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ULTRASTRUCTURAL ASPECTS OF CONIDIOGENESIS OF *FUSARIUM SACCHARI* F.SP. *SUBGLUTINANS*

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Development of conidia from phialides in *Fusarium sacchari* f.sp. *subglutinans* was observed by electron microscopy. The conidiogenous cell wall is composed of an electron-dense outer layer and an electron-transparent inner one. The outer layer first becomes swollen at the apex and then ruptures, forming the collarette. The conidium initial elongates and a new three-layered outer wall forms at its apex. After elongation of the conidium a basal septum forms. The septum splits by abscission to form the inner wall layers of both the conidium and a new conidium initial. New electron-dense outer wall layers form on both of these inner wall layers before the conidium is released. The released conidia remain attached by mucus to form false heads.

The fusaria are pathogens of important agricultural crops, and diseases they cause have been and are studied extensively, but many aspects of their morphology and ultrastructure remain unknown. Few studies on cell wall organization during the ontogeny of *Fusarium* conidia have been reported (Campbell & Griffiths, 1974; Schneider & Seaman, 1982). Although there have been studies on various aspects of *Fusarium* ultrastructure and morphogenesis (Marchant, 1966; Griffiths, 1973*a*,*b*; Campbell & Griffiths, 1974; Van Eck, 1976; Schneider *et al.*, 1977), the ultrastructural aspects of conidium ontogeny have not been investigated.

The major problem with any study of conidiogenesis is to put together a temporal developmental sequence. The development of macroconidia in *F. decemcellulare* was cited by Subramanian (1971) as an example of the phialidic mode of conidium ontogeny. However, on the basis of an EM study of *F. culmorum*, Marchant (1975) concluded that conidium ontogeny in *Fusarium* follows the thallic mode. A more recent EM study of conidiogenesis in *F. sulphureum* (Schneider & Seaman, 1982) demonstrates phialidic conidiogenesis. This paper describes changes in cell walls during the ontogeny of conidia of *F. sacchari* f.sp. *subglutinans*.

MATERIALS AND METHODS

Transmission electron microscopy

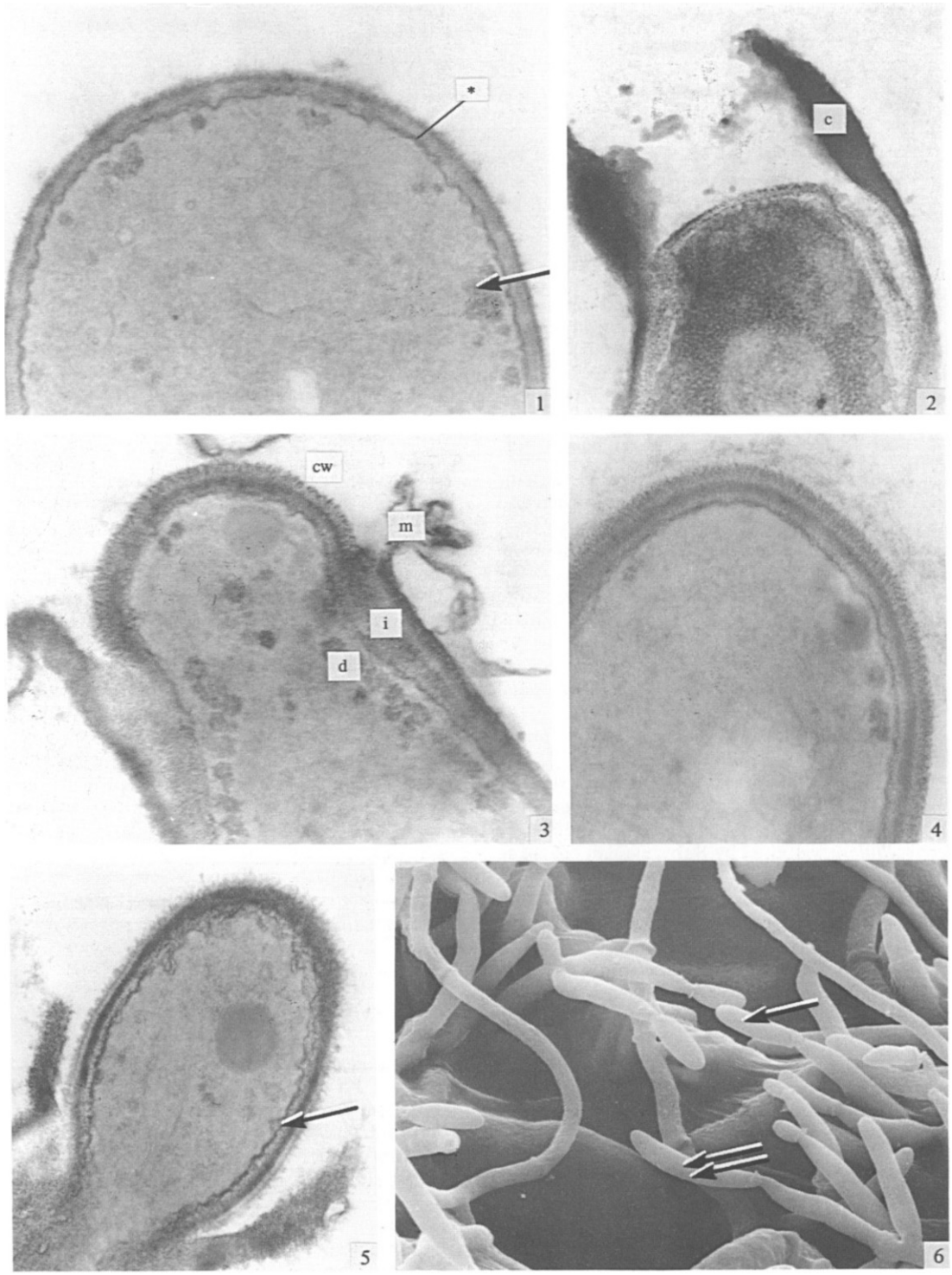
Conidiophores of *F. sacchari* (Butler) W. Gams f.sp. *subglutinans* (Wollenw & Reinking) Nirenberg, were produced on carnation leaf agar (Fischer *et al.*, 1982). The Petri dishes were inoculated, incubated at 27 °C for 48 h then flooded with 2% glutaraldehyde/formaldehyde (Bullock, 1984) for 1 h and washed twice with phosphate buffer (pH 7·2) for 30 min each. The samples were post-fixed with 1% osmium tetroxide for 1 h and washed with distilled water for 5 min. Strips (2 × 10 mm) of agar with fungus growth on them were cut from the colonies and placed on a glass slide. The strips were covered with a thin layer of cooled melted 1·5% water agar. The strips were cut into blocks (2 × 2 mm) and dehydrated in a graded acetone series (50%; 75%; 95%; 100%; 100%) for 15 min each. The dehydration was performed at 4°. After dehydration, the samples were placed in a 1:1 mixture of Spurr's resin and 100% acetone for 1 h, then transferred to 100% resin (twice) for 30 min each. Finally the sample blocks were embedded in fresh Spurr's resin with the longitudinal plane of the conidiogenous cells and differentiating conidia parallel to the cutting surface of the microtome. Sections of 90 nm thickness were stained with 2% uranyl acetate and lead citrate (Reynolds, 1963) and viewed in a Siemens Elmiskop 101 electron microscope.

Scanning electron microscopy

Sections (10 × 10 × 1 mm) of the colony and underlying agar were cut and fixed in 1% OsO_4 vapour for 4 d and air dried. The samples were mounted on specimen stubs, sputter-coated with carbon and gold and viewed with a Cambridge Stereoscan 250S scanning electron microscope operating at 10 kV.

RESULTS

The terminal conidiogenous cells do not differ from the hyphal tips, except for the presence of dense



Figs 1–6. For captions see opposite

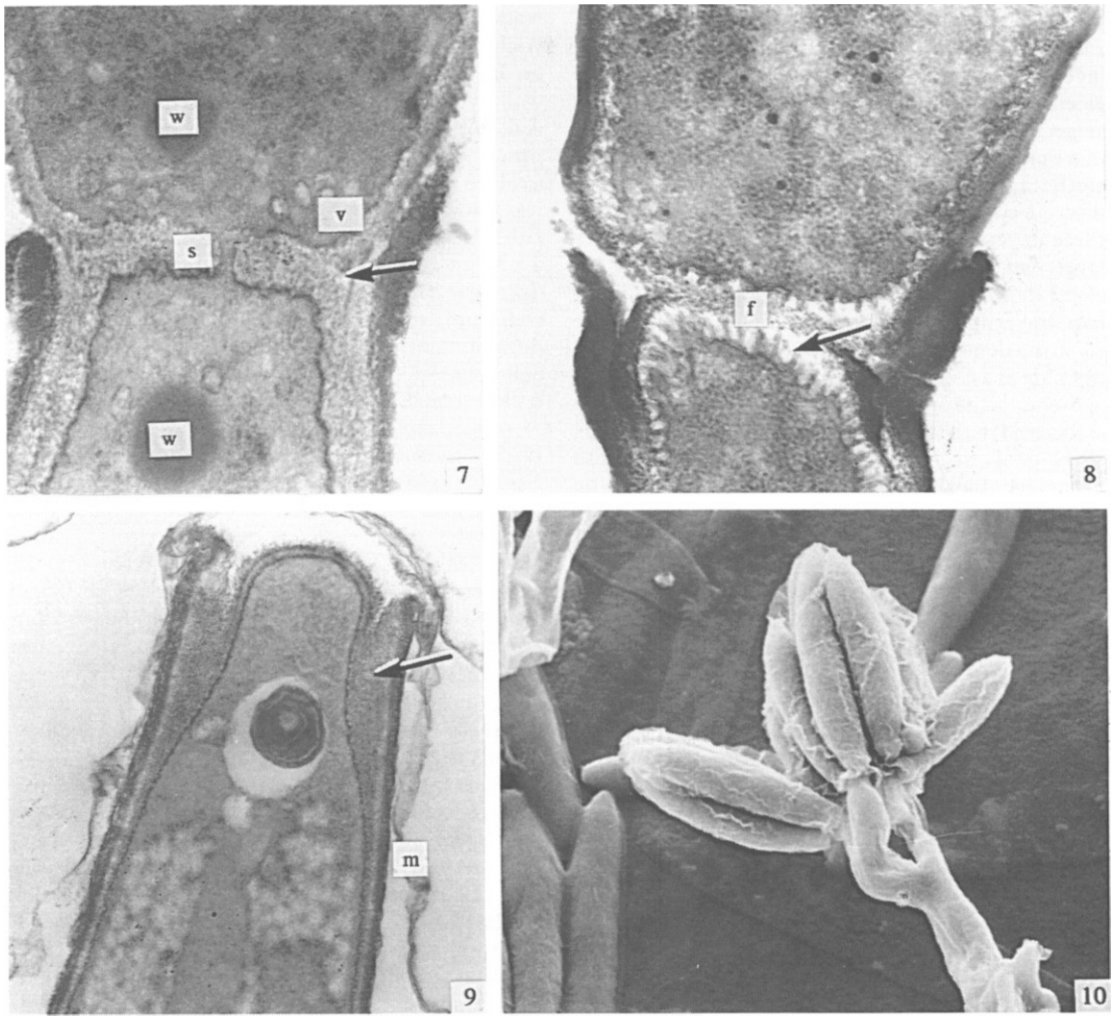


Fig. 7. Pore in the basal septum (s) of a conidium with Woronin bodies (w) nearby and vesicles (v) near the plasmalemma where transverse abscissional splitting begins (arrow). × 60000.

Fig. 8. Deposited outer wall-layer material adjacent to the fissure (f) at the base of the conidium and the apex of the new conidium. Note the loss of contact between the wall and the plasmalemma area where the outer wall layer is deposited (arrow). × 48000.

Fig. 9. Thickening of the inner wall of the phialide as the conidium is produced (arrow). Note the mucus layer (m) attached to the collarette. × 42000.

Fig. 10. SEM of a cluster of microconidia forming on a false head; note the mucus on the conidia that keeps them attached. × 14200.

Fig. 1. Conidiogenous cell with cytoplasmic vesicles containing electron dense bodies (arrow). Note the wave formation of the plasmalemma (*). × 48000.

Fig. 2. L.S. collarette (c). × 48000.

Fig. 3. L.S. of the developing conidium wall (cw) with the fluffy outer layer, electron-dense centre layer and an electron-transparent inner layer; dense bodies (d) mucus (m) attached to the collarette. The inner wall layer of the phialide thickens as conidia are produced (i). × 66000.

Fig. 4. L.S. of the developing conidium wall showing the three layers. × 72000.

Fig. 5. The phialide with an elongating conidium, note the undulation of the plasmalemma (arrow). × 45600.

Fig. 6. SEM showing conidia in different stages of development and the collarettes. Microconidium (arrow), macroconidium (double arrow). × 7100.

bodies in the growth region. The cell wall of *Fusarium* is generally described as having two layers, an outer electron-dense layer and an inner electron-transparent layer (Fig. 1). During the first stage of conidiogenesis, the outer layer becomes more electron-dense and swollen and then ruptures at the apex (Fig. 2). The inner layer forms the discrete conidium wall (Figs 2, 3) which consists of three layers, a fluffy electron-transparent outer layer, an electron-dense central layer and an electron-transparent inner layer (Figs 3–5). Following the rupture of the outer wall layer, the electron-dense remnant remains attached to the phialide as a collarette (Figs 3, 5, 6). A mucus sheath is found at the apex of the phialide which could be associated with the formation of false conidial heads (Figs 3, 9, 10). The conidium extends and the plasmalemma shows wave formation which can be related to cell wall expansion (Figs 5, 8). The conidium is cut off by centripetal invagination of the electron-transparent inner wall layer (Figs 7, 8). An abscissional splitting occurs in the basal septum (Fig. 7) and new electron-dense outer wall layers then form at the base of the conidium and at the apex of the new conidium initial (Figs 7, 8). During conidium development, the apical cytoplasm contains vesicles near the plasmalemma (Fig. 7). With the formation of each conidium, the inner wall layer of the phialide thickens as the new conidium wall develops (Fig. 9).

A polyphialide with a false head of conidia covered by mucus is illustrated (Fig. 10).

DISCUSSION

At the Kanaskis conference (Kendrick, 1971) the phialidic mode of conidiogenesis was defined as one in which the conidia are clad in an entirely new wall which is not derived from any existing layers of the wall of the conidiogenous cell. In general the phialide apex is so small that the details of structure in this area can only be observed with difficulty by light optics. As the ontogeny of conidia is an important criterion in classification of Deuteromycetes, there is a need for studies of this area.

Conidiogenesis in *F. sacchari* f.sp. *subglutinans* reported here follows the phialidic mode and the conidium wall is an extension of the phialide wall. There are different interpretations of the number of matrix wall layers in fusaria as observed in TEM sections. Our results on wall structure indicate the presence of two layers, which is in agreement with results of Schneider & Seaman (1973), Schneider & Wardrop (1979) and Schneider *et al.* (1977). In sections of the developing conidia three layers were observed. Van Eck & Schippers (1974) reported four layers in *F. solani* f.sp. *cucurbitae* conidial cell walls. Griffiths (1973*a*) found striations in the walls of *F. oxysporum*, suggesting that they could be erroneously interpreted as morphologically distinct wall layers. The inner wall layer of the conidiogenous cell (phialide) thickens during the continuous process of conidium formation, and this may play a role in supporting the developing conidia. No sign was found that the thickening of the inner wall layer either blocked or stopped conidium formation. The TEM micrographs show that the second conidium develops a new outer wall layer before the first conidium separates and it occurs without the development of a second collarette, because only one collarette is seen on older phialides. There is a close correspondence between conidiogenesis of *F. sulphureum* (Schneider & Seaman, 1982) and that of *F. sacchari* f.sp. *subglutinans*. Schneider & Seaman (1982) showed that collarettes may disintegrate and Barron (1968) and Hanlin (1971) have noted the absence of distinct collarettes on some phialides of *Fusarium* spp. The shape of the collarette may be associated with the degree of wall elasticity. In *F. sacchari* f.sp. *subglutinans* loss of contact between the wall and the plasmalemma in the outer wall layer-depositing area of the hyphal apex and the conidium base occurs. During late stages of conidium maturation Woronin bodies, usually seen close to the septal pore, probably allow the passage of low-molecular-weight metabolites while blocking that of organelles. Farkas (1979) postulated that the extension of fungal walls is preceded by the concentrated action of cell-wall polysaccharide hydrolases at the given site. The localized release by the action of these enzymes of the wall polymers results in the loss of contact between the plasmalemma and the wall. A similar situation might obtain during conidiogenesis in *F. sacchari* f.sp. *subglutinans*.

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