Electron Microscope Radioautography of ^{14}C Transfer from Rust Uredospores to Wheat Host Cells

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ABSTRACT

Electron microscope radioautograms demonstrate the passage of radioactivity from the pathogen to the host. The label appears to accumulate in the host chloroplasts and cell walls. There is presumptive evidence that at least a portion of the transfer is accomplished via the intercellular mycelium. Label does not accumulate in the haustorial encapsulation, indicating that the encapsulation is not acting as a sink for the radioactive substances involved in the present study. Phytopathology 60: 1850-1851.

One of the prime difficulties in the study of the disease syndrome in obligate parasites is that of determining the role played by each member in the parasitic relationship. Despite the problems inherent in the method, electron microscope radioautography is one of the more useful available techniques whereby the contribution of the host and pathogen can be independently determined. The majority of the studies using tracer methodology have involved labeling of the host (9, 10) followed by whole specimen radioautography, but there are now some reports in the literature in which label was supplied to the uredospore and its subsequent passage into the host determined by radioautography (3, 4, 5). The present paper is a report on an electron microscope radioautographic study of the transfer of ^{14}C from germinating uredospores and its subsequent incorporation into host tissues in the infection center.

Seven-day-old seedlings of *Triticum aestivum* L. f. sp. *vulgare* (Vill., Host) MacKey 'Little Club' were inoculated with isolate 56-51A of *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & H. Henn. Starting 4 days after inoculation and continuing to the 10th day, the host plants and developing infection centers were labeled with ^{14}C by generating $^{14}CO_2$ after the method of Daly et al. (2). Spores were collected on the 11th day and 13th day and used immediately to inoculate the first leaf of nonlabeled, 7-day-old Little Club seedlings. These leaves were harvested 5 days after inoculation with the labeled uredospores and were processed as follows. Infected leaf pieces (ca. 1×3 mm) were killed and fixed in 3% potassium buffered glutaraldehyde (pH 7.2) for 6 hr at 22 C, followed by potassium buffered (pH 7.2) 1% OsO_4 for 12 hr at 4 C, dehydrated in a graded acetone series, and embedded in an Epon-Araldite mixture (6). Thin (silver) sections were cut with glass knives on a Sorvall MT-2 ultramicrotome and placed on Formvar-coated grids. The grids were coated with Ilford L2 nuclear track emulsion (1) and stored in a light-tight lead box for 6-8 weeks. The exposed grids were developed in Microdol-X for 2 min, washed, fixed, stained with Reynolds' lead citrate (8) and examined in an RCA EMU 3G electron microscope. The electron microscope radioautograms thus obtained are referred to as EM-RAG's.

Developed silver grains were present in the mycelium located in the intercellular spaces as well as in the haustoria (Fig. 1-3). Developed grains also were abundant in the host chloroplasts and in the walls of the mesophyl cells (Fig. 1-3). There often seemed to be an accumulation of grains in the host cell in areas of actual mechanical contact between the host wall and fungus mycelium (Fig. 1). Grains appeared to occur with some frequency over host membranes, particularly the tonoplast, and ER, and mitochondria, but considering the geometry of grain scatter (7), it is difficult at this point to determine whether this might simply be fortuitous, in view of the close proximity of these membranes to the wall and chloroplasts in which accumulation is obvious. Some grains were also present in the host nuclei, but none (beyond the expected background grains) was present in the vacuole.

Whole specimen radioautograms (4) demonstrated the transfer of ^{14}C from labeled uredospores to unlabeled host and the subsequent translocation of the ^{14}C from the inoculation site to the remainder of the first leaf and later to the root and second and third leaves. The present EM-RAG's demonstrate the presumed incorporation of the label into specific host organelles. There is some evidence that the label may pass from pathogen to host via the intercellular mycelium at the point of contact between the mycelium and host cell, but the form in which the transfer is accomplished is unknown. The presence of label in host organelles adjacent to haustoria (Fig. 1, 3) and the presence of occasional grains in the encapsulation (Fig. 1) provide presumptive evidence that some material may pass from the haustoria into the host cytoplasm, although the amount of radioactivity present in haustoria is considerably less than that present in the mycelium. At no time have we observed a heavy accumulation of developed silver grains in the haustorial encapsulation. EM-RAG's of infected tissue harvested from leaves labeled with ^{14}C supplied as glucose demonstrated the passage of label from the host through the encapsulation into the haustorium, but also without an accumulation of grains in the encapsulation (*unpublished data*). Results from these two different types of experiments would seem to indicate that, at least at this relatively advanced stage of disease development, the encapsulation is not acting as a sink for the radioactive substances involved, nor as a barrier to their passage from pathogen to host or host to pathogen.

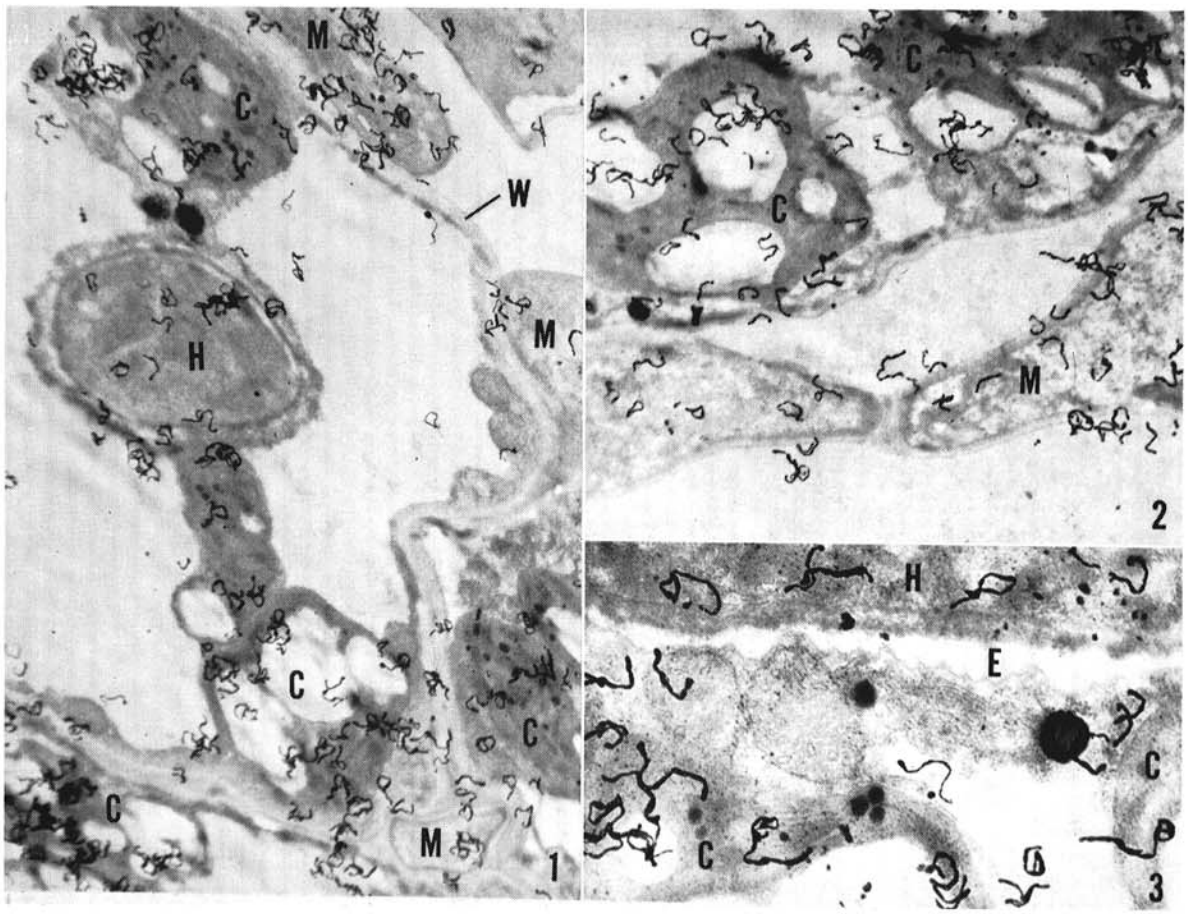

Fig. 1-3. **1)** Haustorium (H) in host cell. Note the silver grains in the chloroplasts (C), intercellular mycelium (M), and in the host cell wall (W) adjacent to the mycelium (×9,300). **2)** Note the label in the chloroplasts (C) and intercellular mycelium (M) and the absence of silver grains (beyond expected background and scatter [7] in the intercellular space on either side of the mycelium (×9,500). **3)** Portion of a haustorium (H) showing the encapsulation (E) which separates the haustorium from the host cytoplasm. Note the absence of label in the encapsulation (×21,000).

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