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DEVELOPMENT OF MACROCONIDIA IN *FUSARIUM*

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Reports on the development of macroconidia in *Fusarium* present contradictory views. The ultrastructure of conidial development in *Fusarium crookwellense* was examined and previously published micrographs of this process in other *Fusarium* spp. considered. Development of the first macroconidium can apparently be either enteroblastic or holoblastic. Enteroblastic development of the first conidium would occur after dissolution of the apex of the conidiogenous cell. If holoblastic development occurs, remains of the outer wall layer constitute the collarette. Ontogeny of all subsequent conidia is holoblastic with enteroblastic proliferation of the conidiogenous cell.

A number of studies on the ultrastructure of *Fusarium* have been published (Campbell & Griffiths, 1974; Garcia Acha *et al.*, 1966; Griffiths, 1973*a*, *b*; Marchant, 1966*a*, *b*). However, only a few reports attempt to describe the formation of the macroconidia.

Light microscope studies of conidiogenesis in Hyphomycetes by Seshadri (Schneider & Seaman, 1982) included *Fusarium* spp. The development of the macroconidium in one of these, *F. decemcellulare* Brick, was discussed by Subramanian (1971). This author suggested a thallic mode of development for the first macroconidium. This observation was supported by immunofluorescence studies of Goos & Summers (1964) on *F. oxysporum* Schlecht. f.sp. *cubense* (E. F. Smith) Snyd. & Hans. A similar type of development was also later suggested for *F. culmorum* (W. G. Smith) Sacc. by Marchant (1975). In contrast, Schneider & Seaman (1982) suggested a completely different mode of development for *F. sulphureum* Schlecht. from those of Subramanian (1971) or Marchant (1975). The former authors suggested that dissolution of the apex of the conidiogenous cell occurs and that this results in collarette formation. The interpretation of Schneider & Seaman (1982) was thus one of enteroblastic as opposed to thallic development.

Marchant (1983) attempted to consolidate the different interpretations of conidium development. Cultural conditions were dismissed as an explanation for the different interpretations and this author suggested that a misinterpretation of results on either one or both the mechanisms was more likely. This report describes the development of the first and subsequent conidia in *F. crookwellense* Burgess, Nelson & Toussoun. An attempt is also made to reconcile the differences in previous reports on conidium development with those found in *F. crookwellense*.

MATERIALS AND METHODS

Fusarium crookwellense (MRC 3852) isolated from necrotic wheat crowns in South Africa was examined. Water agar plates with pieces of carnation leaves (Fisher *et al.*, 1982) were inoculated with a conidial suspension and incubated at 25 °C under a combination of white fluorescent and near ultraviolet light (12 h photoperiod). Colonies were inspected after 48 h at 5 h intervals for the commencement of sporodochium development at the edges of the carnation leaf pieces. Leaf pieces (approx. 2 × 2 mm) were cut and fixed in 3% glutaraldehyde in 0·1 M phosphate buffer (pH 7·0) for 24 h at 4°.

The tissue was rinsed in the buffer solution and fixed in osmium tetroxide (Sabatini *et al.*, 1963) for 2 h before rinsing in the buffer. The material was dehydrated in an ethanol series and embedded according to Spurr (1969). The epoxy resin was polymerized at 70 °C for 8 h.

Ultra-thin sections (60 nm) were mounted on copper grids and stained for 20 min in a saturated uranyl acetate solution followed by 10 min in lead

citrate (Reynolds, 1963). Sections were viewed with a Philips EM300 electron microscope.

RESULTS

Observations of *F. crookwellense* and a re-examination of micrographs previously published (Schneider & Seaman, 1982; Marchant, 1975) lead us to conclude that there are three possible ways for the first macroconidium to develop in *Fusarium* spp. All three modes of development would result in apparently similar collarettes.

When cultures are disturbed, i.e. removal of material from young developing sporodochia for the preparation of slides, in some cases, the first conidium appears to be atypical and subglobose in shape (Figs 1, 2, 9). This conidium is formed holoblastically by apical wall-building. Prior to secession of this first conidium initial, a break can be observed in the outer wall layer of the conidiogenous cell (Fig. 1). After secession, the remains of this outer wall layer form the collarette (Fig. 3).

Production and secession of the first conidium is followed by proliferation of the conidiogenous cell enteroblastically and holoblastic ontogeny of the second conidium (Figs 3–7, 9). This conidium is delimited by the production of the inner wall layer of the newly produced macroconidium that eventually secedes by splitting of the area of contact between the macroconidium and the new conidium initial (Figs 7, 8). Proliferation, conidial ontogeny, conidial delimitation and secession are then repeated in the third, fourth and subsequent macroconidia (Fig. 10). Repeated enteroblastic proliferation results in distinct periclinal thickening at the apex of the conidiogenous cells (Figs 3, 4). Remnants of the outer walls of successive conidia can be seen at the apex of conidiogenous cells (Fig. 4).

The first macroconidium can alternatively develop holoblastically as illustrated by Marchant (1975). Apical wall-building provides length-wise extension of the conidium and diffuse wall-building results in an increase in conidium width (Fig. 11). Secession of this first typical macroconidium leaves a remnant of the outer conidium wall layer which forms the collarette (Fig. 11). The second and subsequent conidia would then develop through enteroblastic proliferation of the conidiogenous cells (Fig. 10).

The formation of a collarette can apparently also be through disintegration of the outer wall layer of the conidiogenous cell prior to the formation of the first conidium. This method of collarette development was proposed by Schneider & Seaman (1982) and is illustrated in their Figs 8–16. Here a septum is laid down close to the apex of the conidiogenous cell (Fig. 12). The outer, original wall breaks down to leave a collarette (Fig. 12). The first conidium develops enteroblastically and secedes. Subsequent conidia would be formed by enteroblastic proliferation of the conidiogenous cells with resulting periclinal thickening of the apex of the conidiogenous cell.

The foot cell appears to form after production of the macroconidium (Figs 7, 8). After the primary stages of macroconidial ontogeny, structures indicating metabolic activity can be found at the base of the developing conidium (Fig. 5). These structures indicate the first signs of foot cell development.

DISCUSSION

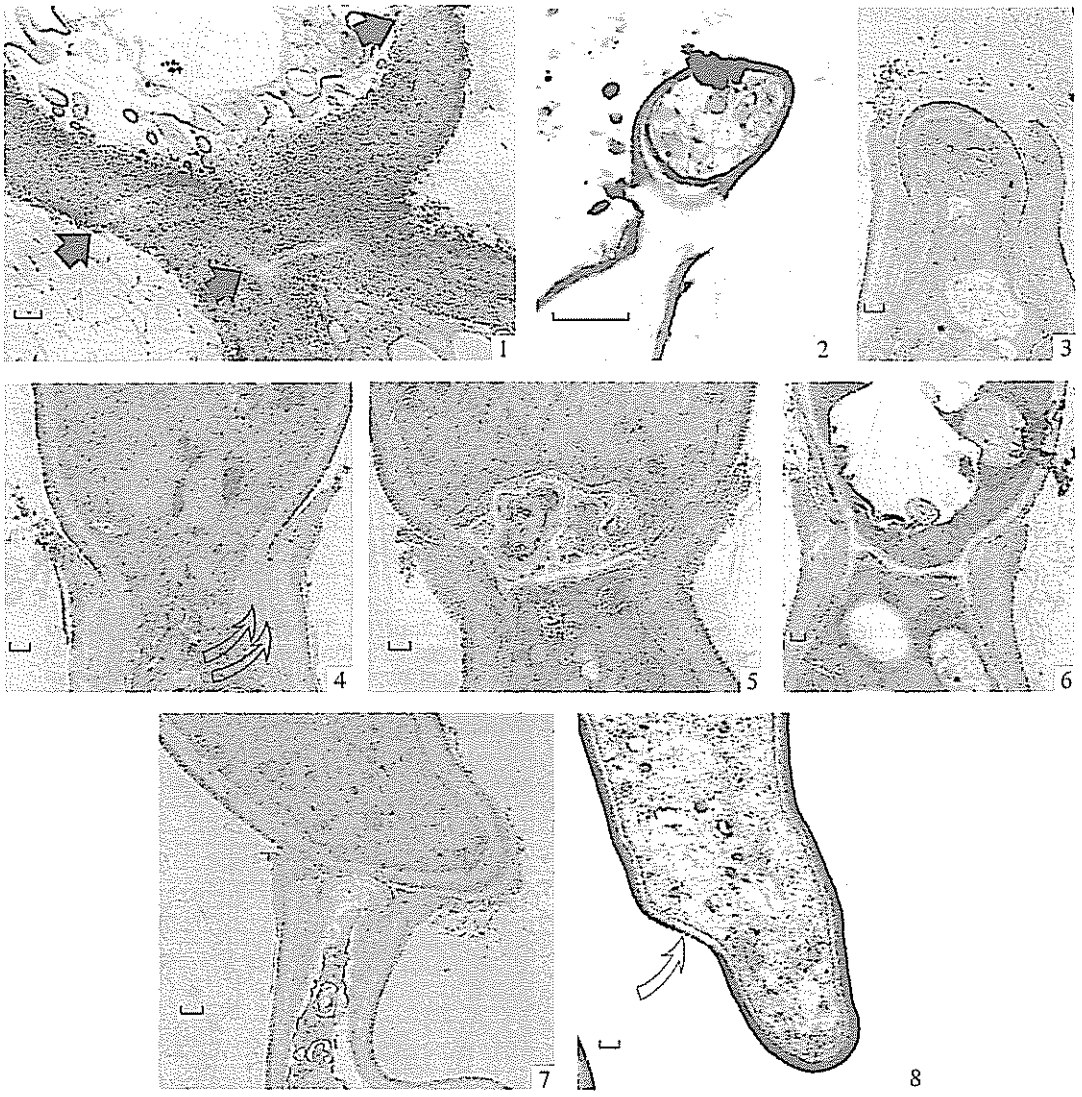
Our observation of *F. crookwellense* and micrographs of *F. culmorum* and *F. sulphureum* published by Marchant (1975) and Schneider & Seaman (1982) have been interpreted using the terminology of Minter *et al.* (1982, 1983).

Subramanian (1971) proposed that the first macroconidia of *F. decemcellulare* are produced thallically. The same interpretation was reported for *F. culmorum* by Marchant (1975, 1983). In contrast, however, Schneider & Seaman (1982) interpreted conidium production in *F. sulphureum* as being enteroblastic with dissolution of the apex of the conidiogenous cell prior to the first conidium production. The latter authors contend that they found conidia to develop in the same way in *F. decemcellulare* and *F. culmorum* but did not provide photographic evidence.

Sutton (1986) in comparing walls of developing conidia in *Trichothecium* and *Fusarium* presented a schematic interpretation of successive conidium development in *Fusarium*. Our observations concur with the interpretation of Sutton and do not include disintegration of the outer wall of the first conidium as proposed by Schneider & Seaman (1982). We find it difficult to reconcile our interpretation of the origin of the collarette with wall disintegration. However, we are not able to provide an alternative interpretation of the micrographs of Schneider & Seaman (1982) and for the present include this mode of development (Fig. 12) as a possible means of collarette origin.

The only significant difference between our interpretation and that of Sutton (1986) is the inclusion of a small subglobose first conidium. In the isolate of *F. crookwellense* that we have studied these conidia apparently occur rarely. They might result from disturbance during conidial development.

Production of the first conidium in *Fusarium*



Figs 1–8. Development of macroconidia in *Fusarium crookwellense*. (Unless otherwise indicated bar = 0·1 μm.)

Fig. 1. Initial of the first conidium produced by means of diffuse wall-building. The break in the conidiophore wall is evident (arrows).

Fig. 2. Conidium initial secedes if disturbed (bar = 1 μm).

Fig. 3. Third conidium initial produced holoblastically by apical wall-building following enteroblastic proliferation. Periclinical thickening is evident.

Fig. 4. Third conidium initial produced holoblastically by apical wall-building following enteroblastic proliferation. Periclinical thickening is evident (arrows).

Fig. 5. Third conidium is delimited with the production of the conidium inner wall layer.

Fig. 6. Inner wall layer of the conidium is liberated from the proliferation. Conidium remains attached to fourth conidium initial. Periclinical thickening extends.

Fig. 7. Third macroconidium is ready to secede. The foot cell is well-developed.

Fig. 8. Scar on the foot cell representing the last area of attachment (arrow).

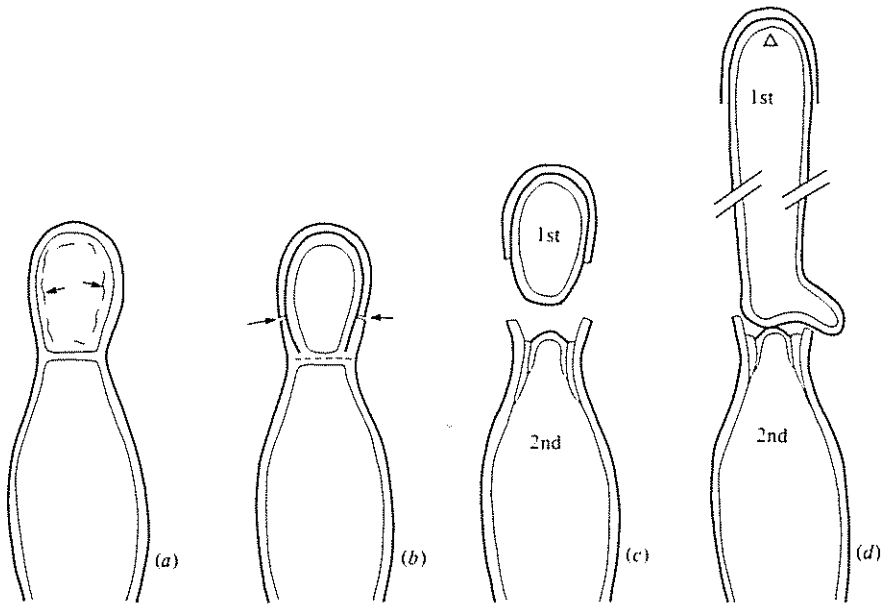


Fig. 9. Production of the first conidium in *Fusarium crookwellense* (*a*) The first conidium is formed holoblastically, the delimiting septum is laid down and followed by diffuse wall-building (arrows); (*b*) Development of the collarette and the cap (arrows); (*c*) Secession of atypical conidium with cap; (*d*) Normal first conidium with cap following apical wall-building (arrow head). The second conidium to be produced holoblastically is present as the initial and attached to the newly formed conidium (commencement of periclinical thickening).

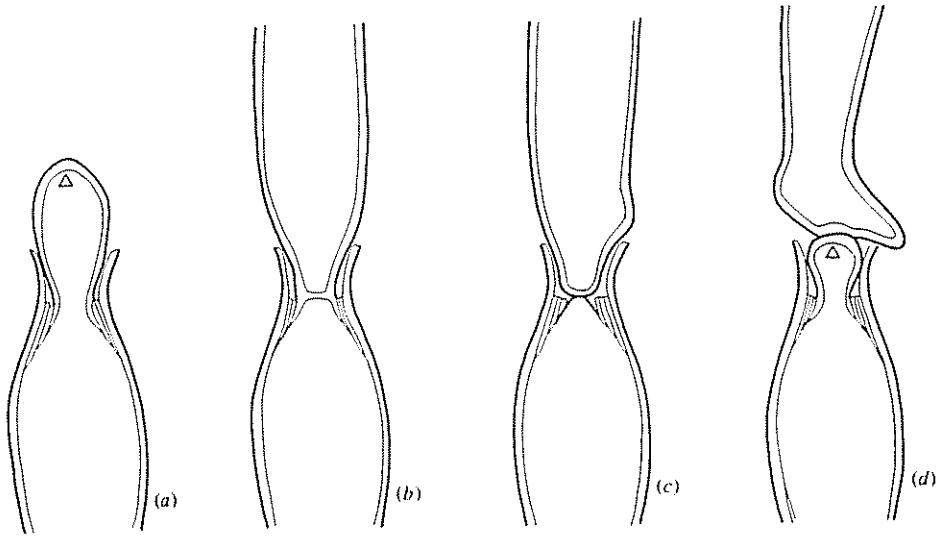


Fig. 10 Production of the third and fourth conidia in *Fusarium crookwellense*. (*a*) Following secession of the second conidium and enteroblastic proliferation of the conidiogenous cell the third conidium is produced holoblastically (apical wall-building, arrow head); (*b*) Third conidium is marked off (delimitation) by the production of the conidium inner wall; (*c*) Third conidium remains attached to the fourth conidium initial. The foot cell starts to develop; (*d*) Third conidium is ready for secession. The fourth conidium is developing and the wall is laid down in the apical region (arrow head).

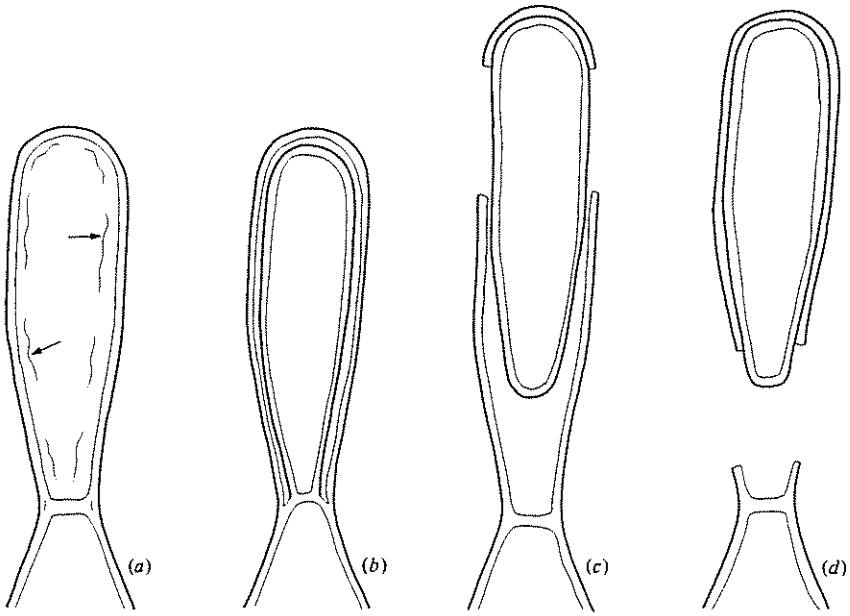


Fig. 11. The first conidium produced in *Fusarium decemcellulare* (after Subramanian, 1971) and *F. culmorum* (after Marchant, 1975). (*a*) Septum laid down away from the apex. Diffuse wall-building activity commences (arrows); (*b*) First conidium produced by a thallic mode; (*c*) Conidial secession; (*d*) Conidial secession.

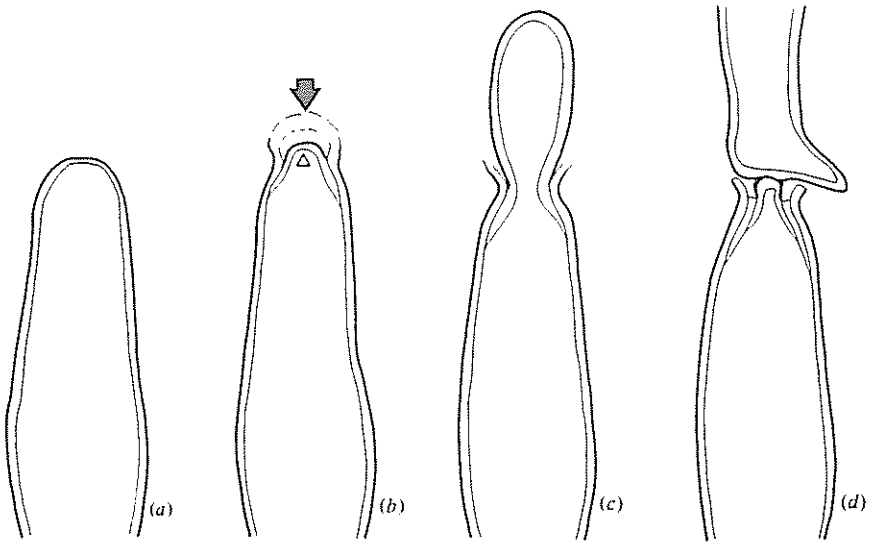


Fig. 12. Production of the first conidium in *Fusarium sulphureum* (after Schneider & Seaman, 1982). (*a*) Septum laid down at the apex of the conidiophore; (*b*) Disintegration of the conidiophore outer wall layer (arrow). Conidium production commences (enteroblastically) by means of apical wall-building (arrow head); (*c–d*) Conidium enlarges, is marked off and stays attached to the second conidium initial.

could be determined by the position in which the first septum is laid down in the conidiogenous cell. If the septum is formed at the apex of the conidiogenous cell, the old cell wall might disintegrate and the first conidium would then be produced enteroblastically as proposed by Schneider & Seaman (1982). The resulting collarette is therefore not a by-product of conidial ontogeny. This form of development is illustrated by Marchant (1975, their Fig. 14 for *F. culmorum*) and by Schneider & Seaman (1982).

If the original septum is laid down further back from the apex of the conidiogenous cell, enteroblastic proliferation is preceded by diffuse wall-building. In terms of Minter *et al.* (1982) this would be thallic development. If the septum is laid down still further away from the apex of the conidiophore, thallic production (Subramanian, 1971; Marchant, 1975) contributes to a greater portion of the first conidium. Only the final stage of conidium development would thus be replaced by apical wall-building. It seems, therefore, that the distance of the first septum from the apex of the conidiogenous cell will determine the relative importance of diffuse and apical wall-building in the production of the first conidium. Conceivably the first septum may be laid down far enough from the apex of the conidiogenous cell to allow the entire first macroconidium to develop. In this case, development of the first conidium is thallic and produced mainly by diffuse wall-building. Production of atypical, subglobose conidia observed in *F. crookwellense* probably represents an intermediate form of conidium development.

After the production of the first conidium, development of all subsequent conidia in the *Fusarium* spp. under discussion appears to proceed by enteroblastic proliferation of the conidiogenous cell. The resulting remnant of the outer conidium wall accumulate at the apex of the conidiogenous cell within the collarette as illustrated in *Trichoderma saturnisporum* Hammill (1974). As proposed by Minter *et al.* (1982), ontogeny of all conidia would then be holoblastic followed by enteroblastic proliferation of the conidiogenous cell. Accumulation of wall material at the apex of conidiogenous cells could not be seen in previously published micrographs but is clearly evident in the micrographs of *F. crookwellense*.

It is possible that the conditions under which *Fusarium* spp. are cultivated determine the placement of the first septum in the conidiogenous cell and subsequently the development of the first conidium. This would explain different types of conidium development reported in the same species by different authors (Schneider & Seaman, 1982; Marchant, 1975). Marchant (1983), however, contends that differences are more likely to be due to different interpretations of electron micrographs. Further studies should include a number of different *Fusarium* spp. and should compare different cultural conditions.

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