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Nuclear division and septation in macroconidia of *Fusarium crookwellense*

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In *Fusarium crookwellense*, a single nucleus moves into young, developing macroconidia prior to conidium delimitation. Two patterns of septation occur. In one, the nucleus divides and a primary septum is laid down in the middle of the conidium. The resulting two nuclei divide synchronously and secondary septa are laid down: one near the apex and the other near the base of the conidium. The result is that both the apical and basal cells contain a nucleus. A second synchronous division of the two nuclei, next to the primary septum, is followed by the formation of a third set of septa resulting in a 5-septate, 6-celled conidium with each cell containing a single nucleus. In an alternative pattern of septation, anucleate apical and basal cells are delimited by septum formation preceding the first nuclear division. Division of the single nucleus is followed by the formation of the primary septum. A second synchronous division is completed by the formation of the last set of septa resulting in a 5-septate, 6-celled conidium with the four middle cells each containing a single nucleus.

In *Fusarium crookwellense* beweeg 'n enkele kern in die jong makrokonidium net voor finale afgrensing van die konidium. Twee verskillende patrone van septasie in makrokonidia is waargeneem. In die een tipe verdeel die kern en 'n primêre septum word neergelê wat die konidium halveer. Die twee kerne verdeel gesinkroniseerd en 'n tweede stel septa word neergelê sodat die apikale- en basisselle afgegrens is, elk met een kern. 'n Tweede gesinkroniseerde verdeling van die kerne langs die primêre septum vind plaas wat weer eens gevolg word deur die neerlegging van septa. Die gevolg is 'n makrokonidium met vyf septa, ses selle met elke sel wat een kern bevat. Alternatiewelik is septa neergelê voordat die eerste kernverdeling plaasgevind het wat kernlose apikale- en basisselle afgrens. Die verdere verloop in hierdie geval was soortgelyk aan dié in die eersgenoemde tipe septasie en verdeling. Hierdie tweede patroon van verdeling het egter tot gevolg gehad dat 'n volwasse makrokonidium slegs vier kerne, een elk in die middelste vier selle, gehad het met kernlose apikale- en basisselle.

Keywords: *Fusarium crookwellense*, macroconidia, nuclear division, septation

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Introduction

Cytology in the genus *Fusarium* has received considerable attention (Hirsch, Snyder & Hansen 1949; Buxton 1954; Garcia Acha *et al.* 1966; Aist & Wilson 1968; Punithalingam 1972, 1975). In these studies several *Fusarium* spp. were included, viz. *F. avenaceum* (Fr.) Sacc., *F. culmorum* (W.G. Smith) Sacc., *F. decemcellulare* Brick, *F. equiseti* (Corda) Sacc., *F. graminearum* Schwabe, *F. moniliforme* Sheldon, *F. oxysporum* Schlecht. emend. Snyd. & Hans. and *F. solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans. These studies reported on the number of nuclei in cells of hyphae, microconidia, macroconidia and chlamydospores. Different numbers of chromosomes were also reported. Only one report (Punithalingam 1975) considered septation in macroconidia in relation to nuclear division. According to Punithalingam (1975) the nucleus within the conidial primordium of the developing macroconidia in *F. oxysporum* undergoes repeated divisions. This is followed by a process where septa are layed down linearly to separate the nuclei.

This paper reports on patterns of septation and how this relates to nuclear division in *F. crookwellense* Burgess, Nelson & Toussoun.

Materials and Methods

Ten isolates of *F. crookwellense* from wheat crowns were selected for this study and representative isolates lyophilized and deposited in the culture collection of the South African Medical Research Council, Tygerberg as MRC 3852, 3927, 3928. Cultures of these isolates on carnation leaf agar (CLA; Fisher *et al.* 1982) were incubated at 25°C under white fluorescent and near ultraviolet light (12-h photoperiod) for 10 days. Macroconidia produced in sporodochia were removed and studied under a light microscope. The numbers of septa in 300 macroconidia from each of the 10 isolates were counted.

Macroconidia of *F. crookwellense* MRC 3852 were spread on sterilized cellophane strips on wateragar plates and incubated as previously indicated. Strips were removed at 5-day intervals 3 days after commencement of incubation until well-developed sporodochia were evident on the surface. Strips containing fungal growth were fixed overnight in a 3:1 ethyl alcohol:acetic acid solution, inverted over glass slides which were lightly coated with Mayers' albumin, covered with blotting paper and weighted down for 12 h. Mycelium, young developing sporodochia and macroconidia remained attached to the slides after removal of the cellophane strips. After hydrolysis (Knox-Davies 1967) and staining with Giemsa (Knox-Davies 1966), slides were covered and viewed using a light microscope.

Results

Number of septa in macroconidia

Macroconidia from each of the 10 isolates of *F. crookwellense* had either three, four, five or six septa. Five septa were most frequently present (87% of 3 000 mature conidia) followed by four septa (9,6%).

Sequence of septum formation

Conidia are produced on monophialides and the shape of a typical macroconidium is not evident during the early stages of development (Figure 1A). The characteristic shape of the macroconidium becomes evident shortly before or during

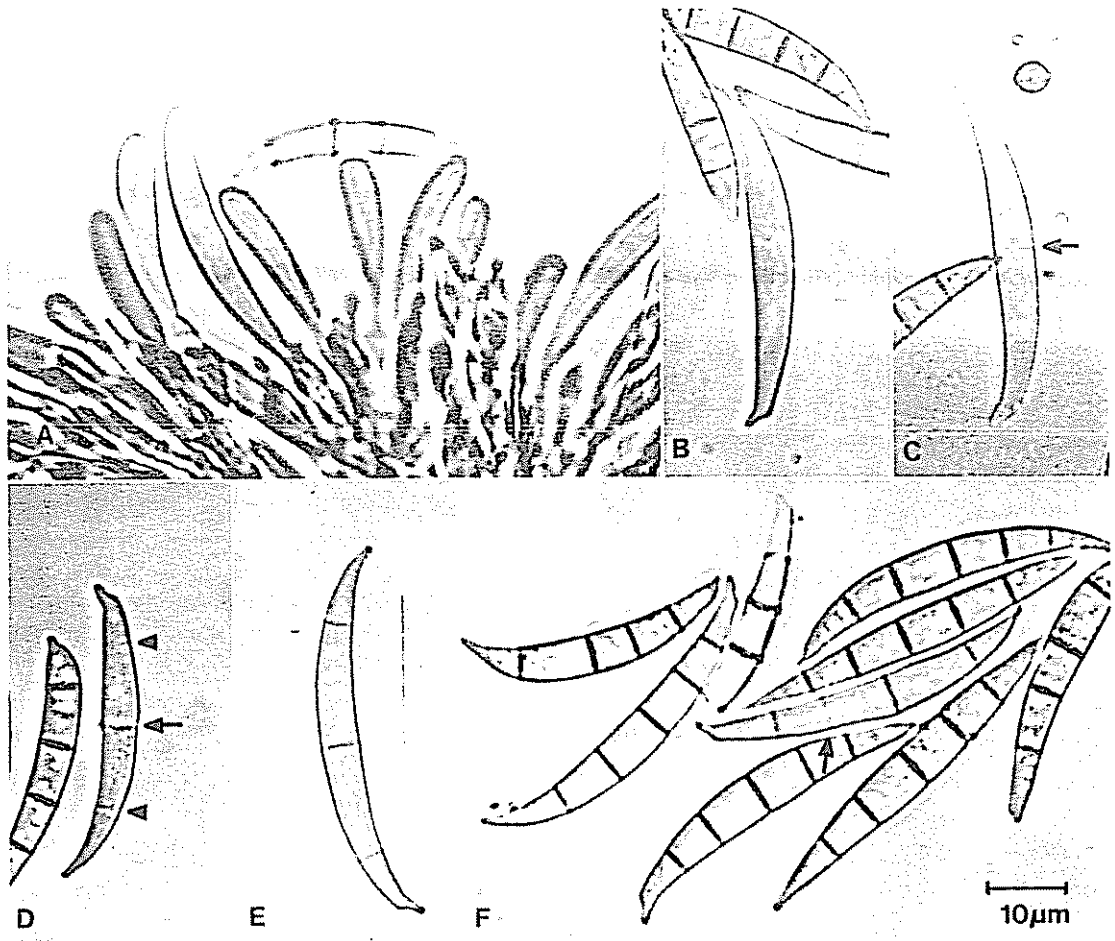

Figure 1 Light microscopy of macroconidia of *F. crookwellense* (Bar = 10 µm for all figures). A. Young developing macroconidia on monophialides. B. Aseptate macroconidium. C. Macroconidium with developing primary septum (arrow). D. Macroconidium with primary septum (arrow) and developing secondary septa (arrow heads). E. Macroconidium with primary and secondary septa. F. Macroconidia with five septa and developing conidium with developing tertiary septum (arrow).

conidial delimitation. At this stage the macroconidium is aseptate (Figure 1B). A septum is laid down approximately midway between the two ends of the macroconidium (Figure 1C). This is followed by the production of two additional septa, one near the apex and the other near the base (Figure 1D) of the conidium, resulting in a macroconidium with three septa (Figure 1E). A further set of septa is then laid down (Figure 1F) giving rise to the most commonly occurring 5-septate macroconidium (Figure 1F). In the situation most commonly encountered septa appear to be laid down from the outside inwards.

Nuclear division

Young conidia remain anucleate until late in their development (Figure 2A). During the early stages of development, a nucleus is present only in the conidiogenous cell (Figure 2A). This nucleus divides and one of the daughter nuclei moves into the young conidium, apparently prior to delimitation (Figure 2B). The macroconidium containing a single nucleus becomes delimited from the conidiogenous cell. This nucleus divides (Figure 2C), the resulting two nuclei separate (Figure 2D), round off (Figure 2E), and the first or primary septum is laid down at the middle of the macroconidium (Figure 2F). The two nuclei divide synchronously (Figure 2G) resulting in four nuclei (Figure 2H). Two secondary septa are laid down and these delimit the uninucleate basal and apical cells (Figure 2I). The two nuclei in the cells adjacent to the primary septum divide again and two tertiary septa are laid down. This results in a 5-septate, 6-celled macroconidium with each cell containing a single nucleus (Figure 2J). In the normal process of nuclear division it appears that only nuclei situated alongside the primary septum retain the ability to undergo subsequent division. Alternatively, septa may be laid down and these delimit anucleate basal and apical cells (Figure 2K). In this case septum formation precedes the first nuclear division. This is followed by nuclear division and the subsequent formation of a septum at the middle of the macroconidium. The two nuclei divide synchronously and a set of septa is laid down.

Cells with two nuclei occur in some macroconidia (Figure 2L). These are, however, only found in either 4-septate or 6-septate conidia and binucleate cells are located next to the septum produced after the first nuclear division.

Discussion

Macroconidia of *F. crookwellense* most commonly have five septa and a definite pattern in which these are laid down (Figure 3). This pattern is quite different from that reported in *F. oxysporum* (Punithalingam 1975). In the normal process of development of *F. crookwellense* macroconidia, septum formation is preceded by nuclear division. Thus a primary septum is laid down following a single division of the one nucleus in the macroconidium. Two secondary and two tertiary septa are then formed after two synchronous divisions of the daughter nuclei.

Occasionally alternative patterns of septation are observed

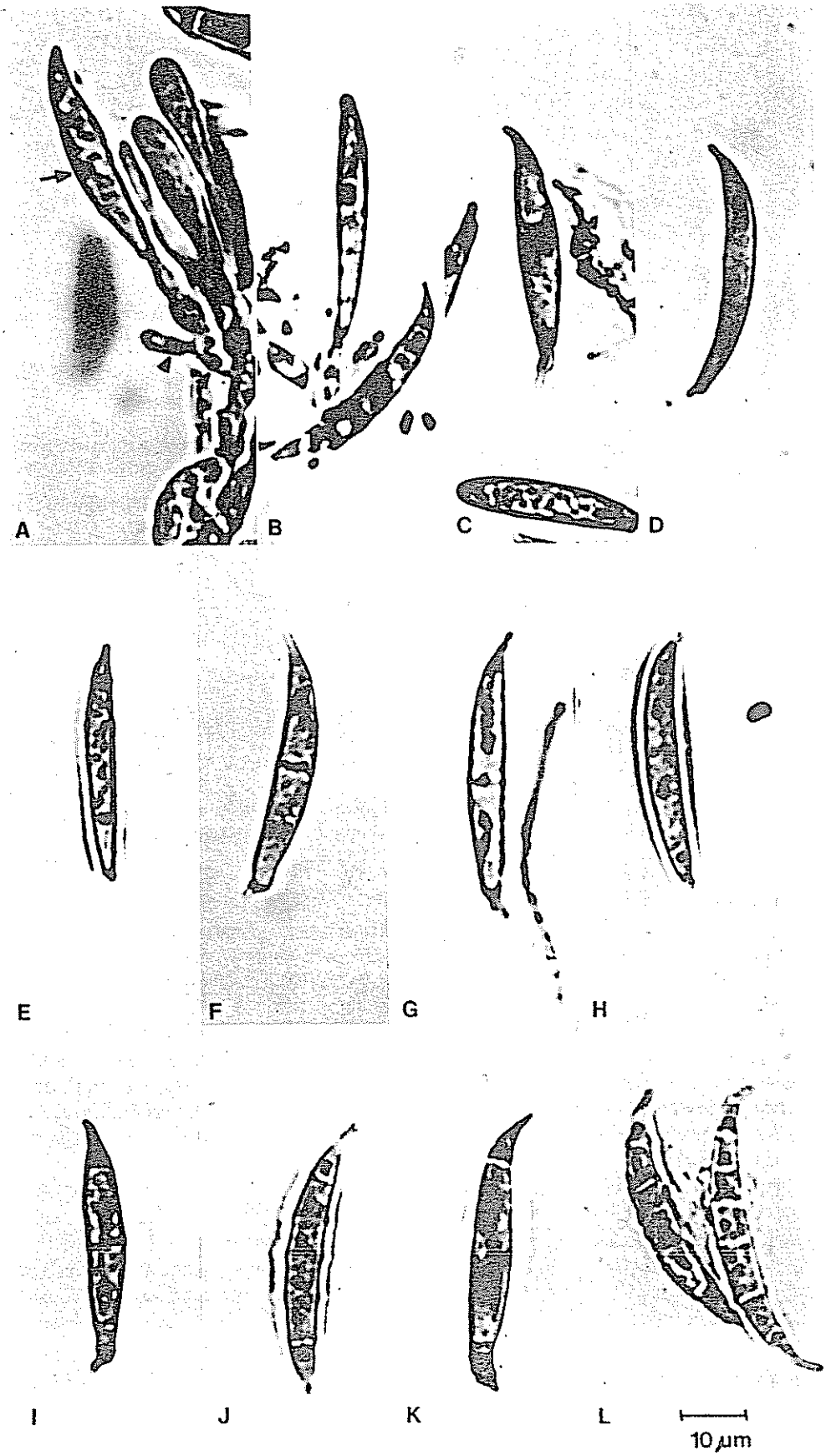

Figure 2 Light microscopy of macroconidia of *F. crookwellense* (Bar = 10 µm for all figures). A. Anucleate undifferentiated macroconidia. Differentiated macroconidium with a single nucleus (arrow). Nuclei in conidiogenous cells (arrow heads). B. Differentiated macroconidium with a single nucleus. C. Single nucleus beginning to divide in aseptate macroconidium. D. Completed nuclear division prior to deposition of primary septum. E. First nuclear division complete and the resulting two daughter nuclei rounded off. Note absence of primary septum. F. Primary septum complete. Beginning of the first synchronous division. G. Late stage of synchronous nuclear division. H. Macroconidium with four nuclei. I. Macroconidium with four nuclei. The single nucleate basal and apical cells delimited by the production of secondary septa. Nuclei in cells next to the primary septum beginning to divide. J. Nuclear division and septation complete, resulting in a 5-septate, 6-celled conidium with each cell containing a single nucleus. K. Apical and basal cells delimited by septum formation preceeding the first nuclear division. L. Cell with two nuclei in a 6-septate conidium, situated next to the first (primary) septum.

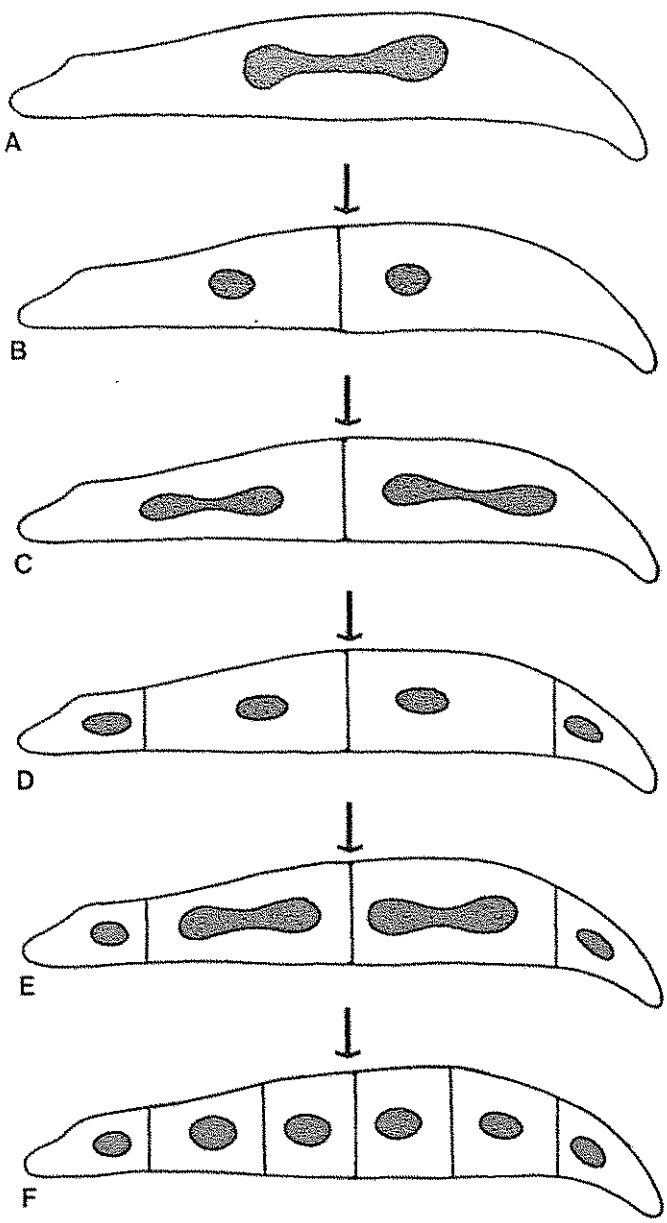

Figure 3 Schematic representation of nuclear division and septation most commonly encountered in *F. crookwellense*. A. First nuclear division. B. Formation of the primary septum. C. The first synchronous nuclear division. D. Formation of secondary septa delimiting the apical and basal cells. E. The second synchronous nuclear division. F. Formation of tertiary septa, resulting in a 5-septate, 6-celled conidium.

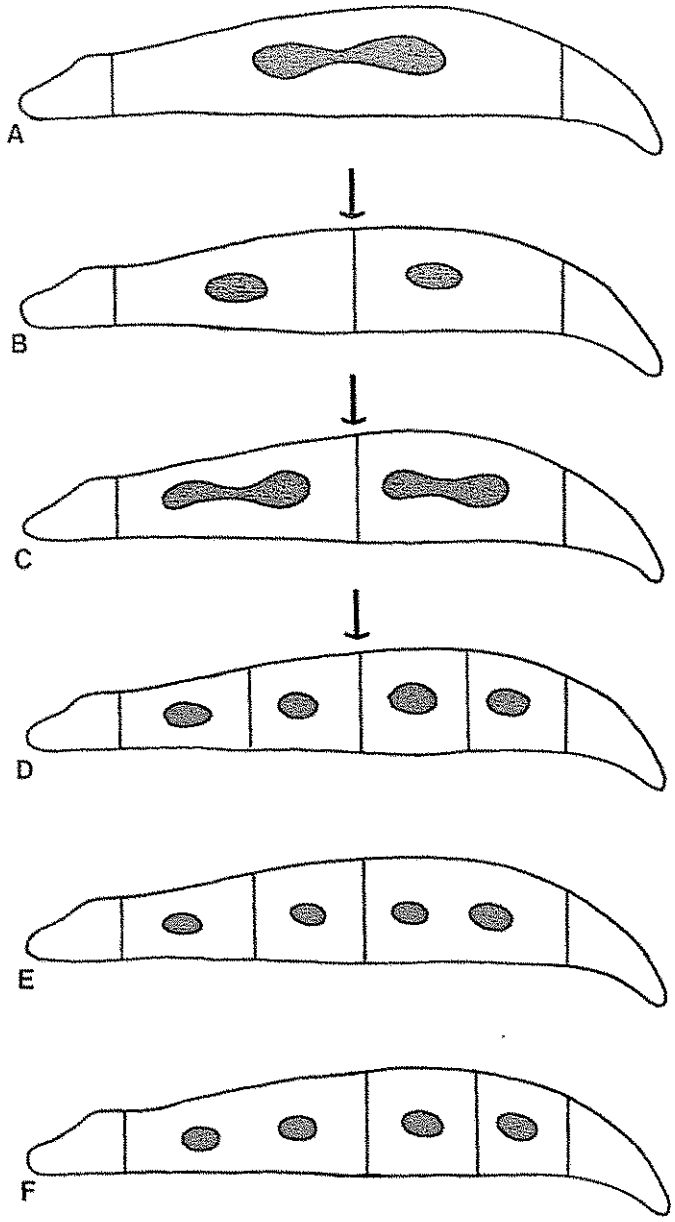

Figure 4 Schematic representation of the alternative forms of nuclear division and septation in macroconidia of *F. crookwellense*. A. Anucleate basal and apical cells resulting from septum formation preceeding the first nuclear division. B. Septum formation at the middle of the macroconidium following nuclear division. C & D. The first synchronous nuclear division complete with the final set of septa laid down. E & F. Cells with two nuclei resulting from failure of final septa to develop.

(Figure 4). The reasons for the premature deposition of septa that delimit the basal and apical cells prior to the first nuclear division (Figure 4A) are unknown. However, anucleate basal and apical cells have been reported previously in *F. culmorum* (Garcia Acha *et al.* 1966) and possibly occur in other *Fusarium* spp.

In some cases cells in macroconidia contained two nuclei. Here macroconidia had either four or six septa and the binucleate cells were situated next to the central septum (Figure 4E & F). Macroconidial cells containing two nuclei have also been reported in *F. culmorum* (Garcia Acha *et al.* 1966). This situation may arise when one of the tertiary septa fails to be produced.

In macroconidia of *Fusarium crookwellense* nuclear division precedes the formation of septa. Following the first mitotic division of a single nucleus which originally entered the developing conidium, a septum is laid down at the middle of the macroconidia. Further septa appear to be laid down from the outside inwards after further mitotic divisions of the nuclei. Only nuclei situated alongside the primary septum appear to retain the ability to undergo subsequent division.

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