Cytology of Ceratocystis ulmi

R. J. Howard and D. P. Maxwell

Undergraduate Student and Associate Professor, Department of Plant Pathology, University of Wisconsin, Madison 53706. Present address of senior author: Department of Plant Pathology, Cornell University, Ithaca, New York 14853.

Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, and by National Science Foundation Grant No. GB-35950.

The authors express their appreciation to G. Gaard for assistance with the electron microscopy, to H. Lyon and S. Vicen for help in figure preparation, to Cornell University for use of darkroom facilities and to Dr. J. R. Aist for his many helpful suggestions.

Accepted for publication 3 March 1975.

ABSTRACT

Light- and electron-microscopic observations of *Ceratocystis ulmi* showed that typical eukaryotic nuclei were present in hyphal tip and older cells. Conidia and the majority of hyphal cells contained a single nucleus. Most organelles typical of other Ascomycetes were also observed in this fungal pathogen.

Phytopathology 65:816-819

Additional key words: Conidia, ultrastructure, vegetative hyphae.

Previous cytological studies of *Ceratocystis ulmi* (Buism.) C. Moreau have produced conflicting interpretations of several aspects of this fungus (10). Controversy exists over whether "microhyphae" (14), "microendohyphae" (6) and "microendospores" (16) are formed. Ouellette and Gagnon (16) were unable to identify nuclei in spores and hyphae of this fungus; however, Pomerleau (17) readily found nuclei in fixed hyphae.

This report compares for the first time the cytology of living and fixed hyphae with the ultrastructure similar hyphae of *C. ulmi*.

MATERIALS AND METHODS.—A pathogenic isolate of *C. ulmi*, supplied by E. B. Smalley, University of Wisconsin, Madison, was maintained at 26 C on potato-dextrose agar (PDA).

Living hyphae were prepared for light microscopy by growing the fungus for 60-80 hours at 26 C on sterilized glass microscope slides coated with PDA (1).

Hyphae were also fixed for light microscopy by immersing slide cultures in Carnoy's fluid for 5 minutes. These cultures were then rinsed in 95 and 70% ethanol, hydrolyzed in 6 N HCl for 15 minutes, rinsed in 20 mM (K^+) phosphate buffer, pH 6.9, containing 0.2 M sucrose, and then rinsed in distilled water and placed in Giemsa stain (9) overnight. Stained material was rinsed and mounted in distilled water and observed immediately. Observations were recorded on Kodak Tri-X and Pan-X films, with phase-contrast and bright-field optics, using Zeiss oil-immersion objectives and a microflash unit (1).

Slide cultures for electron microscopic examination were grown as described above and fixed in 1.5% glutaraldehyde and 1% OsO_4 in 50 mM (K^+) phosphate buffer, pH 6.8, stained in uranyl acetate, dehydrated in an acetone series and embedded in Spurr's medium. Thin sections were cut with glass knives on a Reichert OmU-3 ultramicrotome, stained with Reynolds' lead citrate and examined with a JEM-7 electron microscope.

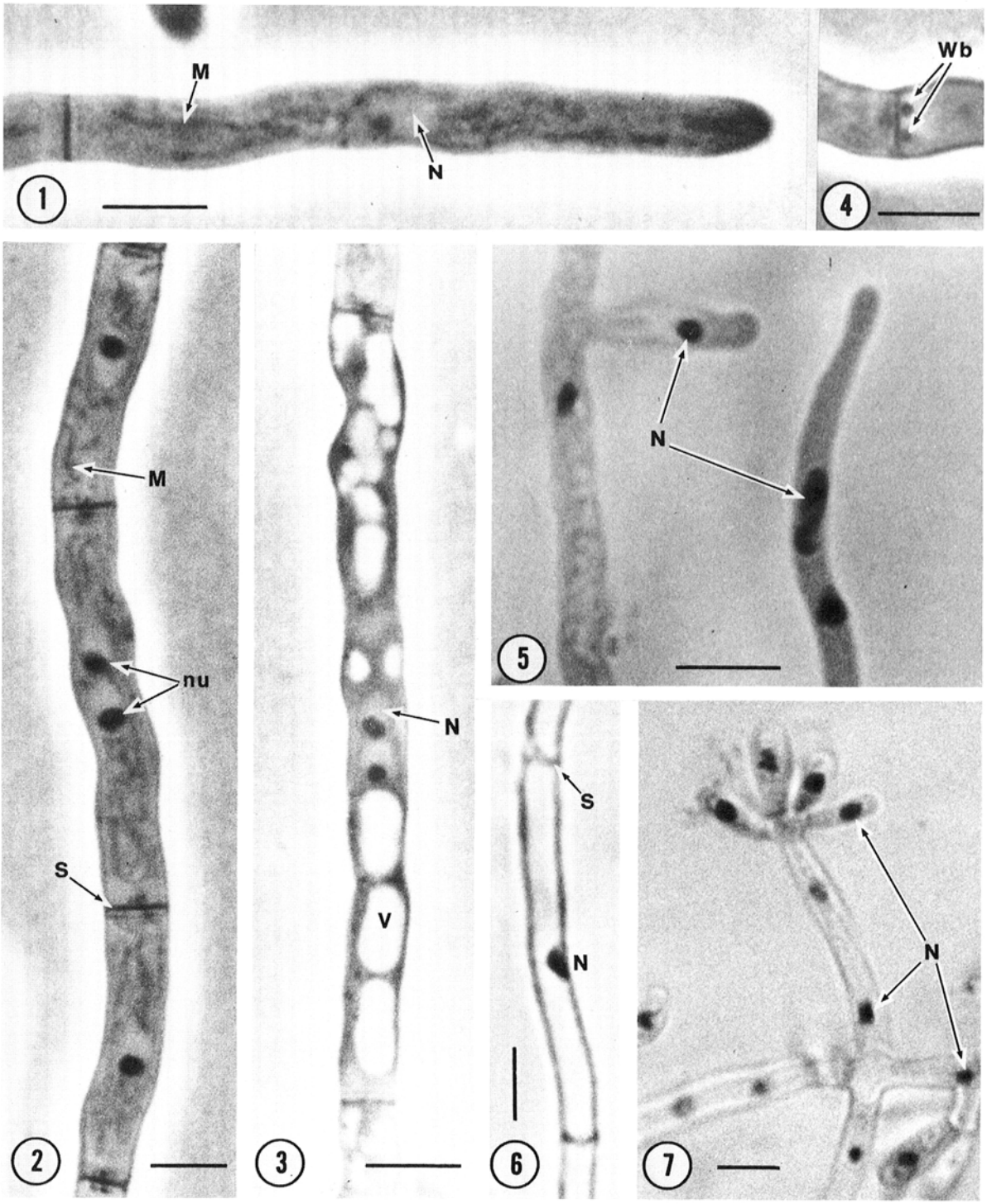
RESULTS AND DISCUSSION.—Typical fungal organelles were observed in hyphal tips, older regions of hyphae, and in conidia by both light and electron microscopy (Fig. 1-13). In living hyphae, oval to spherical nuclei, each containing a single conspicuous nucleolus, were present in both the hyphal tips (Fig. 1) and older cells (Fig. 2, 3). These nuclei were morphologically similar to those described for *C. fagacearum* and *Fusarium oxysporum* (1). An accurate count of numbers of nuclei per cell in living specimens was not possible because the ease with which they could be identified varied. Nuclei and septa in the Giemsa-stained material were readily apparent (Fig. 5-7); the number of nuclei per cell was determined from 70 hyphal cells as: no nuclei, 3%; one nucleus, 81%; two nuclei, 12%; three or four nuclei, 4%. All of 100 conidia observed contained single nuclei (Fig. 7). "Micoendospores" (16) and "microendohyphae" (6) were not seen; whereas, conidiophores similar to those previously reported (7) were observed (Fig. 7). In living hyphae, mitochondria were often more conspicuous than other organelles (Fig. 1, 2). They were long, narrow bodies exhibiting snake-like movements in vegetative hyphae as reported by Armentrout et al, (3). One or more Woronin bodies (Fig. 4, 10) were associated with the septal pore (Fig. 10). Vacuoles were recognizable with both the light- (Fig. 3) and the electron-microscope (Fig. 9). Microbodies and lipid bodies were identified in electron micrographs (Fig.

Fig. 1-7. Light microscopy of *Ceratocystis ulmi*. Scale bar represents 5.0 μm. **1-4)** Living cells of *C. ulmi* as viewed with phase contrast optics. **1)** Hyphal tip cell showing nucleus and nucleolus, mitochondria, and septum. **2, 3)** Older cells with nuclei and nucleoli, mitochondria, vacuoles, and septa. **4)** Septum, with prominent Woronin bodies, between two older cells. **5-7)** Structures of *C. ulmi* stained with Giemsa nuclear stain, and viewed with bright field optics. **5)** Darkly-stained nuclei can be seen in both hyphal tip and older hyphal cells. **6)** Older hyphal cell showing septa and stained nucleus. **7)** An example of a conidiophore bearing conidia. Note the presence of a single nucleus in spores and nuclei in conidiophore and hyphae. Legend: M = mitochondrion, N = nucleus, nu = nucleolus, S = septum, V = vacuole, Wb = Woronin body.

11, 12). A vesicular membrane complex, similar to lomasomes observed in other fungi (2, 4, 5, 13, 18), was commonly found in vegetative hyphae (Fig. 10, arrows).

Each conidium had a truncate region (Fig. 13, arrow), or "birth scar" (8), where it had been attached to a conidiophore. Multiple wall layers were not evident in glutaraldehyde-fixed sections. Ribosomes were abundant throughout the conidial cytoplasm, but endoplasmic reticulum profiles were scarce.

In contrast to the suggestions by Ouellette et al. (6, 14, 15, 16), our observations, as well as those of others (7, 8, 10, 11, 12, 17), clearly indicate that this fungal pathogen is



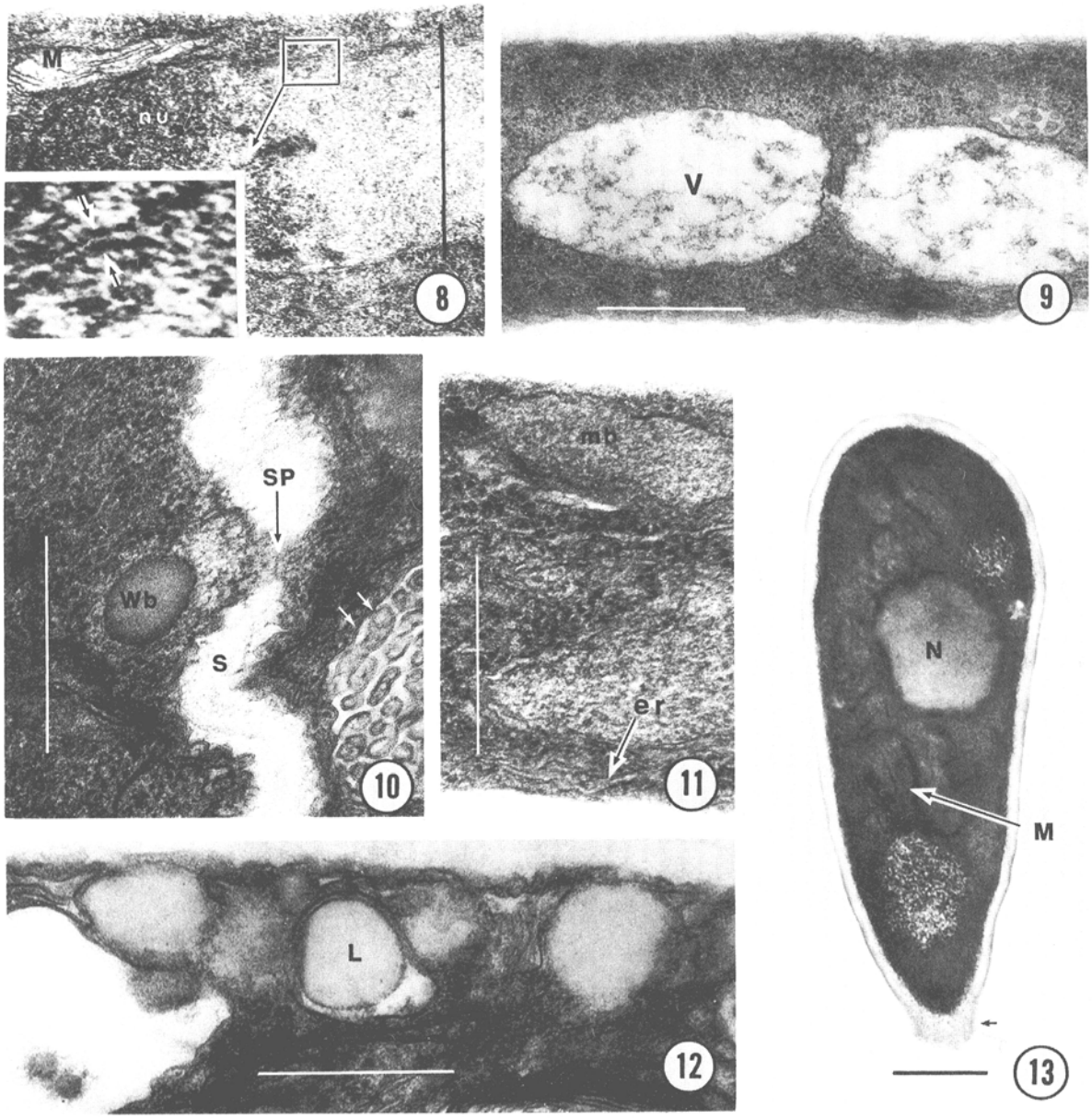


Fig. 8-13. Electron micrographs of thin sections of *Ceratocystis ulmi*. Scale bar represents 0.5 μm. **8)** Longitudinal section of a vegetative hypha showing a mitochondrion and nucleus with nucleolus. Note the nuclear envelope between the arrows (insert). **9)** Longitudinal section of a hypha (1.02 μm in diameter) showing vacuoles and tonoplast. Note the abundance of ribosomes throughout the cytoplasm. **10)** Septal region of vegetative hypha. Note the presence of a Woronin body and a vesicular membrane complex (arrows) or lomasome. **11)** Hyphal cell with a microbody and an endoplasmic reticulum profile. **12)** Hyphal cell showing numerous lipid bodies. **13)** Longitudinal section of a detached conidium showing mitochondria, a single nucleus, and a "birth scar" at the tapered end of the spore (arrow). Legend: er = endoplasmic reticulum, L = lipid body, mb = microbody, M = mitochondrion, N = nucleus, nu = nucleolus, S = septum, SP = septal pore, V = vacuole, Wb = Woronin body.

cytologically similar to other Ascomycetes (1, 3, 4, 18).

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