Development and Ultrastructure Observations of Secondary Hyphae of *Podosphaera leucotricha* on Apple Cultivars of Varying Susceptibility to Powdery Mildew

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Abstract

The development and ultrastructure feature of secondary hyphae of *Podosphaera leucotricha* were studied using light and electron microscopy. The percentage of development and length of secondary hyphae, differed in compatible and incompatible combinations. In compatible host-parasite combinations, hyphal cells of powdery mildew fungus contained a full complement of fungal organelles. There were differences of hyphal ultrastructure in compatible and incompatible host-parasite combinations, the main one was the appearance of dense material inside the nucleus, in the cytoplasm, and a few mitochondria.

Keywords: ultrastructure; hyphae; powdery mildew

INTRODUCTION

Apple powdery mildew is a very common disease caused by the biotrophic fungus *Podosphaera leucotricha* (Ell. et Ev.) Salm. Apple plants are infected by conidia which are of great importance in spreading of fungus from one plant to the next and ensure the repeated infection of host plant. Infection process includes adhesion of conidium, germination, formation of infection structures (penetration peg), development of haustorium, formation of secondary hyphae and conidiophores which produce conidia. In order to understand a mechanism of resistance it is important to characterize each event of infection process, and define the probability of its succession on susceptible and resistant hosts. Little is known of the ultrastructural morphology of hyphae of *P. leucotricha* during their contact with leaf surfaces of resistant or susceptible apple cultivars. For this reason present transmission electron microscopical study of hyphae of this important pathogen in compatible and incompatible combinations with host plant was undertaken.

MATERIAL AND METHODS

Plants. The following apple cultivars were used in the present experiments: resistant (Ducat, Golden Delicious), and susceptible (Aport Alexander, Zailiyskoe). The cultivars were grown in greenhouse. The mature leaves were used for experiments.

Inoculum and inoculation. Conidia of *P. leucotricha* were isolated from leaves of susceptible apple cultivars naturally infected by powdery mildew and suspended in distilled water at a concentration of 8000–10 000 spores/mL. Inoculation was conducted by this aqueous suspension of conidia which applied to leaf surfaces.

Examination of fungal development. Microscopic analysis of powdery mildew development was performed 12, 24, 36, 48, 60 h after inoculation. The length of hyphae was measured.

Transmission electron microscopy. Leaf pieces (1 × 5 mm) containing fungal mycelium at various stages of development were fixed in 2% glutaraldehyde in 0.1M cacodilate buffer (pH 7) for 4 h at room temperature and postfixed in 1% osmium tetroxide in the same buffer for 1.5 h at room temperature, or were fixed in unbuffered 2% potassium permanganate for 3 h at 4°C. Fixed material was then washed by 15 min changes in buffer. Dehydration in a graded ethanol series and in 100% acetone was followed by impregnation in several changes of Epon-Araldite and embedding in fresh resin which was polymerized at 60°C for 48 h. Ultrathin sections were cut with a glass

knife on a Reichert Ultracut Ultratome, collected on formvare coated copper grids, stained with alcoholic uranyl-acetate and lead citrate and examined with Jem-100B transmission electron microscope operated at 80 kV.

RESULTS AND DISCUSSION

Development of secondary hyphae. In the compatible combinations of *P. leucotricha* with apple cultivars, fungal growth is apparent without magnification at 5 day after inoculation, because about 95% of the parasite units produce secondary hyphae. These hyphae are 3.24 ± 0.207 μm in diameter. They elongate, become branched at right angles, spread rapidly, and are capable of initiating secondary infections. The appearance of the secondary hyphae indicates the establishment of a successful host-parasite interaction (KOGA *et al*. 1978).

In the incompatible combinations fungal development is significantly smaller, the growth of mycelia is much delayed. Only 10% of the parasite units produce elongating secondary hyphae.

Ultrastructure of secondary hyphae in compatible host-parasite combinations. The surface of mycelial cell walls in *P. leucotricha* are covered by a thin electron dense sheath (Figure 1), which is often slightly detached on the sections. The same sheaths consisting of polysaccharides, enzymes, or toxic substances surround the hyphae of other fungi (BRACKER 1967). The secondary hyphae of *P. leucotricha* have usually thin walls 0.15 ± 0.009 μm thick (by glutaraldehyde-osmium fixation). The plasmalemma frequently becomes more or less invaginated, especially near the septa of the ascomycete type which are present in hyphae. Diameter of septal pores is 0.18 ± 0.022 μm. Most septal pores are open but some contain a densely staining deposit (plug) or Woronin bodies.

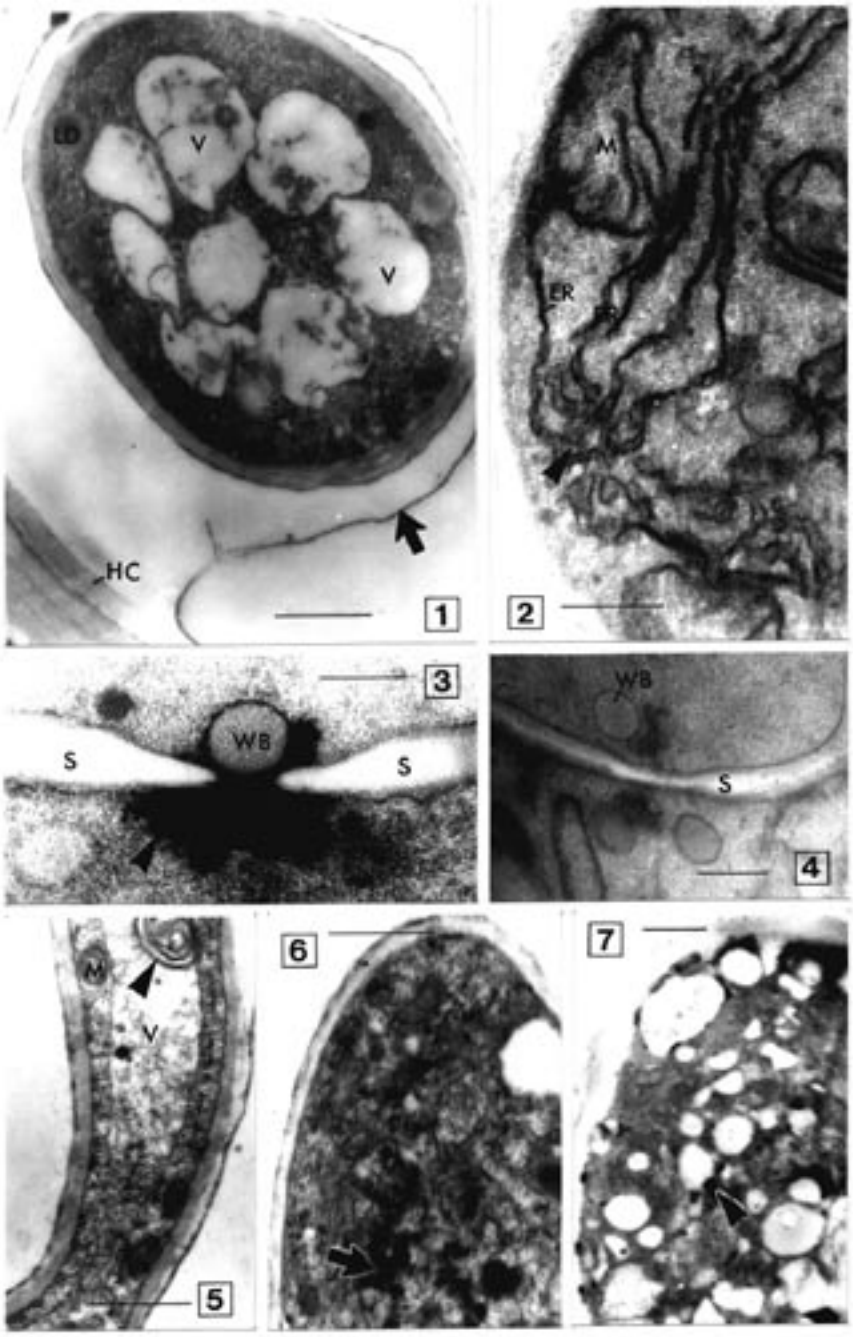
Cytoplasmic content of the secondary hyphal cells differs. The new hyphae have a denser cytoplasm, than the mature hyphae. Cytoplasm of the hyphal cells includes numerous ribosomes, conspicuous cisternae of endoplasmic reticulum of the predominantly agranular type, and mitochondria. The tubular mitochondria are measured 0.88 ± 0.068 μm and 0.25 ± 0.018 μm in diameter. They tend to be distributed peripherally and parallel to the longitudinal axis of cell or hypha. The most mitochondrial profiles, which are observed in thin sections are indeed separate mitochondria, and are not parts of one or two longer branched mitochondria. The content of endoplasmic reticulum suggests significant metabolic activity. The cisternae of reticulum are seemed to spread out from several discrete regions (Figure 2), which are similar to those demonstrated by ROBERTS *et al*. (1996). The endoplasmic reticulum cisternae are sometimes continuous with the nuclear envelope as is observed in other fungi (BRACKER 1967; DUNCAN & HERALD 1974). They have sometimes close contacts with outer membranes of mitochondria, tonoplast of vacuoles, and plasmalemma.

The mycelial cells show a gradation from a highly vacuolate condition in the old or mature hyphae to non-vacuolate new hyphae. Most large sized vacuoles appear as empty but some contain fibrillo-granular material, electron-opaque polyphosphate granules or compound vesicular structures. Both new and mature hyphae have small vesicles (0.07 ± 0.017 μm in diameter). Woronin bodies occur comparatively frequently and are especially common near septa (Figures 3 and 4). They are ellipsoidal and measured 0.31 ± 0.030 μm and 0.22 ± 0.021 μm in diameter. The hyphal cells of *P. leucotricha* have small lipid droplets, and a complete absence of glycogen which is observed in hyphae of *Erysiphe pisi* (MARTIN & GAY 1983), *E. betae* (ZHITNIKOVA *et al*. 1990), *E. graminis* (MISHINA *et al*. 1989). The organelles designated as microbodies are rarely observed in cytoplasm of *P. leucotricha*.

Serial sections confirm that each cell of secondary hyphae contains only one nucleus bounded by a double membrane. It is measured 3.08 ± 0.246 μm and 1.85 ± 0.122 μm in diameter. The chromatin is distributed fairly evenly within the nucleoplasm which has also local regions of heterochromatin. Apparently active nucleolus (1.25 ± 0.115 × 0.93 ± 0.067 μm) with vacuoles often occurs close to the nuclear envelope which contains conspicuous nuclear pores. The nucleolus has fibrous and granular constituens, they are intermixed usually.

Thus, hyphal cells of *P. leucotricha* contain a full complement of organelles, the overall organization in hyphae appear to be similar to that described for other powdery mildew fungi (MARTIN & GAY 1983; ZHITNIKOVA *et al*. 1990).

Ultrastructure of secondary hyphae in incompatible host parasite combinations. In incompatible combinations, hyphal cell wall is thicker (0.18 ± 0.017 μm), than that in compatible combinations. Plasmalemma of many hyphal cells exhibits invaginations, which vary in size and complexity. The mitochondria are elongate or circular in sectional view. The mitochondrial size decreases in incompatible combinations to 0.60 ± 0.076 μm and 0.25 ± 0.018 μm in diameter. Endo-



Figures 1–4. Secondary hyphae in compatible host-parasite combinations

1. Vacuolated secondary hypha with sheath (arrow). Scale bar = 1 μm
2. Discrete region (arrowhead) and cisternae of endoplasmic reticulum. Scale bar = 0.3 μm
3. Septal pore with plug (arrowhead) and Woronin body. Scale bar = 0.3 μm
4. Woronin bodies near the septum. Scale bar = 0.5 μm

Figures 5–7. Secondary hyphae in incompatible host-parasite combinations

5. Hyphal vacuole with granular content and membranes (arrowhead). Scale bar = 1 μm
6. Electron dense deposits (arrow) in cytoplasm. Scale bar = 1 μm
7. Desintegrated hyphal cytoplasm with electron dense deposits (arrowhead). Scale bar = 0.5 μm

Key to symbols: ER – endoplasmic reticulum, HC – cuticle, LD – lipid droplets, M – mitochondrium, S – septum, V – vacuole, WB – Woronin body

plasmic reticulum is present in smaller amounts, than in compatible combinations. Membrane connections between the endoplasmic reticulum and mitochondrial membrane, vacuolar tonoplast and plasmalemma are not definitely discernible. Vacuoles and lipid droplets are visible in many cells. Vacuolar contents appear granular in most sections. Some vacuoles contain also membranes or large polyphosphate globules (Figure 5). Increased electron density of hyphal cells are observed in incompatible combinations. Notable is the association of electron dense material deposition with plasmalemma, tonoplast, cytoplasm (Figure 6), and nuclear envelope.

The nuclear size is identical in both compatible and incompatible combinations. There are electron dense deposits, which accumulated in nucleoplasm. A nucleolus is occasionally visible in sections of hyphal cells.

Some secondary hyphal cells have a disintegrated cytoplasm with lipid globules and electron dense deposits (Figure 7).

In conclusion, there are differences of hyphal ultrastructure in compatible and incompatible combinations of fungus and host, the main one being the appearance of dense spots of unknown electron dense material inside the nucleus, in the cytoplasm, and a few mitochondria.

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