Light and Transmission Electron Microscopy
of White Pine Blister Rust Canker

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ABSTRACT

This report describes some reactions that occur between *Pinus monticola* bark cells and hyphal cells of *Cronartium ribicola*. A striking reaction is the affixation of hyphal cells to host cell walls. This is not a randomly occurring phenomenon, and it is an important host-pathogen relationship. Host cell walls beneath affixed hyphal cells stained lighter than the wall material on either side of this zone of affixation. Evidence is given for the possible presence of cellulase and pectinase activity in the zone of affixation.

Described for the first time is an extracellular gel-like material that differs in electron density from the sheathing layer surrounding hyphal cells. In cross section, this material occurred on both sides of hyphal cells affixed to host cell walls. Strands of this gel-like material connected several hyphae that were near each other but not otherwise in contact. Also, this material was observed bridging spaces between host cell walls and unaffixed hyphal cells.

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Additional key words: host-parasite interactions, pectinase, cellulase

Scanning electron microscopy (SEM) studies of western white pine (*Pinus monticola* Dougl.) bark infected with the blister rust fungus (*Cronartium ribicola* J. C. Fisch. ex Rabenh.) revealed that hyphal cells of the fungus were affixed to host cell walls (14, 16). To obtain some knowledge of reactions that occur at the cell wall interface between the rust pathogen and its host, and how these reactions are involved in affixation, we undertook light and transmission electron microscopy (TEM) studies of *P. monticola* bark infected with *C. ribicola*.

MATERIALS AND METHODS.—A segment of *P. monticola* stem containing a bole canker was excised from a 7- to 8-year-old nursery-reared tree. Infected bark from the peripheral area of the canker was cut into strips approximately 4-6 mm × 10 mm. Each cut was made to the wood, and the whole strip of bark was removed from the woody stem.

Infected bark strips for TEM studies were trimmed to 0.25 to 0.5 mm and placed in 6% glutaraldehyde buffered with 0.1 M collidine for 4 hours at 6 C. Strips were washed with fresh 0.1 M collidine buffer for 20 hours at 6 C and postfixed for 2 hours in buffered 1% osmium tetroxide followed by buffer rinse and dehydration in an acetone series. The strips were embedded in epoxy resin (10). Tissue sections 60 nm thick were supported on carbon-coated, Formvar-covered grids. Sections were stained with saturated uranyl acetate and lead acetate (4) and examined at 50 kV.

Sections 1,500 nm in thickness, cut from the same embedded strips described above, were used for light microscopy. The method used to stain these sections has been described (8).

RESULTS.—Light microscopy disclosed chains of two-to-six host cells within the bark cortical parenchyma. These were young, thin-walled cells that were filling in or had filled in large schizogenously formed intercellular spaces (Fig. 1, a-arrows). SEM investigations revealed similar chains of host cells (16).

Light microscopy revealed two types of cell wall material: that which surrounds living host cells and that which remains after host cells have died (Fig. 1, b-arrows). Fig. 1, a light micrograph of *P. monticola* bark infected with *C. ribicola*, illustrates the extent to which hyphal affixation has occurred. The few hyphal cells not affixed to host cell walls are likely to be those that in SEM appear to bridge intercellular spaces (14, 16). Hyphal cell affixation occurred on both types of cell material (Fig. 1, c and d-arrows).

Transmission electron microscopy disclosed a thin layer, the "capsular sheath" (12), that completely surrounded all unaffixed hyphal cells (Figs. 2, 3, and 4, e-arrows). This observation is in agreement with other

reports (1, 12). For hyphal cells affixed to host cell walls, the capsular sheath becomes indistinguishable at the juncture between the hyphal wall and the zone of affixation. The zone of affixation is the area where hyphal cells are affixed to host cells (Figs. 2 and 3, f-arrows). Fig. 3 is an enlargement of the zone of affixation of Fig. 2.

The portion of host cell wall beneath the affixed hyphal cell stained much lighter than the cell wall material on either side of the zone of affixation (Fig. 2, f-arrows). This reaction was observed in most cases where hyphal cells were affixed to host cell walls. When hyphal cells were not affixed to host cell walls, no light-staining area occurred (Fig. 4, g-arrow).

In cross section, a deposit of gel-like material was observed along both sides of the zone of affixation (Figs. 2 and 3, h-arrows). This gel-like material stained lighter than the capsular sheath; therefore its chemical composition differs from that of the capsular sheath. Affixed hyphae were often connected together by this gel-like material (Figs. 2 and 4, i-arrow). Often, strands of this gel-like material connected several unaffixed hyphae together. This gel-like material would occasionally bridge the space between host cells and unaffixed hyphal cells (Fig. 4, j-arrows). This suggests that the material is deposited prior to any visible reaction between host and pathogen. Sometimes we observed a slight swelling of the capsular sheath where the sheath and gel-like material meet (Fig. 4, k-arrow).

DISCUSSION.—The observations concerning the filling of schizogenously formed host intercellular spaces with young host cells demonstrate the dynamic nature of bark cortical parenchyma, which is constantly changing in respect to the distribution of individual host cells and intercellular spaces. The fate of the carbohydrates, amino acids, lipids, mineral ions, and the other chemical components of host cells that have been crushed or stretched apart is not known. Living cells of the host could absorb this material, it could accumulate in intercellular spaces, or some of it could be used by *C. ribicola* as it invades cortical parenchyma. The last possibility is feasible for the following reasons: (i) *C. ribicola* can be vegetatively cultured on a simple medium containing mineral salts, glucose, agar, and a sulfur-bearing amino acid (7); and (ii) *C. ribicola* can be vegetatively cultured on a membrane placed above noninfected *P. monticola* tissue cultures (6).

It has been hypothesized that the function of the capsular and haustorial sheaths is to protect the rust organism from toxic substances (tannins or polyphenols) released by the host (1). However, the processes that crush host cells and schizogenously form large intercellular spaces in the bark might also rupture segments of the

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Fig. 1-3. Light and transmission electron micrographs of *Pinus monticola* bark infected with *Cronartium ribicola* (FC = fungal cell, HCW = host cell wall). 1) Light micrograph illustrating the extent of hyphal cell-affixation to host cell walls: arrows (a) point to young thin-walled host cells that divided prior to sectioning; arrows (b) point to extracellular host cell wall material; arrows (c) point to hyphal cells affixed to host cell wall material surrounding living host cells; arrows (d) point to hyphal cells affixed to extracellular host cell wall material (×300). 2) Transmission electron micrograph illustrating the reactions between host cell wall and an affixed hyphal cell: arrow (e) points to capsular sheath; arrows (f) point to the lighter staining area on host cell wall directly beneath an affixed hyphal cell; arrows (h) point to gel-like material on either side of affixed hyphal cells; arrow (i) points to gel-like material connecting two affixed hyphal cells together (×8,854). 3) An enlargement of Fig. 2 showing part of the zone of affixation: arrow (e) points to capsular sheath; arrow (f) zone of affixation (width); arrow (h) points to gel-like material on one side of an affixed hyphal cell (×95,388).
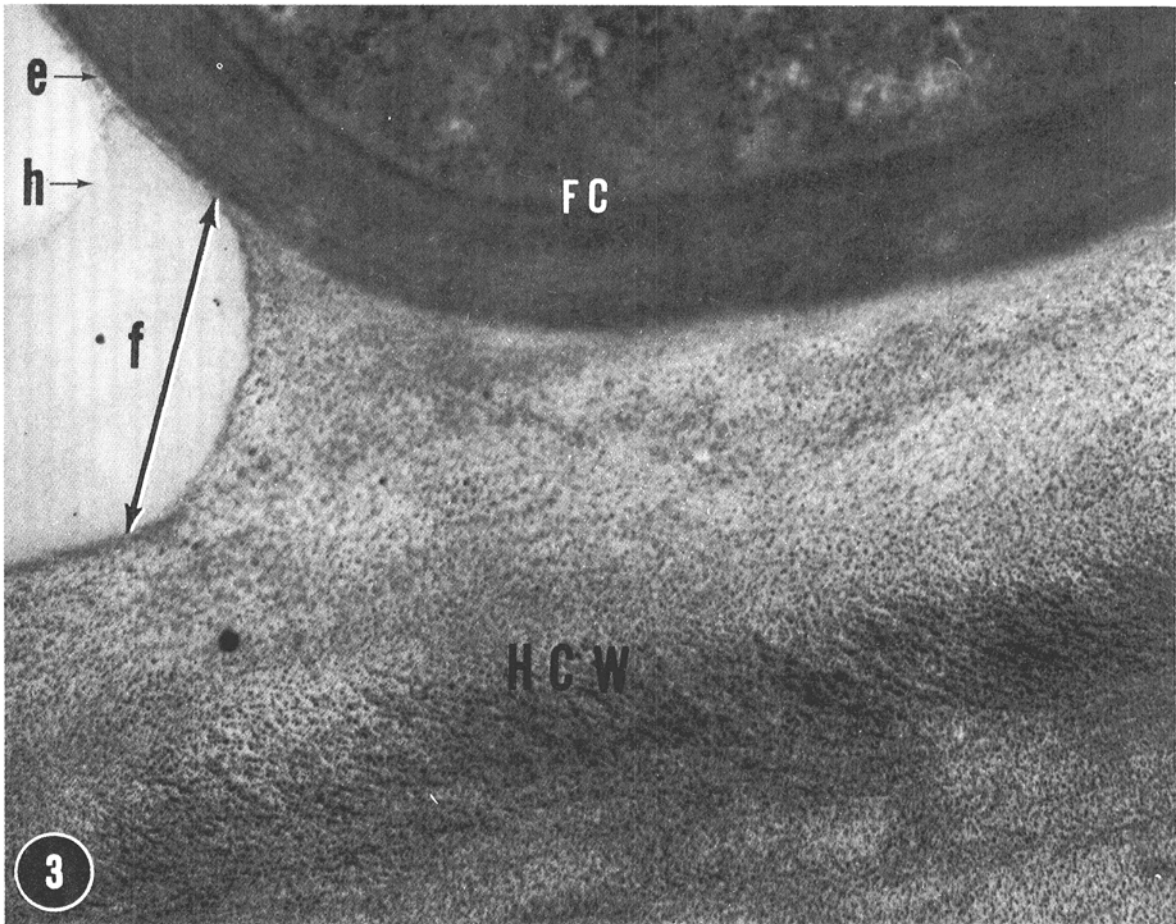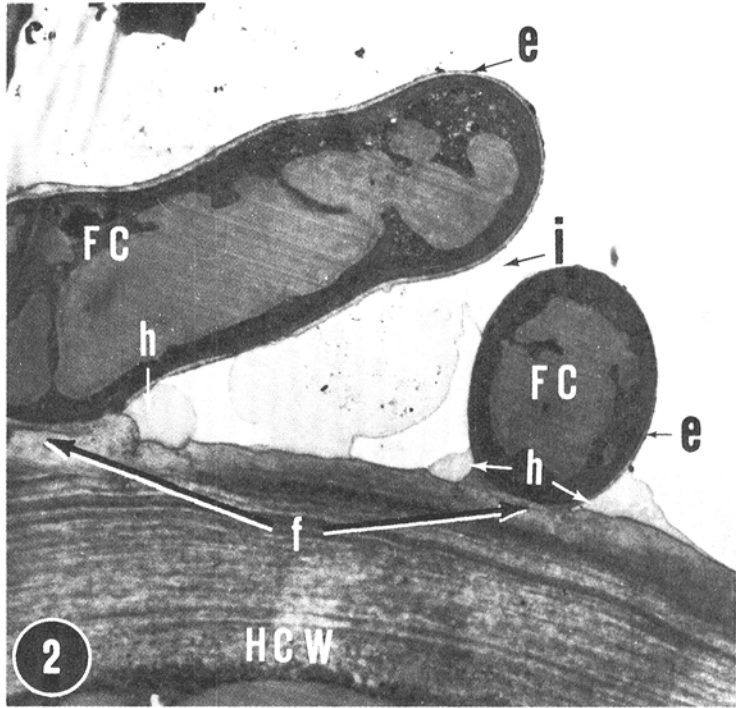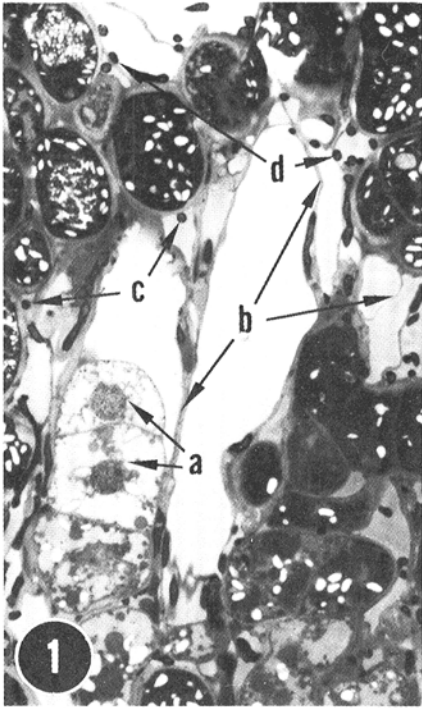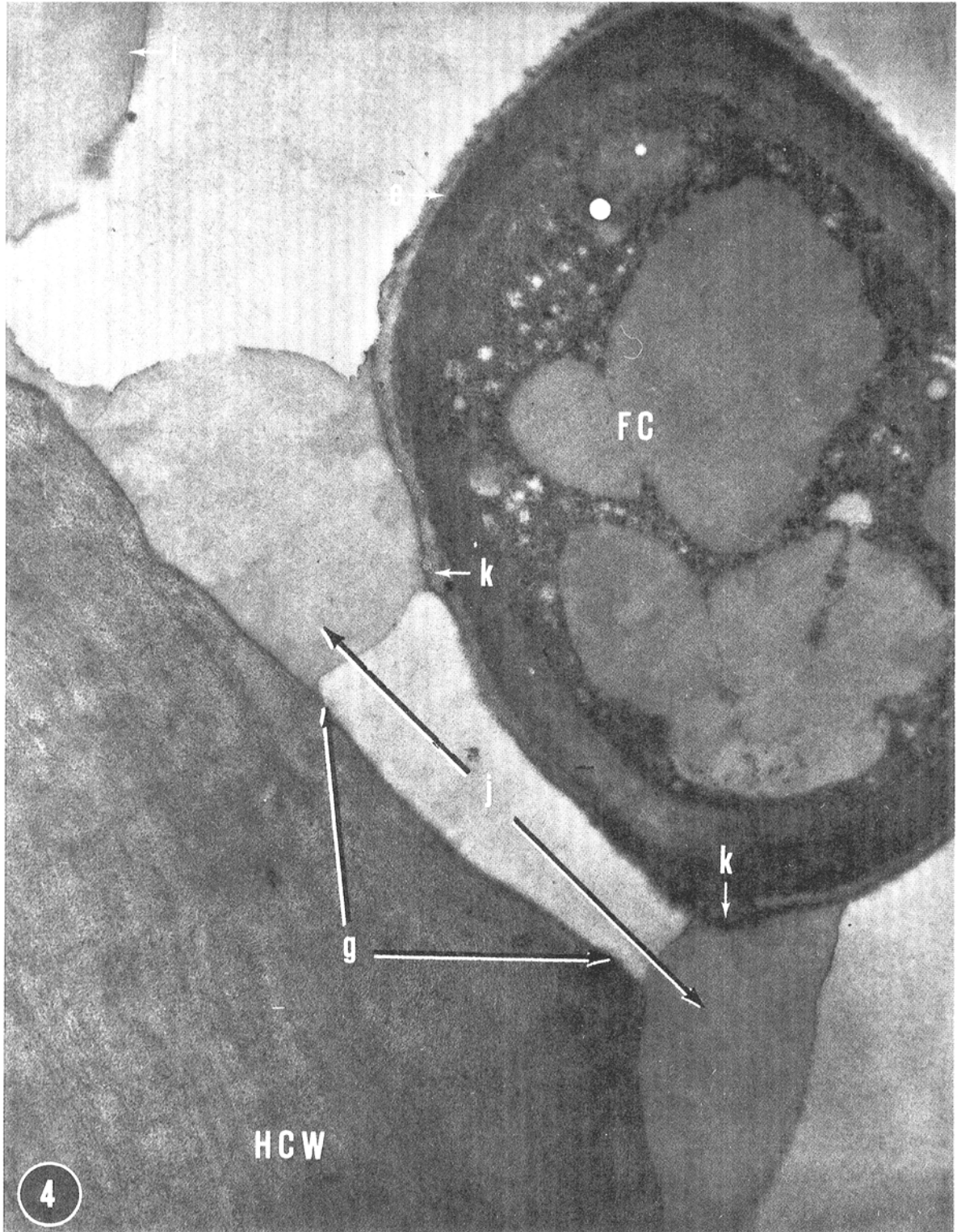
Fig. 4. A transmission electron micrograph illustrating the connecting of unaffixed hyphal cells to host cell wall by strands of gel-like material: arrow (e) points to capsular sheath, arrow (i) points to gel-like material connecting affixed hyphal cells together; arrows (g) point to the absence of a lighter staining area on host cell wall close to an unaffixed hyphal cell; arrows (j) point to pedestals of gel-like material connecting an unaffixed hyphal cell to host cell wall; arrow (k) points to the swollen condition of the capsular sheath where gel-like material and sheath meet (×27,991).

capsular and haustorial sheaths, and thus allow penetration of host toxins.

Others studying the penetration mechanism of haustoria of various rust fungi have observed the affixing of haustorial mother cells to host cell walls (2, 3, 5, 9, 11). Unfortunately, their observations did not extend to other hyphal cells. Two reports contained no evidence of affixation between host cell walls and haustorial mother cells (2, 13). Apparently, the affixing of at least certain rust fungus cells to host cell walls may be a widely occurring phenomenon.

One of two processes probably occurs in the zone of affixation: the synthesis of a mat of fibrous material that cements the two organisms together (12); or pectinases are actively present in the zone of affixation, which may cause host cell walls to swell (16). The latter seems to be the more likely alternative based on the following: a reported significant decrease of 44% of the extractable pectic substances in *P. monticola* bark infected with *C. ribicola* (15), and the swelling of *P. monticola* cell walls following pectinase treatment (16). Because *C. ribicola* does not macerate host tissue, pectic substances apparently are degraded in restricted areas, as is suggested in Fig. 2. In this way, the structural integrity of the host tissues would be maintained.

The dark points in the lower portions of Fig. 3 probably are host cellulose microfibrils. When Fig. 3 is compared with Fig. 4 it is apparent that, in the zone of affixation between the fungus and host (Fig. 3), cellulose microfibrils have been altered, which implies that cellulase activity occurred in that zone.

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