

## DEVELOPMENT OF CONIDIOMATA IN *GLOEOSPORIUM MUSARUM* CKE & MASSEE

J.A. JAYACHANDRA\* AND J. MUTHUMARY

Centre for Advanced Studies in Botany, University of Madras Guindy Campus, Madras-600 025.

(Accepted September, 1998)

The ontogeny of conidiomata in *Gloeosporium musarum* was studied in artificial culture. The conidia are produced in four different ways in culture.

**Key Words:** Conidiomata, Conidia, germination, *Gloeosporium musarum*, phialide.

Anthrachnose, of banana, caused by *Gloeosporium musarum* is more destructive in ripe fruit in storage. Infection occurs during flowering and formation of the young fruit (Chakravarthy, 1957). This disease was originally described by Cooke and Massee (1873) from Brisbane, Australia but now it is found in all parts of the world where bananas are grown.

Developmental morphology has been used as a taxonomic criterion in classification of fungi in recent years. Dodge (1923) made a detailed study of the formation of cavity in pycnidia, Maiello and Petersen (1979) suggested that pycnidial development could be initiated from hyphal ring. Later Punithalingam 1979; Mass et al., 1979; Muthumary and Vanaja 1986; Muthumary *et al.*, 1993; Roux and Van warmelo 1990 and Masilamani and Muthumary 1994, 1995 studied the developmental stages of conidiomata in Coelomycetes. In the present study, the development of conidiomata, germination of conidia and the cytology of the conidium are described.

### MATERIALS AND METHODS

The fungus was isolated from banana fruit cultivated at Martandam, Kanyakumari District, and the Herb MUBL No. 438. The culture was grown on potato dextrose agar (PDA), in petri-dishes at 25°C. The cytology of conidia was studied by using the HCL-Giemsa staining technique (Punithalingam, 1983).

To study the development of conidiomata and conidiogenesis, the agar blocks with the conidiomata were trimmed into 2 to 3 mm cubed pieces and fixed in 2% glutaraldehyde in 0.1M Phosphate buffer (PH

7.5) for 2hr at room temperature. Three washes in 0.1M Phosphate buffer were followed by post fixation in 1% osmium tetroxide in 0.1M phosphate buffer for 12 hr at 4° C. The specimens were washed again in buffer three times. Dehydration in acetone was followed by infiltration and embedding in araldite. Thin sections (0.5 µm) were cut from these blocks and stained with 0.5% aqueous toluidine blue to study the development of conidiomata and conidiogenesis under the light-microscope.

### RESULTS

#### *Germination studies*

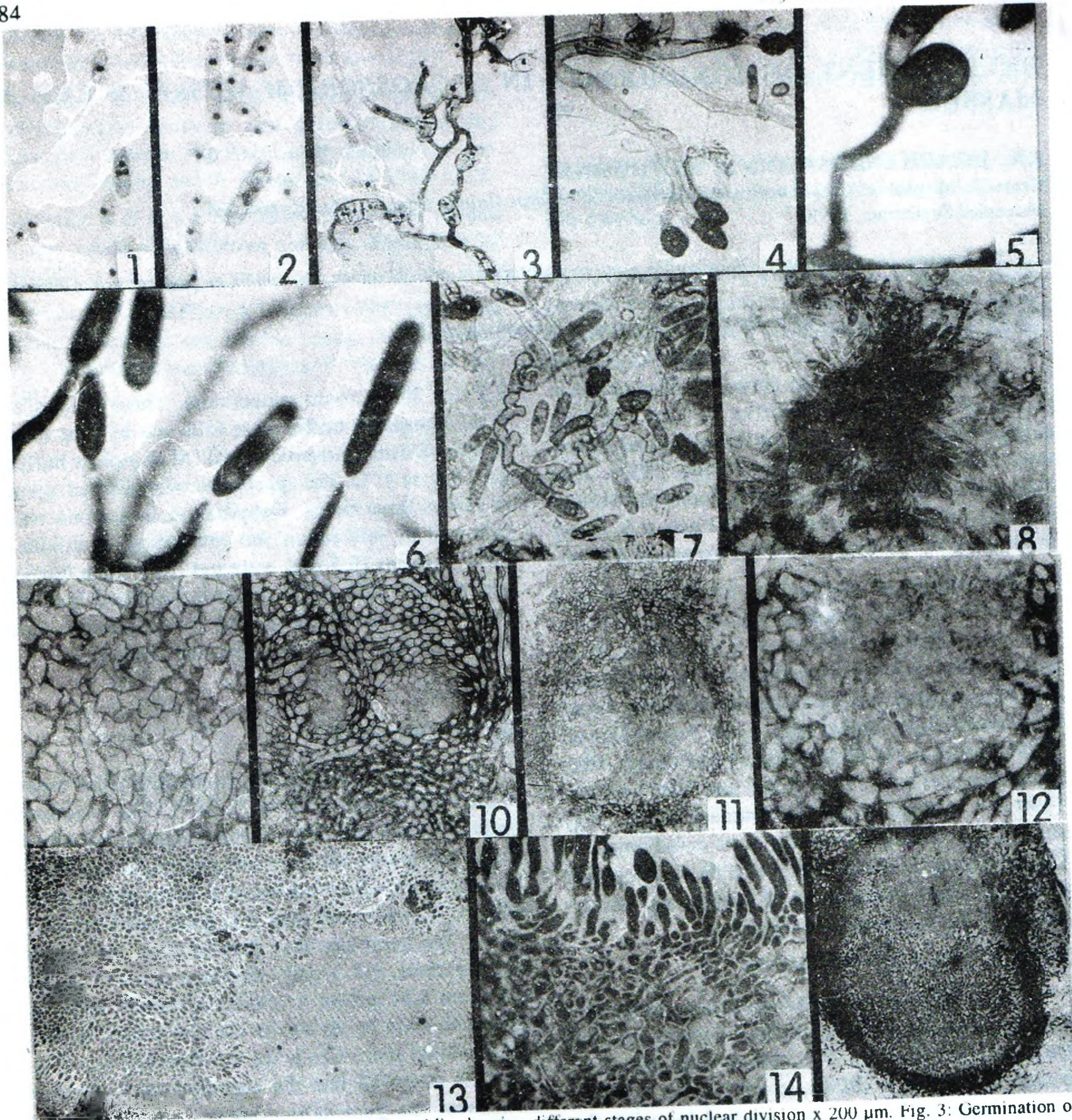
Conidia germinate readily in sterile distilled water or tap water within 4-8 hr at 22-25° C. The conidium initially swells and often become septate. Usually a germ tube grows out from one end of the conidium but septate conidia produce germ tubes at both ends (Fig. 3). Rarely more than two germ tubes are produced. The germ tube may develop from any side of the conidium, but usually develops at one end. As the germ tube grows it comes in contact with the glass and soon its tip swells out into a dark brown, thick-walled spore-like body, the appressorium (Fig. 4). It is observed that the appressorium is formed whenever the germ-tube comes in contact with a hard surface.

#### *Nuclear behaviour*

The HCL-Giemsa staining of the conidia show mostly uninucleate condition. During initiation of germination, the conidia swell and the nucleus starts dividing to form binucleate conidia and further di-

\*Present address: Scott Christian College, Department of Botany, Nagercoil-629 003.





Figures 1-15. Figs. 1-2: HCL-Giemsa stained conidia showing different stages of nuclear division x 200  $\mu$ m. Fig. 3: Germination of conidia. Note the development of septa in the conidia x 200  $\mu$ m. Fig. 4: Hypha end with appressorium x 300  $\mu$ m. Figs. 5-6: Fungus produces conidia on phialides and occasionally appressoria (Fig. 5 x 400  $\mu$ m) (Fig. 6 x 500  $\mu$ m). Fig. 7: Meristogenous development x 300  $\mu$ m. Fig. 8: Phialides produced on sclerotia x 250  $\mu$ m. Fig. 9: Section of young primordial initials x 350  $\mu$ m. Figs. 10-11: Sections of primordia showing dissolution of cells to form the cavity. (Fig. 10 x 300  $\mu$ m) (Fig. 11 x 150  $\mu$ m). Fig. 12: Section of young primordia showing the undifferentiated central cells x 350  $\mu$ m. Figs. 13-14: Sections of mature conidiomata showing stages of conidiogenesis (Fig. 13 x 200  $\mu$ m) (Fig. 14 x 500  $\mu$ m). Fig. 15: Section of conidiomata x 125  $\mu$ m.

vides to from tetrad conditions (Fig. 3). Some of the conidia showed multinucleate condition (Figs 1-2). The germinating conidia often showed septation and formed two celled condition (Fig. 2).

#### *Cultural characters*

On PDA, the fungus develops numerous, evenly distributed, orange pink, gelatinous conidial masses. The conidia are borne on phialides. In some places,



instead of phialides, dark brown appressoria are formed (Fig. 5). The conidia produced from phialides are uniform in shape and size, and are  $11-17 \times 5-6 \mu\text{m}$ . The phialides are  $20-25 \times 3-5 \mu\text{m}$  (Fig. 6).

#### *Development of the pycnidial conidiomata*

Soon after the germination, there is a tendency towards an increase in mycelium, accompanied by well pronounced sclerotia where the phialides are visible very clearly (Figs 7-8). Later, well developed sclerotia are formed. Sections of young globose primordia showed pseudoparenchymatous tissue arrangement (Fig. 9). Further during the development, the cells at the centre of the primordia showed signs of disintegration to form the cavity (Figs. 10-11). The primordium increases in size considerably and the cavity becomes enlarged (Fig. 12). The cells lining the cavity start producing conidia and during further development well developed pycnidia are observed in the cultures (Figs. 13-15).

In culture, the fungus produces conidia on phialides and occasionally appressoria also (Fig. 5-6). Then, well pronounced sclerotia are produced in the cultures and phialides are still visible on these sclerotia. Sections of these globose pseudoparenchymatous sclerotia show the presence of thin walled cells at the centre which start disintegrating to form a cavity (Figs. 10-11). As the primordium increases in size considerably, the cavity becomes enlarged, in a pycnidial conidiomata. During further development, the cells lining the cavity start producing conidia. (Figs. 13-15).

The conidia are produced in four different ways in culture. i) On phialides, which are solitary and producing a plurality of conidia. the phialides arising from any point on the surface or immersed hyphae; spore mass orangish pink, spores borne in a hyphomycetous fashion. ii) On regularly arranged palisade-like phialides arising at discrete points on the surface of the culture or aerial hyphae. iii) On scattered hemispherical or irregular stromatic or sclerotial structures of the surface mycelium; they are pseudoparenchymatous and phialides are arranged on the periphery of the sclerotia. iv) Typical pycnidia where the conidiomata in section shows well developed cavities inside which the conidia are produced from phialides; the phialides line the centre cavity of the pycnidia.

## DISCUSSION

The fungus *G. musarum* shows the presence of sclerotial mass, still with the phialides, indicates the tendency of the fungus to produce acervuli on the natural host, i.e. banana skin, it produces typical acervular fructification. However, in culture, the fungus produces well developed pycnidial conidiomata. Wardlaw (1961) when studying the fruit rot of banana, in Gros Michel variety reported the tendency of the fungi to produce sclerotial type of fructifications which were pycnidial conidiomata.

Kempton (1919) who reported the development as simple meristogenous in *Phoma herbarum* West, *Sphaeropsis citricola* Mc Alphine, compound meristogenous method in *Phoma cichorii* Passr, and the combination of both meristogenous and symphogenous methods in *Macrophomina citrulli* and *Gloeosporium refromaculans*. Appressoria were first described for *Fusicladium tremulae*. Frank and *Gloeosporium lindemuthianum* Sacc. & Magn. by Frank (1883 a, b). Later, Halsted (1893) found that *Colletotrichum* and *Gloeosporium* isolates from 24 different hosts produced appressoria. These specialized cells formed before the penetration of the host tissues, have since been demonstrated frequently in *Colletotrichum* and many other fungi.

Roux and Van Warmelo (1990) concluded that the development of conidiogenesis cells from the individual hyphae is important because in this process the pseudoparenchyma in the form of hyphae, produce the conidiogenous apparatus, which in turn produces the conidia and then disintegrates to enlarge the evolving locule. The mode of formation of the conidiogenous apparatus could also be used to determine whether one is dealing with a stromatic or a pycnidial conidioma.

We are thankful to Prof. A. Mahadevan, Director, Centre for Advanced Studies in Botany, University of Madras for providing the laboratory facilities.

## REFERENCES

- Chakravarty T 1957 Anthracose of banana (*Gloeosporium musarum* Cke & Masses) with special reference to latent infection in storage *Trans Br mycol Soc* **40**(3) 337-345.
- Cooke M C & G Massee 1897 *Grevillea* **XVI** 3.



- Dodge B O 1923 origin of the central and ostiolar cavities in pycnidia of certain fungal parasites of fruits *J agric Res* **23** 743.
- Frank A B 1883a Uber einige neue U Wehiger-bekannte pflanzen-krankheiten II Ber Deut *Boton Ges* **1** 29-34.
- Frank A B 1883b Uber einige nene U Weniger Bekannte pflanzen-kheiten II Ber Deut *Boton Ges* **1** 58-63.
- Halsted B D 1893 A study of fruit decay *J Agric Exp Sta* **13** 322-333.
- Kempton F H 1919 Origin and development of the pycnidium, *Bot Gaz* **68** 233-261.
- Maiello J M & J L Peterson 1976 Pycnidium ontogeny in *Phyllosticta antirrhini*, *Mycologia* **68** 1121-1125.
- Mass J L, F G Pollack & F A Clecker 1979 Morphology and development of *Pilidiella quercicola*, *Mycologia* **71** 91-102.
- Masilamani S & J Muthumary 1994 Development of conidiomata in *Ciliochorella mangiferae*, *Mycol Res* **98**(8) 857-861.
- Masilamani S & J Muthumary 1995 Pycnidium ontogeny in *Coleophoma cylindrospora*, *Mycol Res* **99**(6) 693-696.
- Muthumary J & R Vanaja 1986 Development of Conidiomata in *Coniella fragariae*, *Trans Br mycol Soc* **87**(1) 109-113.
- Muthumary J, JA Jayachandra & Preetha Bhagavathy 1993 Development of conidiomata in the *Phyllosticta* state of *Guignardia magiferae* Roy and observations on the fine structure of the conidium, *Mycotaxon* **XLVII** 147-155.
- Punithalingam E 1979 Graminicolous *Ascochyta* species Mycological pappers on. 142 Commonwealth Mycological Institute Kew.
- Punithalingam E 1983 The nuclei of *Macrophomina phaseolina* (Tassi) Goid, *Nova Hedwigia* **38** 339-367.
- Roux C & K T Van warmelo 1990 Conidiomata in *Bartaliia robillardoides*, *Mycol Res* **94** (1) 109-116.
- Wardlaw C W 1961 *Banana Diseases* Great Britain Hopetoun Edinburgh.