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DELIMITATION OF *FUSARIUM CROOKWELLENSE* MACROCONIDIA

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Delimitation of second and subsequent *Fusarium crookwellense* macroconidia, determined by means of TEM and SEM, appeared to occur in the absence of a well-defined, double-layered septum produced by invagination of the inner wall layer of the conidiogenous cell. Rather, the delimiting layer appeared to be continuous with the inner wall layer of the maturing conidium. Repeated production of conidia resulted in a build-up of proliferation layers (periclinal thickening) leaving a small translocation channel. At this stage the conidiogenous cells could regenerate themselves by means of percurrent proliferation.

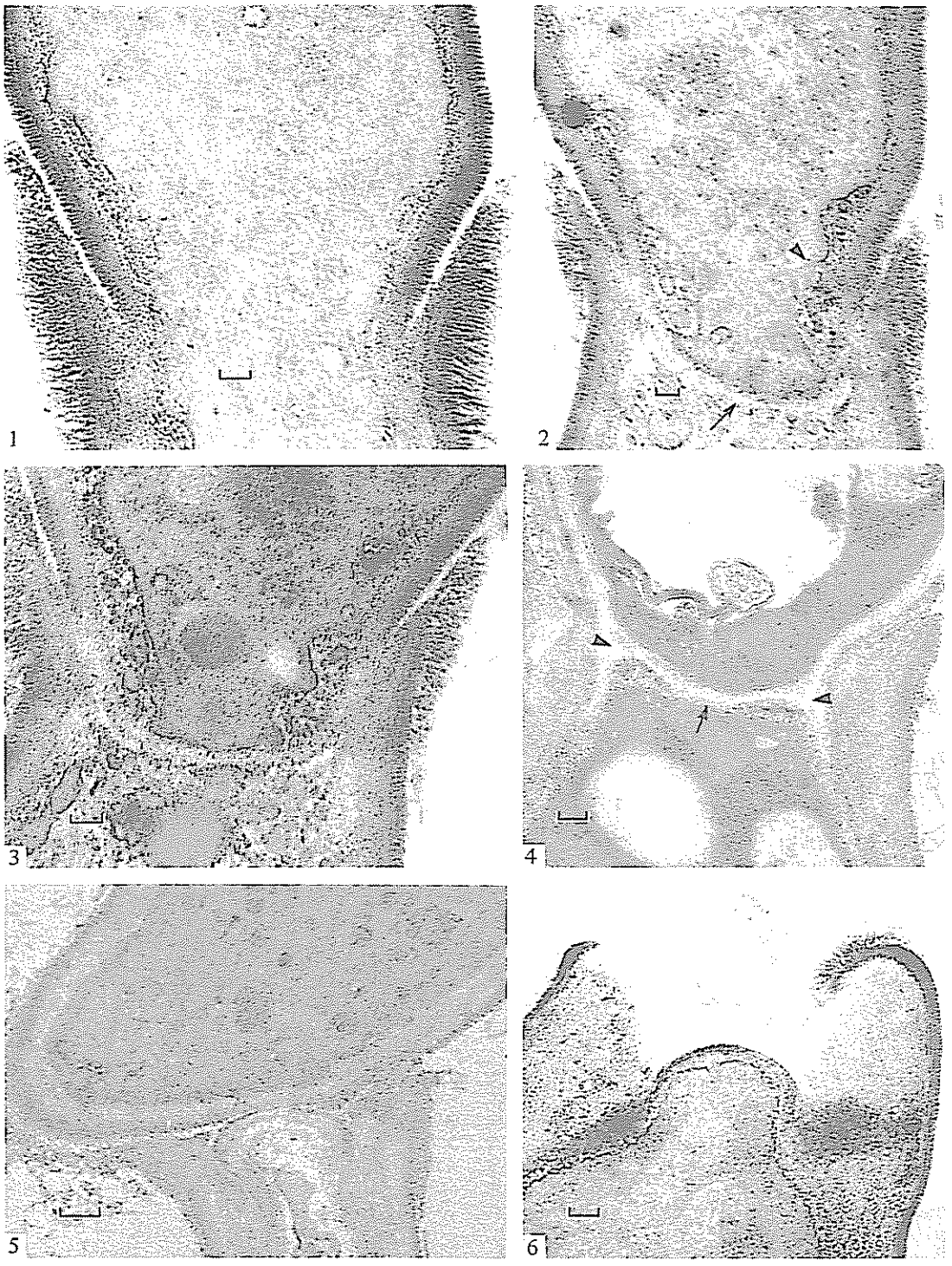
Terminology introduced by Minter, Kirk & Sutton (1982, 1983) to describe stages of conidial ontogeny have motivated a study of these processes in *Fusarium* spp. The stages involved in the ontogeny of *F. crookwellense* Burgess, Nelson & Toussoun macroconidia have recently been illustrated (van Wyk *et al.*, 1987). Conidial delimitation, one of five stages in conidium production introduced by Minter, Kirk & Sutton (1982, 1983), is thought to be accomplished in Deuteromycetes by the formation of a perforated, double-layered septum (Cole & Samson, 1979). In a schematic representation of conidium production in *Fusarium*, Sutton (1986) also illustrated delimitation in this genus by means of a perforated, double-layered septum.

Since the first report of perforated septa in filamentous fungi by de Bary (Reichle & Alexander, 1965), several types of septa have been recognized (Bracker, 1967). The typical septum of Ascomycetes and of Deuteromycetes with ascomycetous affinities is a simple plate with a central pore (Bracker, 1967), although a multiperforate septum was found in large hyphae of *F. solani* (Reichle & Alexander, 1965). A double-layered plate with a single pore has been illustrated in hyphae (Reichle & Alexander, 1965; Wergin, 1973) and macroconidia (Stålhammar-Carlemalm, 1976; Marchant, 1983) of *Fusarium* spp. These septa are presumably formed by invagination of the inner wall layers of developing cells.

In preliminary studies it appeared that the mode of conidial delimitation in *F. crookwellense* was possibly related to the occurrence of periclinal thickening. The aim of this study was therefore to examine processes leading to the delimitation of macroconidia in this fungus.

MATERIALS AND METHODS

Fusarium crookwellense was isolated from necrotic wheat crowns in South Africa and a lyophilized single-conidial culture was deposited in the culture collection of the South African Medical Research Council (MRC 3852). 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 with a 12 h photo-period. After 48 h, colonies were inspected 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 °. Tissue was rinsed in the buffer solution and fixed in osmium tetroxide (Sabatini, Bensch & Barrett, 1963) for 2 h before rinsing in buffer. Material was dehydrated in a graded ethanol series and embedded according to Spurr (1969). The epoxy resin was polymerized at 70° for 8 h. Ultrathin sections (60 nm) were mounted on copper grids and stained



Figs 1–6. Conidial delimitation in *Fusarium crookwellense* (TEM, bar = 0·1 μm).

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.

Agar disks from cultures bearing young developing sporodochia were fixed in 1·5% glutaraldehyde in 0·1 M phosphate buffer for 1 h followed by 1% osmium tetroxide for 2 h, dehydrated in a graded acetone series, critical-point dried, coated with gold palladium and viewed with an ISI scanning electron microscope.

RESULTS

A delimiting layer was not evident at the base of a developing second or subsequent conidium until the conidium was fully developed (Fig. 1). At this stage a diffuse abstriction layer at the base of the conidium was observed (Fig. 2). This layer became prominent and wall-building activity appeared to be enclosed at the base of the new conidium (Fig. 3). On completion of the delimitation layer, newly-produced conidia remained attached to the conidiogenous cells by an area of contact with newly-initiated conidia (Figs 4,5). Enteroblastic proliferation of the conidiogenous locus preceded ontogeny of subsequent conidia. Proliferation resulting in the production of a new conidium included the production of a new plasmalemma with the new conidial inner wall layer produced to the outside of the plasmalemma (Fig. 6).

Production of conidia from the first conidiogenous locus continued until only a small pore was left at the apex of the conidiogenous cell (Figs 7, 8) as a result of periclinal thickening. At this stage conidium production was regenerated by means of percurrent proliferation of the conidiogenous cell. In this way a new conidiogenous cell, able to resume conidium production at a higher level, was produced (Fig. 11). At the base of the new conidiogenous cell a double-layered septum apparently formed by invagination of conidiogenous cell inner wall layers was laid down (Figs 7, 8). This septum represents a structure different from that involved in conidial delimitation.

Septa present in hyphae (Fig. 9) and macroconidia (Fig. 10) were typical of those normally encountered in Deuteromycetes and which are commonly referred to as perforated, double-layered septa.

The different stages involved in conidial delimitation in *F. crookwellense* as reported here, are schematically illustrated in Fig. 12.

DISCUSSION

A well-defined double-layered septum formed by invagination of conidiogenous cell inner wall layers was not observed to delimit second and subsequent macroconidia in *F. crookwellense*. If such a double-layered septum was involved in delimitation, schizolytic cleavage (Cole & Samson, 1979) would result in conidial secession. Consequently, the conidiogenous cell apex would be sealed off by the basal part of the original septum (Fig. 13A). This remaining half septum then would serve as a conidial apex during production of subsequent conidia (Hughes, 1971). Thus no septal remnants would be evident at the apex of the conidiogenous cell after the production of a series of successive conidia. In this study of conidial delimitation in *F. crookwellense*, periclinal thickening was, however, clearly evident at the apex of conidiogenous cells. This suggests that schizolytic separation of a true septum formed by the invagination of conidiogenous cell inner wall layers was not involved in the delimitation process.

We believe that when the first macroconidium develops through enlargement of the apical cell of the conidiophore (Marchant, 1975; van Wyk *et al.*, 1987), a 'normal' perforated double-layered septum, produced before differentiation, would delimit this conidium. However, the electron micrographs suggest that, in general, second and subsequent macroconidia are delimited through a process of conidial completion. Here, one half of the delimitation layer, which differs from a true

Fig. 1. Absence of a delimitation layer at the base of a nearly mature conidium.

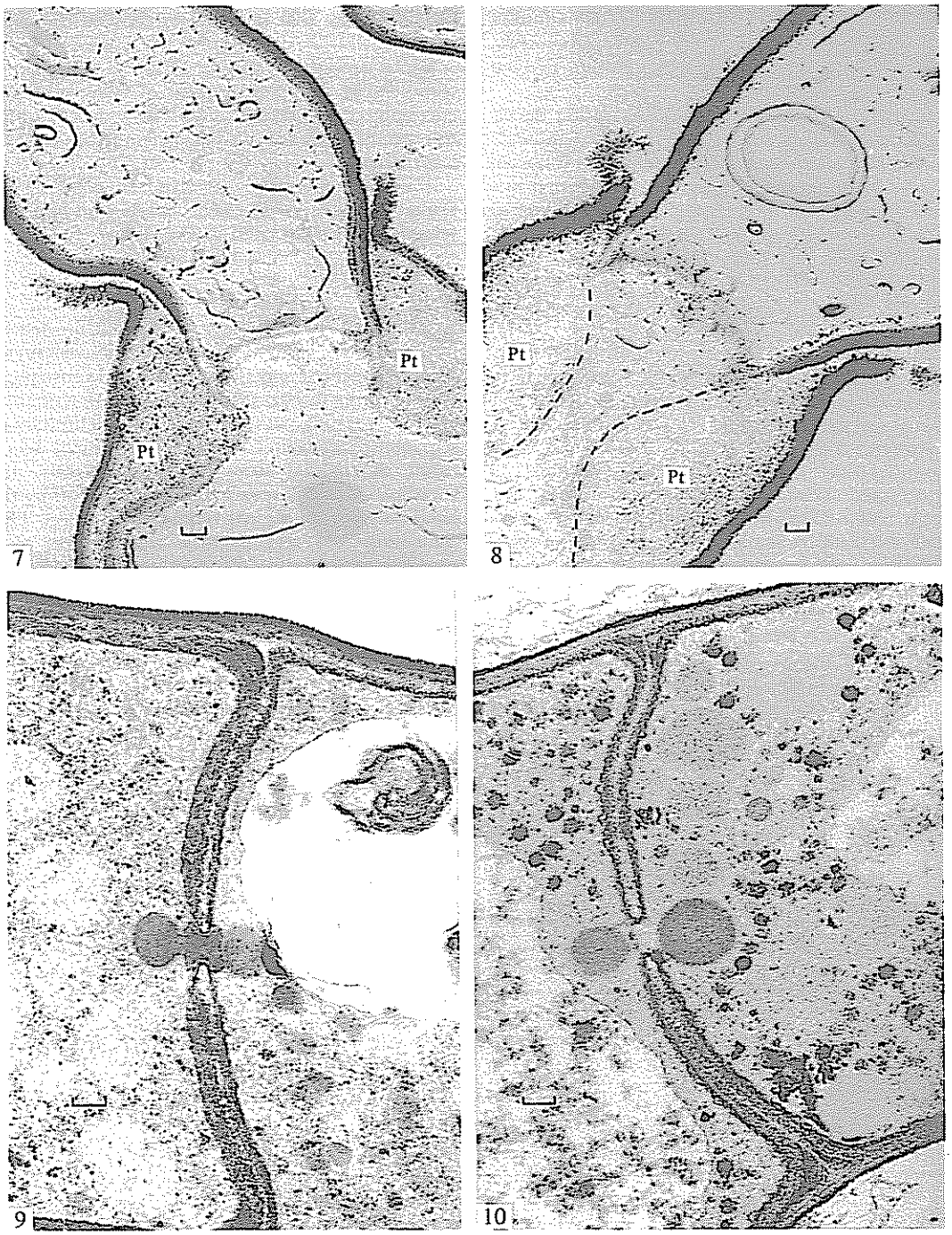
Fig. 2. Diffuse layer representing the onset of conidial delimitation (arrow). Note wall-building activity at the base of the delimited conidium (arrowhead).

Fig. 3. Prominent delimitation layer continuous with the inner wall layer of the conidium to be delimited.

Fig. 4. Final stage of delimitation. The inner wall layer of the conidium has separated from that of the conidiogenous cell (arrow heads) and a new conidium apex has been initiated (arrow).

Fig. 5. The conidium is fully developed and remains attached to the newly initiated conidial apex.

Fig. 6. Initiation of a new conidium apex.



Figs 7–10. Double-layered septa in *Fusarium crookwellense* (TEM, bar = 0·1 μm).
Figs 7, 8. Delimitation of the new conidiogenous cell by means of a double-layered, perforated septum after percurrent proliferation. Note extensive periclinal thickening (Pt) at the apex of previous conidiogenous cell.
Fig. 9. Perforated, double-layered septum in a macroconidium (phragmospore).
Fig. 10. Perforated, double-layered septum in a hypha.

Fig. 11. Percurrent proliferation of the conidiogenous cell in *Fusarium crookwellense* (SEM, bar = 1 μm).

septum in that it does not originate from the invagination of conidiogenous cell inner wall layers, is produced by the conidium. The basal half of the delimitation layer is formed at a later stage and is produced by the conidiogenous cell as a new conidial apex. The delimitation layer is thus comprised of the apex of a new conidium and the base of the previously-formed conidium. Although there is an impression of a double-layered structure, it does not appear to be formed by the invagination of inner wall layers of the conidiogenous cell.

If the conidial apex was derived from a double-layered septum produced by invagination, it would have been secured to the conidiogenous cell inner wall layer. This mechanism of delimitation as illustrated in Fig. 13A, is in agreement with the description of this process by Hughes (1971) for annellidic conidium development. In view of the fact that the new conidium apex in *F. crookwellense* was apparently not part of such a septum, this apex would need to secure itself to the conidiogenous cell inner wall layer. We argue that this securing of the conidial apex to the inner wall layer of the conidiogenous cell is expressed as periclinal thickening (Fig. 13B).

It is not suggested that the mechanism of delimitation illustrated here is necessarily the only

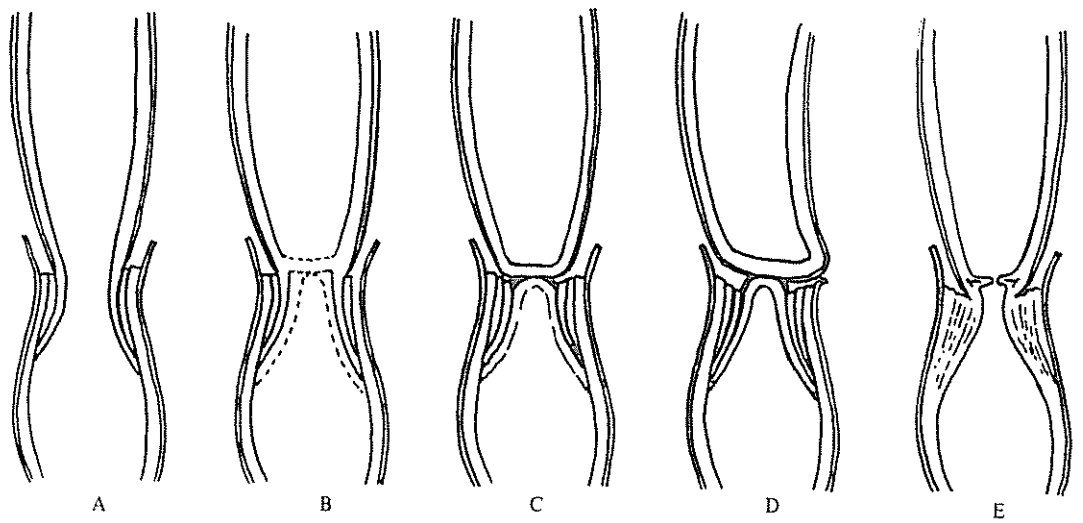


Fig. 12. Schematic representation of conidium delimitation in *Fusarium crookwellense*. (A–D). The process involved in delimiting second and subsequent macroconidia without the formation of a double-layered septum as suggested for *Fusarium crookwellense*. (E). Delimitation of the percurrently proliferating conidiogenous cell by means of a perforated septum.

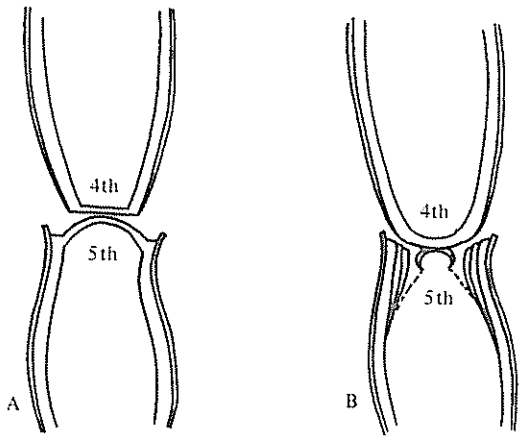


Fig. 13. Schematic representation of two possible mechanisms of conidial delimitation. (A). Remaining half septum acting as a conidium apex (5th conidium) after secession of the fourth conidium; (B). As reported for *F. crookwellense*. New conidium initial (5th conidium) being secured to the conidiogenous cell inner wall layer with a resultant build-up of proliferation layers (periclinal thickening).

form of delimitation that could occur for macroconidia in *Fusarium* spp. However, we have found that periclinal thickening is present in a wide range of *Fusarium* spp. that we have examined. It thus appears that the mechanism of delimitation observed in *F. crookwellense* is the predominant one for delimitation of macroconidia in *Fusarium* spp.

Schneider & Seaman (1982, Fig. 22) illustrate a perforated, double-layered septum at the base of the conidium. We suggest that in such cases, proliferation will not result in a build-up of proliferation layers (periclinal thickening) following secession. Tiedt, Jooste & Hamilton-Attwell (1986, Fig. 7) also illustrate a perforated, double-layered septum as a delimitation layer in *F. sacchari* f. sp. *subglutinans* (Wollenw. & Reinking) Nirenberg. These authors, however, give no indication of whether their micrograph pertains to macro- or microconidia. In view of the fact that periclinal thickening was not evident in their illustration, it is possible that this micrograph could illustrate development of a microconidium. We believe that microconidia and macroconidia in *Fusarium* spp. are delimited differently and are presently comparing these processes. Indeed, *F. crookwellense* was specifically chosen for this study of conidial delimitation because this fungus only forms macroconidia. Periclinal thickening, resulting from a build-up of proliferation layers at the apex of the conidiogenous cell eventually blocks this apex. In some cases it appears that these blocked conidiogenous cells can be revitalized by percurrent proliferation of the conidiogenous cell. Subsequent conidia are then produced at a higher level. Here, a septum with a single pore, similar to those produced in hyphae and septate conidia, is laid down at the base of the new, revitalized conidiogenous cell. Delimiting septa at the base of such conidiogenous cells thus appear to differ from the layer produced during the delimitation of second and subsequent macroconidia. The role of different types of delimitation layers in phialidic Deuteromycetes is not entirely clear and thus deserves further study.

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