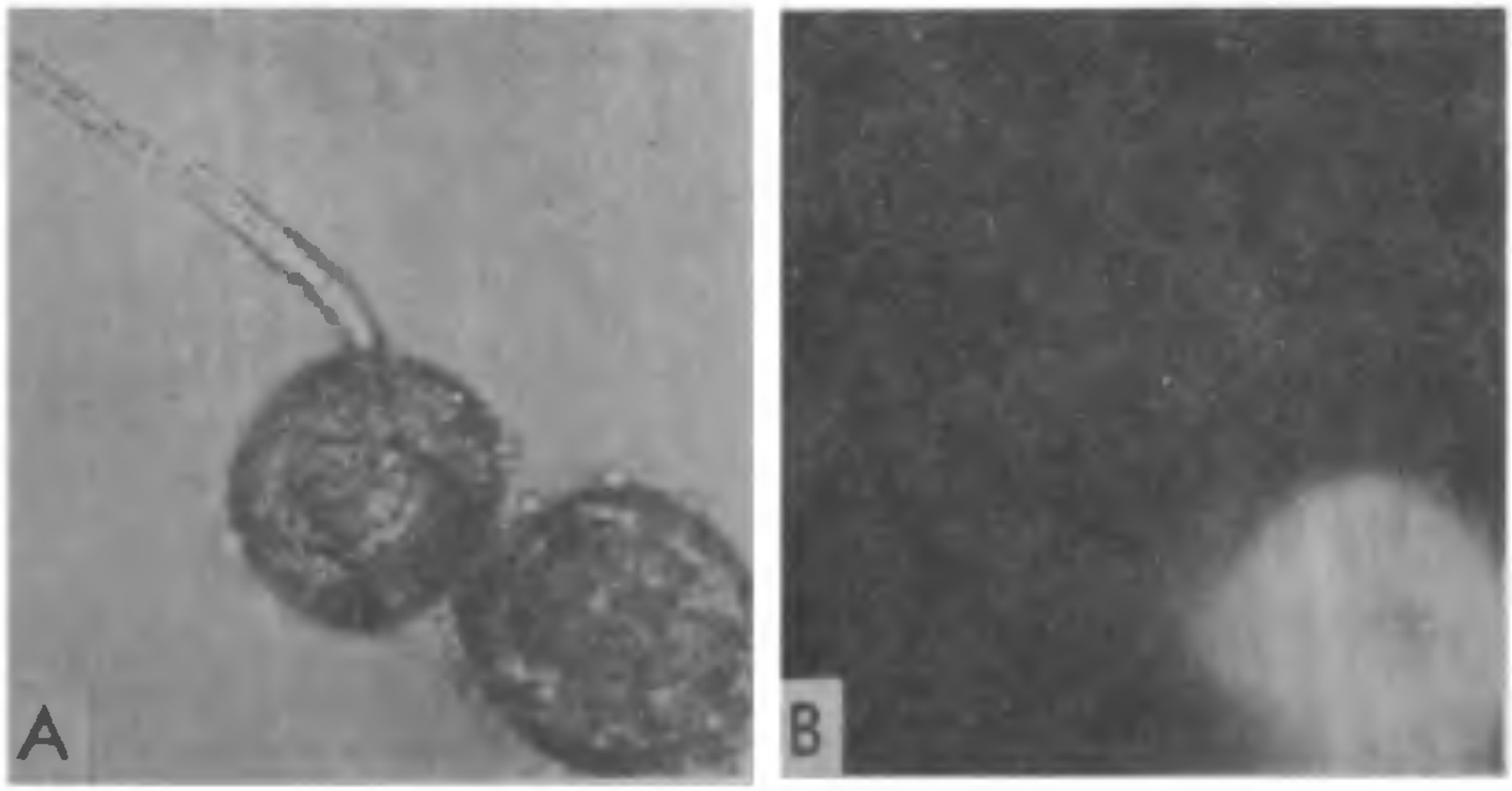

Figure 2. Germinated and ungerminated oospores of *Peronosclerospora sorghi* on water agar (×750) under (A) bright-field and (B) fluorescence microscopy.

To examine the possibility of staggered germination, oospores sown on water agar with furfural/ seedlings were removed after 3–4 days from the agar surface, rinsed 8–10 times in sterile distilled water, and then transferred to petri dishes containing water agar with furfural/seedlings.

In the test for staggered germination, up to 2% of the oospores germinated when they were plated for the second time on water agar with furfural/ seedlings.

Non-fluorescent but non-germinated oospores of *P. sorghi* are in a state of dormancy. The occurrence of staggered germination of oospores confirms this view. Kaveriappa[8] also suggested that all the viable oospores do not germinate simultaneously.

Wu and Warren[6] observed natural autofluorescence in the cytoplasm of conidia, pycnidiospores, ascospores and mycelia of 12 species of fungi incubated on agar plates, soil or in suspension. The authors concluded that natural autofluorescence in fungal materials indicated their death, and found a relationship between autofluorescence and viability. The same procedure is useful for determining viability of the oospores of *P. sorghi*. The technique may be suitable for determining viability of other downy mildew fungi.

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IN VITRO GROWTH OF SOME FUNGI ISOLATED FROM WHEAT PHYLLOPLANE IN RELATION TO SO_2 TREATMENT

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SULPHUR dioxide adversely affects microbes on aerial plant surfaces and suppresses the pathogenicity of many plant pathogens[1]. A number of field observations have been made on the effects of SO_2 on saprophytic and parasitic micro-organisms with special reference to diseases of plants grown in SO_2-polluted localities. Singh[2] observed that the population of some test fungi inoculated on the phylloplane of wheat decreased on prolonged exposure to SO_2 at $2669 \pm 105\ \mu g/m^3$ concentration. However, little is known about effects of SO_2 on growth of phylloplane fungi. The present communication deals with effects of SO_2 on growth of some phylloplane fungi *in vitro*.

The experimental system was designed according to the method described by Magan and Lacey[3]. The following fungi, isolated from the phylloplane of wheat, were selected for the study: *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Cladosporium cladosporioides*, *Curvularia lunata*, *Drechslera australiensis*, *Epicoccum purpurascens*, *Fusarium oxysporum*, *Penicillium chrysogenum* and *P. citrinum*. Mycelial blocks (5 mm each) cut from actively growing margins of colonies of the fungi were transferred separately onto petri plates, in triplicate, containing 20 ml of potato dextrose agar (PDA) medium. The lids of the plates were raised slightly using sterile paper clips, and the plates containing the mycelial blocks were placed in a fumigation chamber. Dishes of glycerol/water were placed in the bottom of the chamber to provide required humidity. The petri plates were exposed to sterilized air–SO_2 mixture for 10, 30 and 60 min, with the concentration of SO_2 adjusted to $2669 \pm 105\ \mu g/m^3$ air. Plates exposed to gas-free air served as control. The plates were incubated at $24 \pm 2°C$ after the treatment and growth was measured after 24 h of incubation. Percentage growth stimulation/inhibition was cal-