Cell Wall Ingrowths of Nonhaustorial Hyphae of Peronospora trifoliorum

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Contribution No. 548. Department of Plant Pathology, Kansas Agricultural Experiment Station. Supported in part by a National Science Foundation Traineeship.

Grateful acknowledgement is made to D. J. Pihlaja for assistance in electron microscopy.

ABSTRACT

The cell wall of *Peronospora trifoliorum* mycelium in *Medicago sativa* leaves has finger-like projections extending as deep as 1 μm into the fungal cytoplasm. The fungal plasma membrane follows the contours of these wall ingrowths and its surface area thereby is increased considerably.

Phytopathology 65:638-639

Peronospora trifoliorum d By., the causal fungus of downy mildew of alfalfa (*Medicago sativa* L.), is an intercellular obligate plant parasite that does not require haustoria for growth and reproduction. Fraymouth (3) found a few haustoria of *P. trifoliorum* in alfalfa leaves collected in May or June, but found none in infected leaves collected in August. To date we have observed no haustoria in mildewed leaves from several alfalfa plants inoculated in the laboratory. This paper reports on the ultrastructure of nonhaustorial hyphae of this fungus.

Potted alfalfa plants with 5 days regrowth were placed in an 8-hour photoperiod with 5.4×10^3lx of fluorescent and incandescent lighting at 18 C for 5 days. They were then sprayed until run-off with a sporangial suspension (10^5 sporangia/ml) of *P. trifoliorum*, placed in the dark at 100% relative humidity for 16 hours at 18 C, and then returned to the above conditions for 11-16 days.

Intact infected leaves were cleared with chlorine gas, stained for 1-2 hours at 50 C in 0.1% aniline blue in lactic acid, and destained in lactic acid at 50 C for 2-24 hours. Mycelium was abundant but haustoria were not observed in those whole-leaf preparations. Other infected leaves were cut into small pieces, fixed in FAA, dehydrated in an

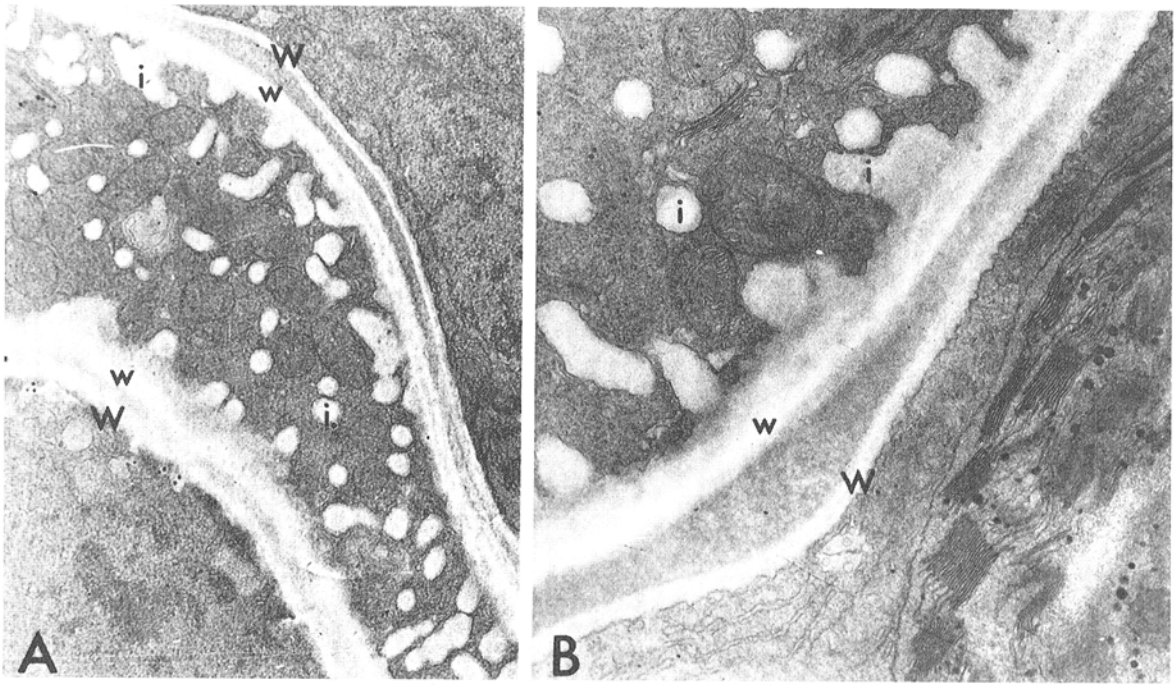

Fig. 1-(A, B). Longitudinal sections near the surface of intercellular *Peronospora trifoliorum* hyphae in alfalfa leaves showing hyphal wall ingrowths (i) longitudinally near the hyphal wall (w) and transversely within the hyphae. W = host cell wall. **A)** (× 12,740). **B)** (× 27,440).

alcohol dehydration series, paraffin-infiltrated and blocked for sectioning. Sections 10-μm thick were stained in a fast-green and safranin staining series (4), modified by staining with aniline blue in water before the fast-green step. Vegetative hyphae were found throughout the leaf conforming to intercellular spaces, but haustoria were not observed.

For electron microscopy, plugs were cut from the infected and healthy leaves with a punch made from an 18-gauge hypodermic needle. The tissue was immediately fixed in either 4% unbuffered $KMnO_4$ for 30 minutes at 0 C, or in 1% glutaraldehyde in 0.05 M *s*-collidine buffer pH 7.4 for 2 hours, and the latter postfixed for 1 hour in 1% osmium in 0.05 M *s*-collidine buffer pH 7.4 at 0 C. Ethyl alcohol was used in the dehydration series. The tissue was stained 30 minutes in saturated uranyl acetate in the 70% alcohol step. The tissue was embedded in Araldite Epon embedding medium (5), sectioned with glass knives on a Reichert ultramicrotome, and examined with an RCA EMU-4 electron microscope.

The fine structure of the vegetative hyphae of *P. trifoliorum* was similar to that reported for other Oomycetes (2, 7), except the *P. trifoliorum* hyphal wall had finger-like ingrowths extending as far as 1 μm into the fungal cytoplasm (Fig. 1). The ingrowths were present in all hyphae observed in tissue fixed in either permanganate or glutaraldehyde and gave a granular appearance to the hyphae through the light microscope. The fungal plasma membrane conforms to the contours of the wall, and thus the ingrowths thereby extend its surface area considerably.

The role of the fungal wall ingrowths is unknown, but they resemble those in transfer cells of higher plants (6), which are believed to facilitate transmembrane flux of solutes. Dick et al. (1) demonstrated with microvilli that the larger the surface area of the cell membrane, the greater the potential flux across it. Perhaps the relatively large plasma membrane area in *P. trifoliorum* hyphae is partly responsible for this obligate parasite's ability to function without haustoria.

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