Scanning Electron Microscopy of Pinus monticola Bark Infected with Cronartium ribicola

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ABSTRACT

This paper demonstrates the usefulness of scanning electron microscopy to help elucidate the physical association of *Cronartium ribicola* to its host *Pinus monticola*. Mycelia had smooth to slightly roughened surfaces and were of various diameters. Mycelia either bridged intercellular spaces or were appressed to the host's cell walls. Mycelial projections into host cells or haustoria were not found. The sheathing material of the mycelia appeared to be intimately involved with the contiguous surfaces of pathogen and host cells. Host cell walls contiguous to the pathogen showed no evidence of lysis.

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Additional key words: invasion, establishment, host-pathogen interaction, western white pine, physiology of disease.

The physical associations of mildew and rust fungi to their host cells have been described (8) and illustrated in a number of transmission electron micrographs (1, 2, 5, 6, 7, 9). We are attempting to elucidate by scanning electron microscopy (SEM) some interactions of *Cronartium ribicola* J. C. Fischer ex Rabenh. and *Pinus monticola* Dougl.

In this preliminary work, two fresh bark samples were excised from a 5-year-old tree infected with *C. ribicola*. One sample of invaded bark was taken from the outer periphery of the yellow margin of a blister rust canker and one sample of noninvaded bark was taken 5 cm (2 inches) above this canker margin.

The samples were dehydrated in a standard ethanol series followed by a similar series of increasing concentrations of Freon TF (4), and preserved for SEM by the critical point drying method of Cohen & Garner (3). Before examination with a scanning electron microscope, they were cemented to specimen holders with conductive silver paint and coated in a vacuum evaporator with a thin layer of gold (20- to 30-nm thick).

Direct treatment of fresh plant tissues in an ethanol dehydration series is not generally considered a process for preserving delicate cellular structures. However, this method of tissue dehydration avoided chloroplast collapse as well as complete lysis of the cytoplasm in both invaded and noninvaded bark cortical parenchyma (Fig. 1-4). As a result we considered these findings to be accurate and not the consequence of artifacts.

Our comparisons of the radial surface of these samples (Fig. 1 & 2) revealed how easily *C. ribicola* mycelium can be found in the peripheral areas of a blister rust canker. Strands, such as those in Fig. 1, were interpreted as pine cell protoplasts that were most likely stretched over the sample surface during cutting of the fresh bark.

Mycelia were found either bridging the numerous intercellular spaces of the cortex or closely appressed to the outer surfaces of the parenchymatous cell walls. Mycelial projections into host cells or bulbous or fingerlike haustoria were not found in this study. Mycelia were of various diameters as illustrated in Fig. 4 & 5. The mycelium and its unidentified associated coverings had a smooth to slightly roughened topography (Fig. 5 & 6).

Substances deposited at the junctions of mycelium and the outer surfaces of cell walls were evident throughout the invaded sample (Fig. 4-6). This finding extends the observations of Boyer & Isaac (2), who illustrated their report with transmission electron micrographs showing a sheathlike structure associated with the intercellular mycelium of *C. ribicola*. Based upon our observations, we believe the sheathing substances cement the mycelium to the host cell walls. Texture of the sheathing material, as well as the relationship of the contiguous cell surfaces of fungus and host cell wall, are shown in Fig. 6 in which a hypha has been torn from the host cell wall. Absence of lysis of host cell walls, also illustrated in Fig. 6, was evident throughout this sample.

The use of SEM has provided some insight into the physical relationship of *C. ribicola* to bark-cortical parenchyma of *P. monticola* by illustrating the spatial relationships that occur between pathogen and host. We are expanding our work to include comparisons of the invading periphery and sporulating areas of cankers having different symptoms.

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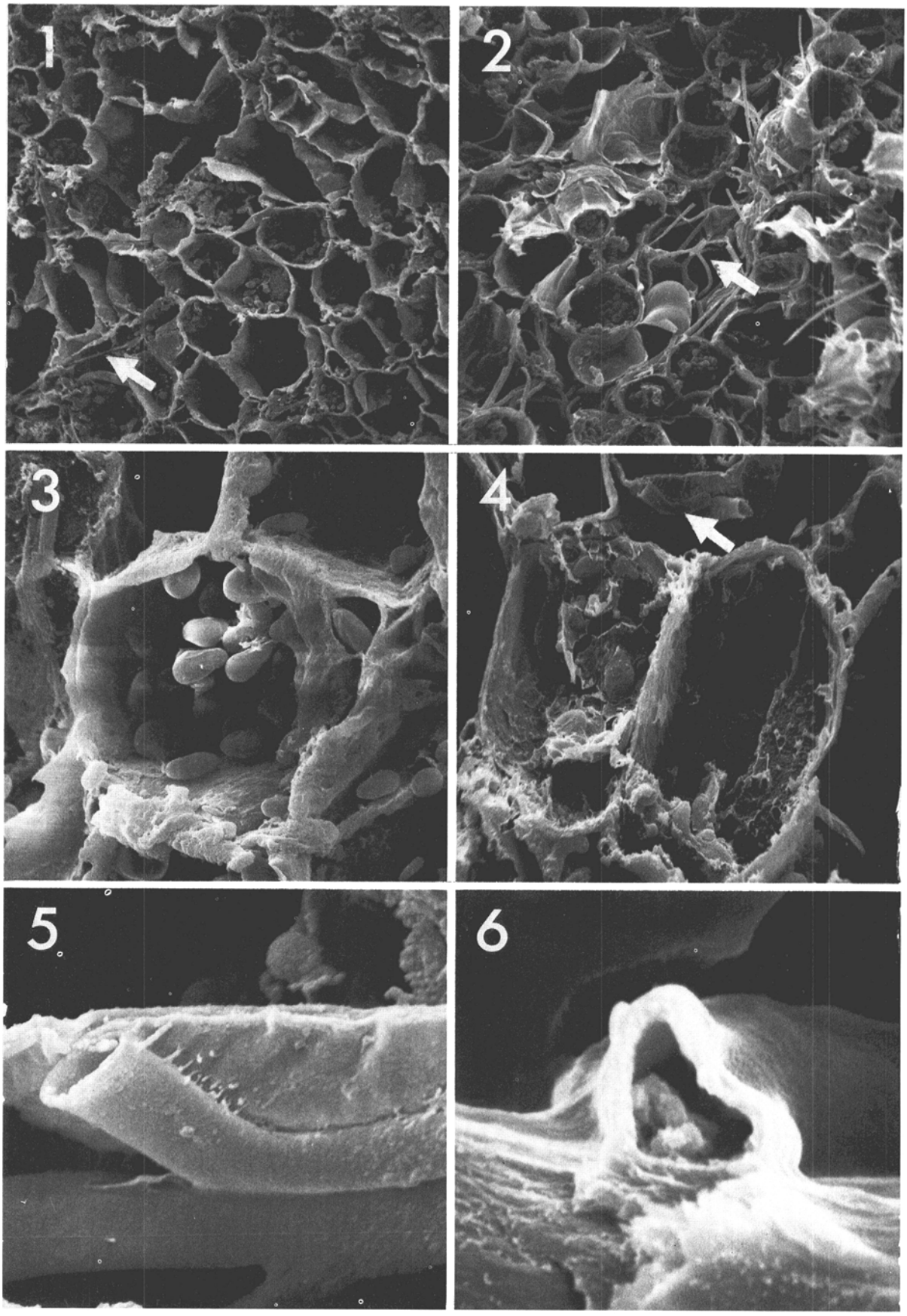
Fig. 1-6. Scanning electron micrographs of invaded and noninvaded western white pine bark. **1)** Noninvaded bark with arrow pointing to protoplast strands (× 200). **2)** Invaded bark with intercellular mycelium (arrow) of *Cronartium ribicola* (× 200). **3)** A single pine cell from noninvaded bark with chloroplasts and remaining protoplasmic materials (× 1,000). **4)** A segment ot mycelium (arrow) appressed to a host cell wall (× 560). **5)** The physical relationship between the mycelium segment of Fig. 4 and the host wall (× 5,600). **6)** An end view of a hyphae appressed to the host cell wall showing the continuation of the sheathing material around the mycelium as well as the relationship of the sheath to the host cell wall (× 10,000).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