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Conidial Nucleation in Stemphylium botryosum

by

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Abstract

Conidial nucleation in an isolate of *Stemphylium botryosum* obtained from lucerne was investigated using the HCl-Giemsa technique. Conidiophores arose as anucleate buds into which somatic nuclei migrated. After attaining varying stages of complexity the terminal cell, which was strictly monokaryotic, developed into a conidium. Division of the young conidial nucleus and subsequent cell wall formation resulted in a multicellular conidium. Conidial cells contained either one, two or four nuclei. As all the conidial nuclei are derived from the single nucleus of the terminal conidiophore cell, the whole conidium is homokaryotic. There is thus no mechanism for the perpetuation of heterokaryons through the conidia.

Introduction

Stemphylium botryosum is a common fungus of worldwide distribution which is found on a wide variety of host plants. This study was undertaken to investigate the transmission of genotypes through the conidia.

Two closely similar genera, *Helminthosporium* spp. and *Alternaria tenuis* (Hughes, 1953) were investigated by different workers. Hrushovetz (1956) showed that, in *Helminthosporium sativum*, heterokaryosis could be perpetuated through the conidia, as several possibly dissimilar nuclei entered the developing conidium and continued to divide within it. Knox-Davies & Dickson (1960) showed that the same mechanism operated in *H. turcicum*. Although the same mechanism for the perpetuation of heterokaryons did exist in *Alternaria tenuis* (Hartmann, 1966), it was, however, also possible for only one nucleus to migrate into the young conidium. All the nuclei within a conidium would, therefore, be identical and the conidium would be homokaryotic.

Van Warmelo (1970) showed that nuclear migration between hyphae of *Stemphylium botryosum* could occur. There is thus a mechanism for the production and cytoplasmic maintenance of heterokaryons.

Materials and Methods

The strain of *Stemphylium botryosum* used in this study was isolated from lucerne and is the same isolate as was used in a previous investigation on somatic divisions (van Warmelo, 1970). Cultivation of the fungus and staining of the nuclei was done in the manner described before. The use of agar pieces cut from a culture instead of air-dried mycelium (Ward & Ciurysek, 1962) or Cellophane

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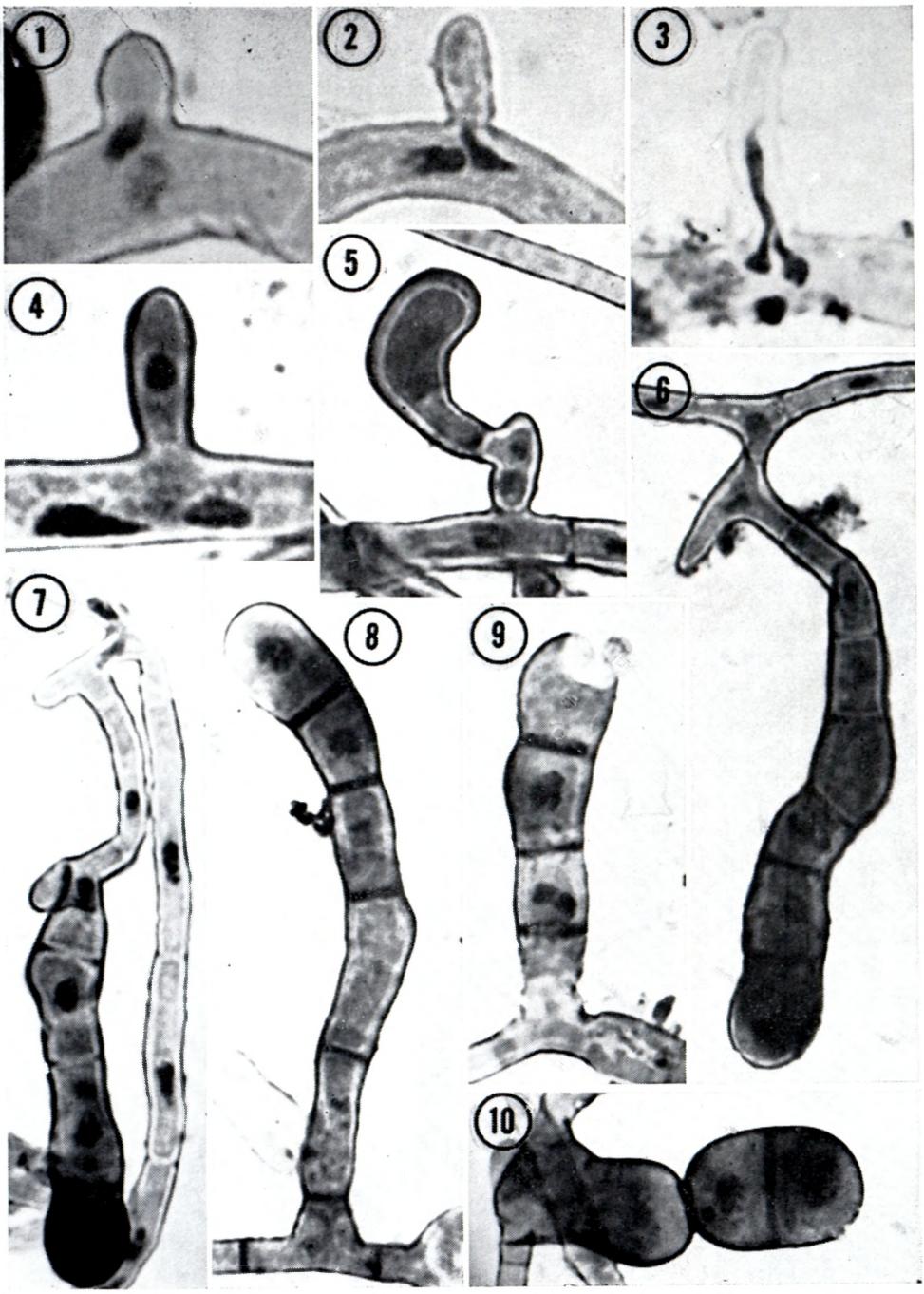
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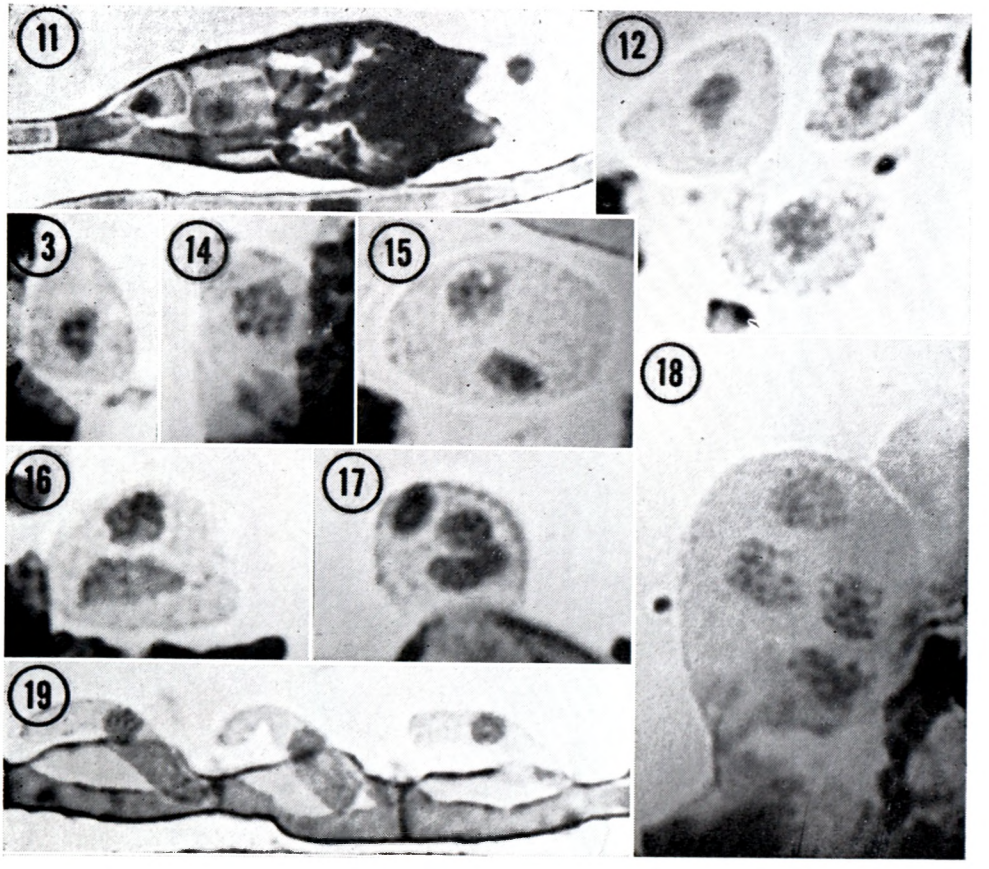

PLATES 1 and 2.

Fig. 1. Anucleate bud arising from dikaryotic hyphal cell (3000x).
Fig. 2. Single nucleus migrating into a bud (3000x).
Fig. 3. Two nuclei migrating into a bud (3000x).
Fig. 4. Bud with central nucleus (3000x).
Fig. 5. Pigmented secondary branch arising from primary branch (2000x).
Fig. 6. Multicellular pigmented secondary conidiophore with terminal conidial primordium (2000x).
Fig. 7. Multicellular pigmented tertiary conidiophore (2000x).
Fig. 8. Primary branch developing into a pigmented conidiophore (2000x).
Fig. 9. Conidiophore with terminal nucleus in prophase (2000x).
Fig. 10. Bicellular developing conidium (2000x).
Fig. 11. Young multicellular conidium (2000x).
Fig. 12. Uninucleate conidial protoplasts (2000x).
Fig. 13. Conidial protoplast with nucleus in early prophase (2000x).
Fig. 14. Late prophase or metaphase nucleus (2000x).
Fig. 15. Dikaryotic conidial protoplast (2000x).
Fig. 16. Dikaryotic protoplast with nuclei in prophase (2000x).
Fig. 17. Quadrinucleate conidial protoplast (2000x).
Fig. 18. Conidial protoplast with four nuclei in interphase (2000x).
Fig. 19. Squashed conidiophore showing protoplasts with stained nuclei (2000x).

films (Roane, 1952) was found to be more convenient as sporulating areas could be identified on the surface of the agar and selectively removed for examination after staining.

In the following descriptions the nomenclature for the nuclear status of cells is according to Jinks & Simchen (1966).

Results

Branch formation

Hyphal branches originated from relatively unpigmented hyphae as anucleate lateral buds from individual cells which usually contained two nuclei (Fig. 1). With continued elongation of the bud, one or occasionally both nuclei migrated into the developing hypha (Fig. 2, 3, 4). A basal septum then formed to cut off the newly formed mono- or dikaryotic cell from its parental cell.

Subsequent development could give rise to either a new hypha or to a conidiophore.

Development of the conidiophore

Conidiophores were found to develop in a variety of ways. The primary branch described above could develop into a pigmented conidiophore of which the terminal cell became the conidium (Fig. 8).

The primary branch could, however, give rise to a secondary branch which could then develop into a pigmented conidiophore with an apical developing conidium (Fig. 5, 6).

Instead of developing into a conidiophore a secondary branch could form a tertiary branch which would become pigmented and develop into a conidiophore, as described above (Fig. 7).

At a certain stage, for which no definite stimulus was identified, the single nucleus in the terminal cell of the conidiophore, the young conidium, began to divide. It can be seen that cells of the conidiophore became dikaryotic, while the young conidium, with its nucleus in prophase (Fig. 9), remained monokaryotic. After division of the young conidial nucleus a septum was formed which separated the two daughter nuclei (Fig. 10). This process of nuclear division and septum formation was repeated until a multicellular monokaryotic homokaryotic conidium was formed (Fig. 11, 12). As development of the conidium progressed further nuclear divisions took place which were not followed by septum formation. (Fig. 13, 14). These divisions gave rise to dikaryotic cells (Fig. 15). A further division could follow to give rise to quadrinucleate cells (Fig. 16, 17, 18). No higher nuclear number per cell was seen during the investigation.

Not all the cells of a single conidium contained four nuclei at maturity and the conidium was thus a mono-, di-, multikaryotic homokaryon.

The heavily pigmented walls of the conidiophores and conidia made observation of the nuclei extremely difficult. It was found, however, that on squashing, the cell walls ruptured and released the intact protoplasts with the clearly visible stained nuclei (Fig. 16, 19).

Discussion

From the results it is evident that the mature conidium of *Stemphylium botryosum* contains several cells, each with a variable number of nuclei. All these nuclei are, however, genetically identical as they are all derived from the one nucleus present in the terminal cell of the conidiophore. The mechanism of nucleation resembles that of *Alternaria tenuis* (Hartmann, 1966) except that only one nucleus was present in the young conidium. Heterokaryosis cannot, therefore, be perpetuated through single conidia.

It is not known whether these differences in nucleation are of any taxonomic or phylogenetic importance.

The reason for the variable number of nuclei in the conidial cells must also remain unexplained. Multinucleate cells in both sexual and asexual spores have been reported in a number of different fungi (Carr & Olive, 1958; Hall, 1963; Knox-Davies, 1966; van Warmelo, 1966; Rogers, 1967).

Nuclear migration between hyphae of *Stemphylium* has already been demonstrated (van Warmelo, 1970). It is, therefore, probable that the mycelium is heterokaryotic. Each conidium, with the exception of the rare parasexual diploid or aneuploid nucleus, would contain haploid nuclei of one genotype. This would also explain the observed reduction in sporulation vigour if the genotype favouring sporulation differs from the genotype favouring growth in culture. Continued culture would thus be, in effect, an unconscious selection for the "culture genotype."

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