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, Zailyskoe). 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 cacodylate 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 Ultrcut Ultratome, collected on
formvar 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 es-
buildment of a successful host-parasite interaction
(Koga et al. 1978).

In the incompatible combinations fungal develop-
ment 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 elec-
tron dense sheath (Figure 1), which is often slightly
detached on the sections. The same sheaths consisting
of polysaccharides, enzymes, or toxic substances sur-
round 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-os-
mium 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 cis-
ternae of endoplasmic reticulum of the predominantly
agranular type, and mitochondria. The tubular mito-
ochondria 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 mi-
tochondria, and are not parts of one or two longer
branched mitochondria. The content of endoplasmic
reticulum suggests significant metabolic activity. The
cisterneae 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 cisterneae 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 ma-
ture 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 sec-
dondary 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 enve-
lope which contains conspicuous nuclear pores. The
nucleolus has fibrous and granular constituents, 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; Zhitniki-
ova et al. 1990).

Ultrastructure of secondary hyphae in incompatible
host parasite combinations. In incompatible combina-
tions, 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

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.

References