A Rapid Staining Method for *Erysiphe graminis* f. sp. *hordei* in and on Whole Barley Leaves with a Protein-Specific Dye

Gerhard Wolf and Friedrich Frič

Institut für Pflanzenpathologie und Pflanzenschutz, University of Göttingen, Grisebachstrasse 6, D-3400 Göttingen, West Germany.
Present address of the second author: Institute of Experimental Biology and Ecology, Bratislava, CSSR.
Accepted for publication 4 November 1980.

ABSTRACT

Wolf, G., and Frič, F. 1981. A rapid method for staining *Erysiphe graminis* f. sp. *hordei* in and on whole barley leaves with a protein-specific dye. Phytopathology 71:596-598.

Whole barley leaves infected with *Erysiphe graminis* f. sp. *hordei* were fixed with and cleared in ethanol/chloroform/trichloroacetic acid and stained with Coomassie Brilliant Blue R-250. Conidia, germ tubes, and haustoria as well as reactions of the host cell could be visualized by this protein-specific dye.

Additional key words: haustorium, halo, host cell wall reaction.

Routine experiments to follow the infection process in powdery mildew-infected cereal leaves require a method that allows optimal staining not only of germinating spores and mycelium on the leaf surface but also of haustoria in the epidermal cells. The most commonly used method to date is the staining of whole leaves after treatment with lactophenol or staining the stripped epidermis mainly with cotton blue or methyl blue in lactophenol (1,4,6,8).

Both methods have disadvantages. In the method for whole leaves the haustoria are not clearly stained, and when using isolated epidermis the process of stripping is laborious and practically impossible with older leaves. The use of polychromatic dyes has an additional limitation; they also stain the plant cell wall and make it difficult to obtain a high-contrast picture of haustoria.

The following method overcomes the problems mentioned above.

Infected whole leaves or 1–2 cm fragments are cleared for 10–60 min (or longer as required according to the age of the leaves) at 70 C or overnight at room temperature in an ethanol-chloroform (75:25, v/v) mixture containing 0.15% trichloroacetic acid, with frequent changes of the solution. The cleared leaves are then stained in a dye solution prepared 1 day prior to use and consisting of one volume 15% trichloroacetic acid in water and one volume 0.6% Coomassie Brilliant Blue R-250 (Serva, Heidelberg, West Germany) in 99% methanol. Young leaves are usually stained within 15–30 min; however, leaves of some cultivars and older leaves require a longer staining period. It is advisable to run preliminary tests to determine the period required for optimal staining. It should be noted that a light blue background due to staining of chloroplasts in the mesophyll cells cannot be avoided. This does not affect the visibility of the haustoria.

Stained preparations are easily preserved in a solution of glacial acetic acid-glycerol-water (5:20:75, v/v) either submerged in small containers or on slides where the coverslips are sealed with, for example, fingernail varnish. Such preparations are stable for at least 1 yr if kept in darkness.

Conidia, germ tubes, and also haustoria are clearly visible after staining by this procedure (Fig. 1A and B). In addition, this method preserves the structural integrity of the infection structures so that details such as the extrahaustorial membrane and the haustorial nucleus can be clearly observed (Fig. 1A). The good staining properties of haustoria are based on their high cytoplasm content in contrast to the low cytoplasm content of the leaf epidermal cells.

Changes of the host cell can also be visualized. For example, the concentration of dye in the host cell underneath the appressorium shows that in the area of the phenomenon known as 'halo,' an accumulation occurs that is not only of silicon and manganese (7) but also of proteins. Moreover, reactions of the cell wall beneath or adjacent to the appressorium may be observed in a resistant cultivar (Fig. 1C).

Although commonly used for the specific staining of proteins after gel electrophoresis, Coomassie Brilliant Blue until now has rarely been applied for cytological studies of botanical material (2,3).

In our opinion, this dye is particularly suitable for investigations of the host-parasite complex cereal-powdery mildew, not only because of the detailed staining of haustoria but also because this dye is specific. In contrast to polychromatic dyes, staining with Coomassie Brilliant Blue offers more information on the accumulation of proteins in host and/or parasite structures.

0031-949X/81/06059603/$3.00/0

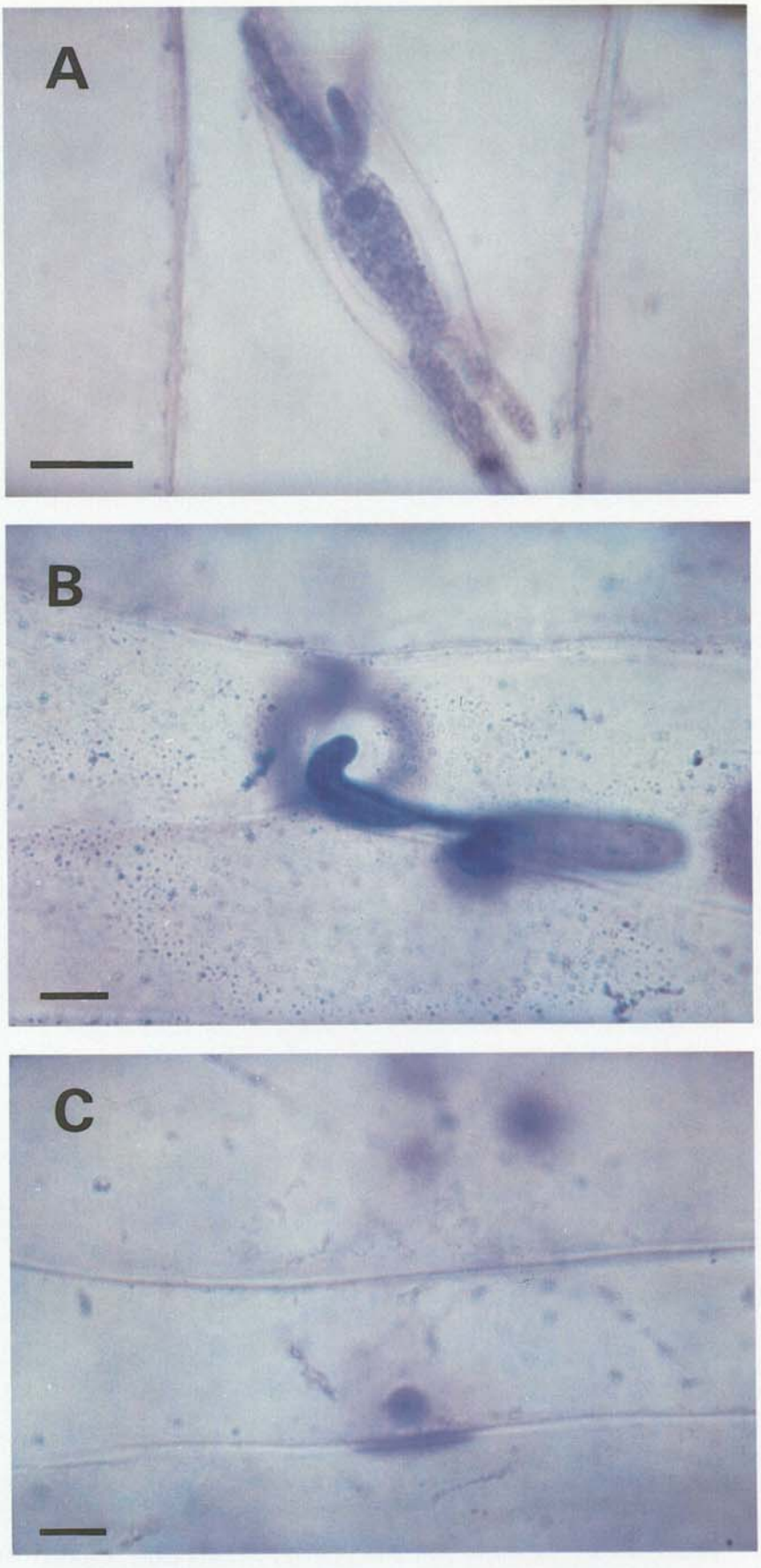

Fig. 1. Photomicrographs of barley leaves infected with *Erysiphe graminis* f. sp. *hordei*. **A,** Haustorium 48 hr after inoculation of a 6-day-old leaf of a susceptible cultivar. Note the high contrast of the nucleus and the extrahaustorial membrane. **B,** Germinating conidium from a secondary infection of a susceptible cultivar showing the appressorium with the induced 'halo' of the epidermal cell. **C,** Accumulation of protein beneath the appressorium and the adjacent cell wall in a leaf of a resistant cultivar. The fungus was removed before staining. Specimens were stained with Coomassie Brilliant Blue and observed with a Zeiss microscope type Universal using the objectives Neofluar 40/0,75 and Planapo 63/1.4 oil. Bars $\simeq$ 100 μm.

This method has been successfully applied in studies on the reaction of cultivars resistant to powdery mildew and in investigations of the mode of action of fungicides (Wolf and Kollar, *unpublished*). It can also be used for studies with several other fungus-host combinations.

LITERATURE CITED

1. Carver, T. L., and Carr, A. J. H. 1977. Race non-specific resistance of oats to primary infections by mildew. Ann. Appl. Biol. 86:29-36.
2 Cawood, A. H., Potter, V., and Dickinson, H. G. 1978. An evaluation of Coomassie Brilliant Blue as a stain for quantitative microdensitometry of protein insections. J. Histochem. Cytochem. 26:645-650.
3. Fisher, D. B. 1968. Protein staining of ribboned epon sections for light microscopy. Histochemie 16:92-96.
4. Ghemawat, M. S. 1977. Polychromatic staining with toluidine blue O for studying the host-parasite relationship in wheat leaves of *Erysiphe graminis* f. sp. *tritici*. Physiol. Plant Pathol. 11:251-253.
5. Hyde, P. M. 1977. The effect of leaf age on infection of wheat seedlings by *Erysiphe graminis* and on subsequent colony development. Phytopathol. Z. 88:299-305.
6. Kunoh, H. 1972. Morphological studies of host-parasite interactions in powdery mildew of barley, with special reference to affinity between host and parasite. Bull. Fac. Agric., Mie Univ. 44:141-224.
7. Kunoh, H., Ishizaki, H., and Kondo, F. 1975. Composition analysis of 'Halo' area of barley leaf epidermis induced by powdery mildew infection. Ann. Phytopathol. Soc. Jpn. 41:33-39.
8. White, N. H., and Baker, E. P. 1954. Host pathogen relations in powdery mildew of barley. I. Histology of tissue reactions. Phytopathology 44:657-662.