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Ultrastructure of Urediniospore Formation in the Rust Fungus *Coleosporium ipomoeae* as Revealed by High Pressure Freezing Followed by Freeze Substitution

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The rust fungus *Coleosporium ipomoeae* is an obligate parasite that cycles between *Pinus* species and the wild morning glory, *Ipomoea hederacea*. In this study a combination of high pressure freezing (HPF) followed by freeze substitution (FS) was used to prepare the uredinial stage of *C. ipomoeae* on wild morning glory leaves for study with TEM. Although HPF/FS previously has been shown to provide outstanding preservation of ultrastructural details of host-pathogen interactions in plant leaves infected by various rust fungi [1,2,3], this is the first report of its use to study spore development within a rust-infected leaf.

Stems of *I. hederacea* bearing leaves infected by *C. ipomoeae* were collected in the field near Athens, GA in August and placed in water for transport to the lab where 1-2 mm^2 pieces of leaf tissue bearing uredinia were excised and frozen using a Balzer's HPM010 High Pressure Freezing Machine. Samples were then processed for study according to the procedures of Mims et al. [2]. Thin sections were cut using a diamond knife, collected on slot grids [4], post-stained with uranyl acetate and lead citrate and examined using a Zeiss TEM 902A microscope operating at 80 kV. Infected leaf samples also were prepared for study with SEM according to the procedures of Enkerli et al. [5] and examined with a JEOL 5800 microscope operating at 15 kV.

Uredinia developed just beneath the abaxial surfaces of infected leaves. Mature urediniospores were exposed following the rupture of the leaf epidermis (Fig. 1). Examination of thin sections of infected leaves revealed that urediniospores developed in chains from a basal layer of binucleate sporogenous cells. A sporogenous cell gave rise to a binucleate spore mother cell that then divided to form a small basal cell that eventually disintegrated and a binucleate terminal cell that then differentiated into a urediniospore (Fig. 2). HPF/FS provided excellent preservation of ultrastructural details of urediniospore development including details of spore ornamentation formation (Fig.3). Our samples were virtually free of freeze damage and specimen contrast was excellent. Although some separation of cytoplasm from fungal walls was observed, most cytoplasmic organelles as well as nuclei were well preserved (Fig. 4). Our results show that HPF/FS can be a very valuable tool for the study of fungal spores produced inside infected host leaves.

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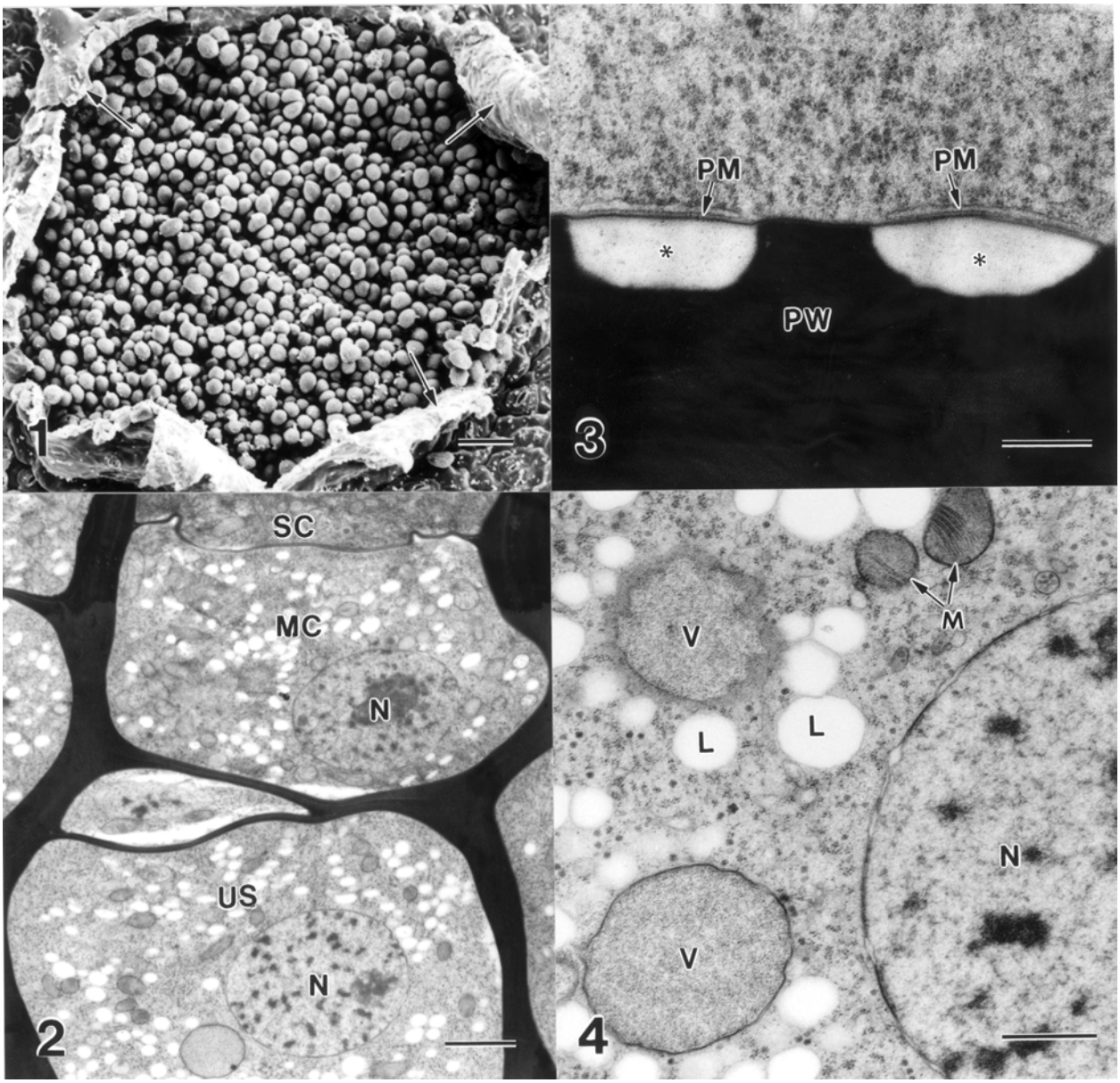

FIG. 1. Scanning electron micrograph of mature urediniospores of *C. ipomoeae* on the abaxial surface of an infected leaf. The ruptured leaf epidermis is visible at the arrows. Bar = 50 μm.

FIG. 2. Transmission electron micrograph showing the tip of a sporogenous cell (SC), a spore mother cell (MC) and a young urediniospore (US). Nuclei are visible at N. Bar = 1 μm.

FIG. 3. Section showing an early stage in the development of spore surface markings. The wart-like markings (asterisks) initially develop on the outer surface of the spore plasma membrane (PM) beneath the primary wall (PW) of the spore. Bar = 0.3 μm.

FIG. 4. Typical appearance of organelles in a young urediniospore. Visible are part of a nucleus (N), mitochondria (M), vacuoles (V) and lipid bodies (L). Bar = 0.5 μm.

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