Conidia of Botrytis cinerea: Labeling by Fluorescent Vital Staining

Abstract. *As a model system, conidia of* Botrytis cinerea *have been vitally stained with optical brighteners to facilitate their location in plant tissue.*

Some optical brighteners are stable, nontoxic, fluorescent biological markers of potential use in labeling certain bacteria, fungi, and algae (*1–3*), and offer considerable advantages over normal stains for particular applications. This report describes their use in tracing the movement of fungal spores in plant tissue, with *Botrytis cinerea* Pers. ex Fr. and tomato stem as a model system.

Investigations of the entry and movement of conidia of *B. cinerea* in tomato petiole stubs have hitherto relied upon standard histological stains and techniques but none of these have been entirely successful because of the great difficulty in detecting stained conidia against the background of stained host tissue (*4*). From a range of 14 optical brighteners two were selected for further tests, namely "Uvitex S2R High Conc." (Ciba Clayton) and "Tinopal 4BMT" (Geigy)—both stilbenic derivatives, but whose structural formulas were not released. These were made up as 3 percent solutions (weight to volume) in 20 percent aqueous glycerol (by volume) (this being selected as the most suitable of 20 solvents tried). Since certain constituents of the commercial formulations were insoluble, filtration was necessary, and the final concentration of active ingredient was approximately 2 percent. The solutions were finally sterilized by autoclaving at 15 lb/in.^{2} for 15 minutes.

Initially the solutions were incorporated in basal media (1 : 10, by volume) but, although hyphae of *B. cinerea* were successfully stained, results with conidia *in situ* were poor. Detached conidia, however, could be stained readily; these were taken from cultures of *B. cinerea* on sterile tomato leaves, suspended in water, centrifuged and resuspended in 15 ml of brightener solution. After standing overnight, during which time germination did not occur, the spores were washed in distilled water by repeated centrifugation and resuspension, until the supernatant showed no fluorescence (normally three times). This treatment did not impair subsequent germination.

From the final centrifugate, a suspension of 0.1 ml of wet conidia in 50 ml of distilled water was applied during bright sunny weather to freshly cut petiole stubs of growing tomato plants, when conidia were rapidly taken into the xylem vessels (*4*). Tissue samples were fixed in a mixture of formalin, propionic acid, and ethanol at 24 hour intervals, dehydrated and embedded in paraffin wax by standard methods, and sectioned at 10 μ. Prior to mounting, portions of the ribbon were examined on slides by fluorescence microscopy using ultraviolet or ultraviolet/blue light (blue light alone was not suitable). The labeled conidia were readily located with a × 10 objective, and ribbons containing them were permanently mounted with a gelatin fixative and distilled water; they were then dried, dewaxed in xylene, and finally mounted in Fluormount (E. Gurr Ltd.) for further examination. No deterioration in fluorescence of labeled conidia was noted after processing, and in the preparations the position of the conidia in the host could be detected with ease (Fig. 1). Throughout all inoculations with labeled conidia, pathogenicity was unimpaired. This method has also been used successfully with spores of species of *Penicillium, Verticillium,* and *Aspergillus.*

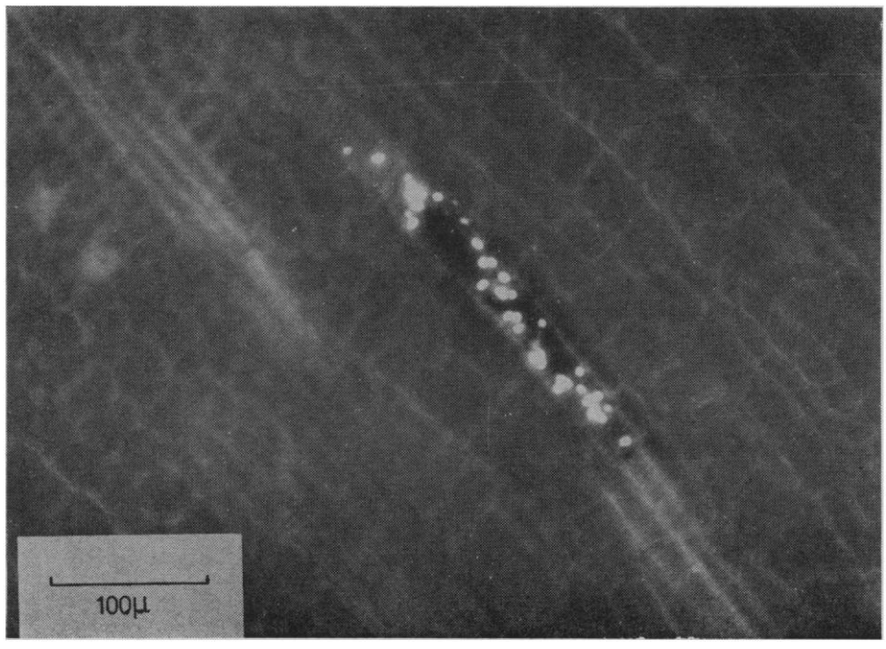

Fig. 1. Labeled conidia of *Botrytis cinerea* in tomato petiole xylem.

In addition to the mycelium of *B. cinerea,* mycelia of other sporing and nonsporing fungi have been stained satisfactorily by growing them on agar media incorporating brightener. Under these conditions, differential staining of various areas of cell wall and cytoplasm has been noted. Bacterial plant pathogens of the genera *Pseudomonas* and *Pectobacterium* have also been stained by this method and, after inoculation, their movement in plant tissue has been traced. In no case has growth on brightener agar had any apparent deleterious effect on viability. While there is some indication that all organisms are not successfully stained by the same compound, vital fluorescent staining with optical brighteners may prove a valuable technique in such problems as spore dispersal, the initial penetration phase of parasitism, and the movement of vascular pathogens.

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References and Notes

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5. The gifts of samples of optical brighteners from Geigy (U.K.) Ltd. and Ciba Clayton Ltd. are gratefully acknowledged.

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