CONIDIUM DEVELOPMENT IN PHOMA ARACHIDICOLA WITH SPECIAL REFERENCE TO DELIMITATION

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ABSTRACT

Conidium development in *Phoma arachidicola* is described from ultrastructural studies. Special attention is given to delimitation, a process which involves the production of an amorphous wall layer, in the neck of the pycnidium, from which the conidium inner wall is derived. The apex of the new conidium initial is formed independently from the delimitation layer. Development of these new apices is accompanied by an accumulation of conidial outer wall layers in the neck of the conidiogenous cell. This periclinal thickening eventually blocks the conidiogenous cell and conidium production from this locus is terminated.

ONTWIKKELING VAN KONIDIUMS VAN "PHOMA ARACHIDICOLA" MET SPESIALE VERWYSING NA AFSNOERINGSMEGANISMES

Die ultrastrukturele ontwikkeling van konidiums van *Phoma arachidicola* met spesiale verwysing na konidiumafsnoering, word beskryf. Die afsnoering van konidiums word voorafgegaan deur die vorming van 'n breë, amorfe, laag waaruit die konidiumwand ontwikkel, in die nek van die piknidium. 'n Konidium word geïnisieer deur die vorming van 'n nuwe apeks onder hierdie gedifferensieerde konidiumwand. Proliferasie wat met die vorming van die nuwe apeks gepaard gaan, lei tot periklinale verdikking in die nek van die konidiogene sel. Met die herhaalde vorming van konidiums raak hierdie verdikking waarskynlik so uitgebreid dat geen verdere spoorvorming plaasvind nie.

1. INTRODUCTION

Developmental stages during production of conidia in *Phoma* spp. have been illustrated by Boerema (1965), Sutton & Sandhu (1968), Boerema & Bollen (1975) and Jones (1975) and discussed by Sutton (1964) and Boerema (1970). These reports have resulted in the acceptance of a phialidic mode of conidial development in *Phoma*. Phia-

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lidic conidiogenous cells have also been observed in *Phoma arachidicola* (Marasas et al. 1974).

Different stages involved in phialidic conidial development have been emphasized by Minter et al. (1982, 1983a, 1983b) and this has led to an improved understanding of conidial development in the Deuteromycetes. One of these developmental stages, delimitation, is thought to be accomplished by the production of a double-layered, perforated septum (Cole & Samson 1979). However, it has recently been suggested (Van Wyk et al. 1988) that delimitation in *Fusarium crookwellense* Burgess, Nelson & Toussoun, does not involve the production of a structure equivalent to septa in hyphae or conidia. Instead, a fragile wall layer eventually seceding with the conidium appears to be formed. The aim of this study was to examine conidium development in the coelomycete *P. arachidicola* and particularly to compare conidial delimitation to that recently observed in *F. crookwellense*.

2. MATERIALS AND METHODS

An isolate of *Phoma arachidicola* (PREM 44889) from peanut plants with typical web blotch symptoms (Marasas et al. 1974) was used in these studies. The fungus was grown on potato-dextrose agar (PDA) at 20 °C under a combination of white fluorescent and black light (12 h photoperiod).

Agar blocks (approx. 2 x 2 mm) containing pycnidia were fixed in 3 % glutaraldehyde in 0,4 M phosphate buffer for 4 h. The material was rinsed in phosphate buffer and postfixed for 4 h in 2 % osmium tetroxide at room temperature before a second rinse in phosphate buffer. Material was dehydrated in a graded alcohol series and embedded in epoxy resin (Spurr 1969). The impregnation with pure epoxy was done overnight in vacuum and the resin was polymerized at 70 °C. Sections (ca. 60 nm) were made with a LKB Ultratome III using a diamond knife. The sections were stained for 25 min in a saturated solution (ca. 60 %) of uranyl acetate, and for 10 min in lead citrate (Reynolds 1963). Sections were examined with a Phillips EM300 electron microscope.

3. RESULTS

The first conidium was produced by means of apical wall building following disintegration of the apex of the conidiogenous cell (phialide) (Figures 1 & 2). Disintegration of the apex was preceded by the production

of an amorphous layer (Figure 1) which eventually became compressed to form a thin conidium wall (apex of conidium), presumably the inner wall (Figure 2). Delimitation of the conidium involved the production of an amorphous wall layer (Figure 3) from which the conidium inner wall was differentiated (Figure 4). At this stage proliferation occured and a new conidium apex was produced. The remaining amorphous wall layer (Figure 4) was eventually compressed into a thin layer (Figure 5) creating the illusion of a double-layered septum. During the first stages of development of the delimitation layer, the base of the conidium was still situated in the neck of the conidiogenous cell (Figure 3). As proliferation proceeded, the amorphous wall layer was compressed and the final double-layered septum (Figures 5 & 6) was situated well above the apex of the collarette. The base of the conidium had thus been pushed out of the neck of the conidiogenous cell (Figure 5,6).

Repeated production of conidia resulted in the accumulation of proliferation remnants (Figures 7 & 8) seen as periclinal thickening finally leaving only a small pore through which further conidia could be produced (Figure 8). The process of conidial delimitation is schematically illustrated in Figure 9.

4. DISCUSSION

Conidial development in *P. arachidicola* observed in this study is similar to that reported for other *Phoma* spp. (Boerema 1965; Boerema & Bollen 1975; Sutton & Sandhu 1968; Jones 1975). Four of the stages of conidial development proposed by Minter et al. (1982, 1983a, 1983b), i.e. proliferation, wall building, delimitation, and secession were evident in *P. arachidicola*. The delimitation stage was, however, of special interest. Delimitation of conidia is usually thought to occur through production of a double-layered septum arising from invagination of conidiogenous cell inner walls. This septum eventually splits schizolytically to leave one half of the septum at the conidiophore apex after secession (Cole & Samson 1979). The half septum then becomes the apex of the next conidium to be produced. The other half of the septum forms the base of the seceding conidium which is usually truncate.

Double-layered septa resulting from the invagination of inner wall layers have been shown in many fungal hyphae and septate conidia (Cole & Samson 1979). However, Van Wyk et al. (1988) recently presented evidence that this type of septum is apparently not involved in the pro-

cess of delimitation in macroconidia of *Fusarium crookwellense*. Rather, during delimitation of newly formed macroconidia in this fungus, a layer is produced from the conidium which completes conidium development. An additional layer produced later at the apex of the conidiogenous cell, and serving as the apex of a new conidium, seals off the phialide apex. In *P. arachidicola* a dense layer is derived from an amorphous area completing conidium development. An additional layer, sealing the phialide apex, is initiated from the amorphous area serving as an apex for the new conidium initial. The latter layer is differentiated at a later stage than the former conidium bound layer.

The results of this study suggest that, as in the case of *F. crookwellense*, each new conidium produced by *P. arachidicola* involves the initiation of a new conidial apex. Proliferation preceding ontogeny results in an accumulation of proliferation remnants (periclinal thickening) which eventually terminate the production of conidia at the neck of the conidiogenous cell. A similar building-up of wall layers within the apex of the phialide has also been demonstrated in other *Phoma* sp. (Boerema 1970).

In *F. crookwellense*, blocked conidiogenous cells can apparently be revitalized by means of percurrent proliferation of this cell resulting in a new fertile locus at a higher level (Van Wyk et al. 1988). Percurrent proliferation of conidiogenous cells was, however, not observed in *P. arachidicola*. Percurrent proliferation of conidiogenous cells appear to be essential for the revitalization of old, non-functional conidiogenous cells. In the absence of this revitalization, conidiogenous cells in *P. arachidicola* would produce a set number of conidia and then cease to function.

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FIGURES 1-8
CONIDIUM DEVELOPMENT IN PHOMA ARACHIDICOLA
(TRANSMISSION ELECTRON MICROSCOPY)
(bar = 1 µm in all figures)
(Co = Conidium; Ph = phialide)

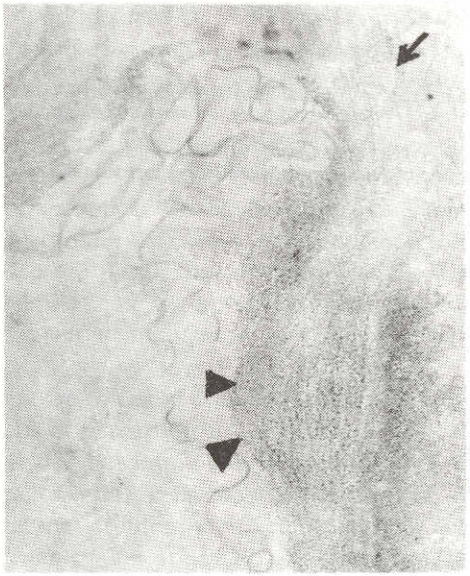


FIGURE 1
APICAL AREA OF A
CONIDIOGENOUS CELL

Note disintegration of outer wall (arrow) and thickening of inner wall layer (arrowheads).

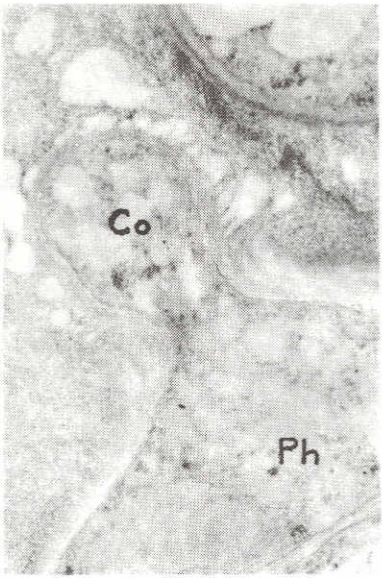


FIGURE 2
CONIDIUM DEVELOPMENT
IN AN ADVANCED STAGE

The conidium is fully developed but no delimitation structure has yet been produced.

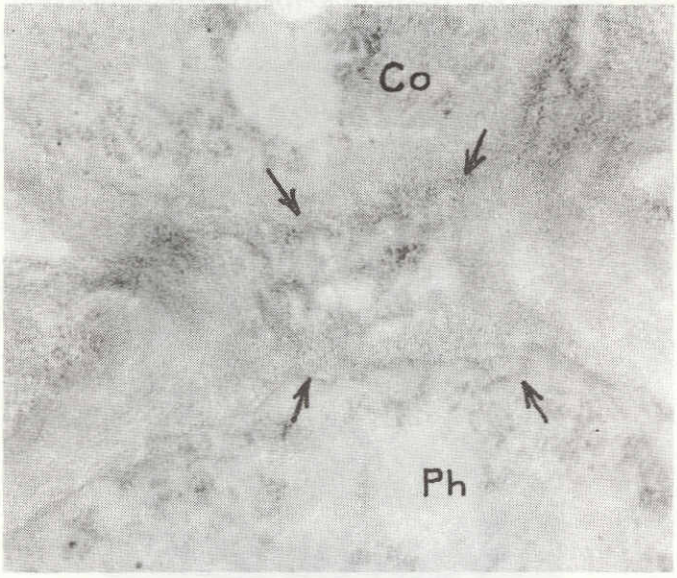


FIGURE 3
ENLARGEMENT OF THE AMORPHOUS WALL LAYER FORMED IN THE NECK OF THE CONIDIOGENOUS CELL (arrows)

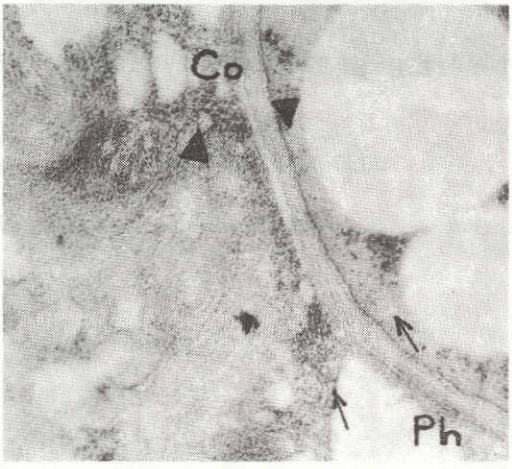


FIGURE 4
CONIDIUM INNER WALL (arrow heads) IS DIFFERENTIATED FROM THE AMORPHOUS WALL LAYER (arrows)

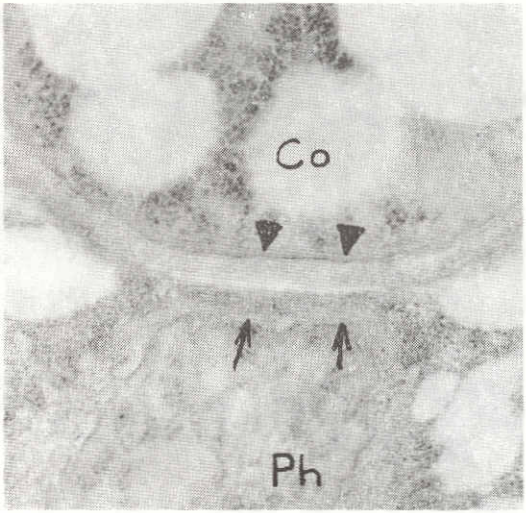


FIGURE 5
CONIDIUM INNER WALL (arrow heads)
AND THE WALL OF CONIDIUM APEX
(arrows) COMPLETE

At this stage the delimitation zone appears as a double layered septum.

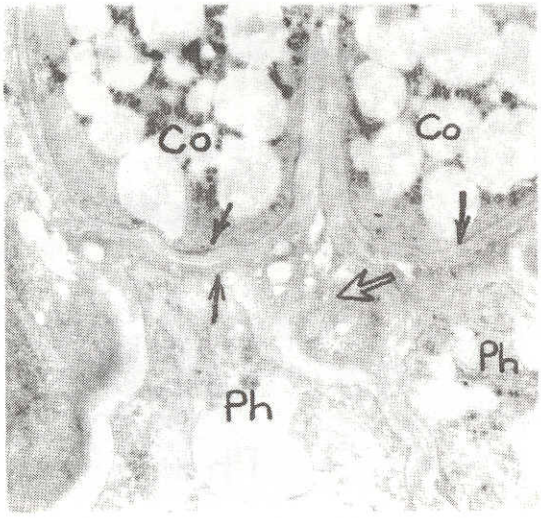


FIGURE 6
CONIDIA IN DIFFERENT STAGES
OF DELIMITATION

Note amorphous wall layer in neck of conidiogenous cell (arrow), final layers in position above the neck of the conidiogenous cell (arrow heads) and position of collarettes (white arrow).

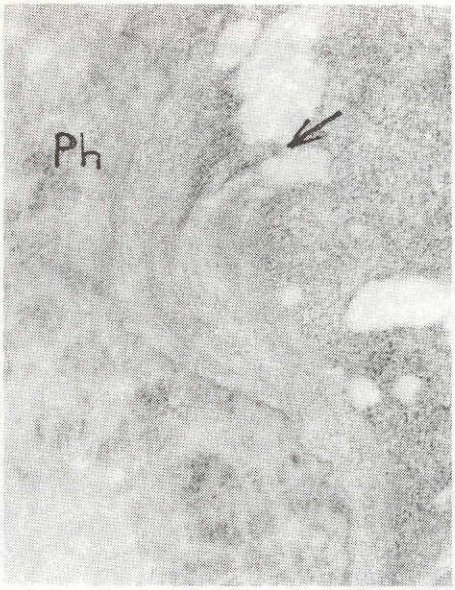


FIGURE 7
EARLY STAGE OF PERICLINAL
THICKENING (arrow)

One half of the phialide apex illustrated.

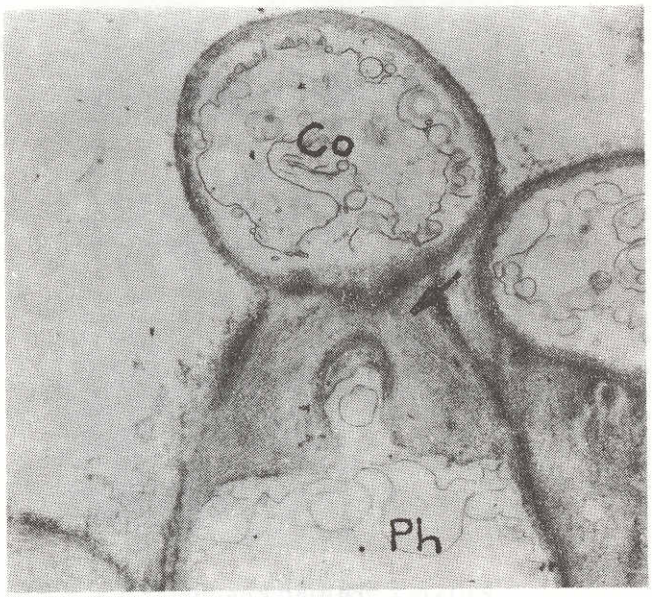
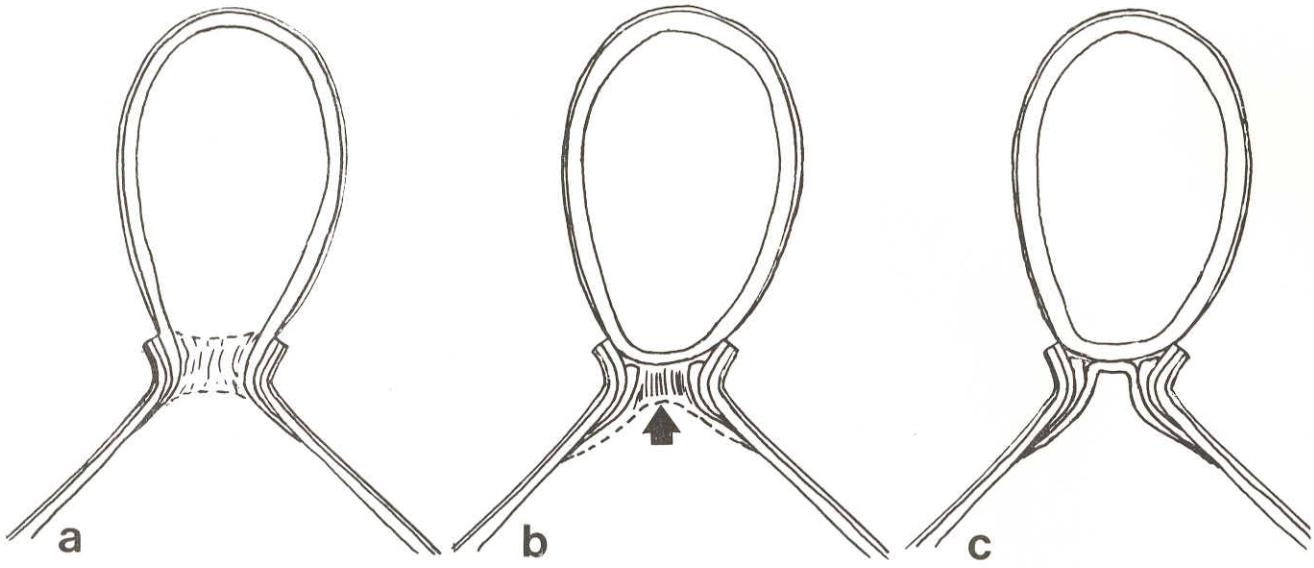


FIGURE 8
ADVANCED STAGE OF PERICLINAL THICKENING

Note newly initiated apex of conidium initial (arrow).



a. Conidium development has been completed and an amorphous wall layer formed.

b. Conidium inner wall has differentiated from the amorphous wall layer. New conidium apex (arrow) is formed.

c. Delimitation has been completed with the formation of a thin, dense wall layer. At this stage the delimitation zone appears as a double layered septum.

FIGURE 9
DIAGRAMMATIC PRESENTATION OF CONIDIAL DELIMITATION IN *PHOMA ARACHIDICOLA*