OBSERVATIONS ON THE CHLAMYDOSPORE GERMINATION OF ENTYLOMA VIGNAE, THE CAUSAL AGENT OF COWPEA SMUT[1]

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ABSTRACT - The chlamydospores of *Entyloma vignae* Batista, Bezerra, Ponte and Vasconcelos, collected from the infected leaves of cowpea (*Vigna unguiculata* (L.) Walp.) were germinated and produced nonseptate promycelia of variable length. The promycelium was characterized by branching at the distal end bearing clusters of 10 to 20 primary sporidia. The adjacent compatible primary sporidia exhibited fusion in pairs forming typical H - shaped structures. The secondary infective mycelium inducing leaf lesions had originated from the fused primary sporidia. The mycelium in the culture produced clusters of chlamydospores which were nonpathogenic.

Index terms: *Vigna unguiculata*, spore germination.

OBSERVAÇÕES SOBRE GERMINAÇÃO DE CLAMIDÓSPOROS DE ENTYLOMA VIGNAE, O AGENTE CAUSADOR DE CARVÃO EM FEIJÃO-CAUPI

RESUMO - Obteve-se a germinação de clamidósporos de *Entyloma vignae* Batista, Bezerra, Ponte e Vasconcelos coletadas das folhas de feijão-caupi (*Vigna unguiculata* (L.) Walp.) infectadas. Os clamidósporos germinaram, produzindo promicélio não septado, com comprimento variável. O promicélio apresentou ramificações terminais com grupos de esporídios primários variando de 10 a 20. Os esporídios primários, adjacentes e compatíveis, exibiam fusão entre pares, formando estruturas típicas de letra "H". O micélio secundário, induzindo lesões nas folhas originou-se de esporídios primários, após a fusão. O micélio em cultura produziu clamidósporos não patogênicos.

Termos para indexação: feijão-de-corda, *Vigna unguiculata*, germinação de esporos.

INTRODUCTION

The causal agent of the cowpea smut fungus was first identified as *Entyloma vignae* Batista et al. 1966. The biology of the fungus has not yet been studied in detail. While repeated inoculation experiments with the fungus grown in culture medium had not been successful, uniform infection was obtained when the diseased leaf material was incorporated in the soil (Prabhu et al. 1975). This necessitated the study on the factors affecting spore germination and infection *in vivo* and *in vitro*. The present paper reports the first part of this study involving description of different phases in the life cycle of *Entyloma vignae* from the chlamydospore germination to the formation of infective mycelium.

MATERIAL AND METHODS

The leaf bits showing typical lesions of smut were surface sterilized for two minutes in 0.1 per cent mercuric chloride solution and washed twice in sterile water. The surface sterilized leaf bits were macerated and a thick suspension of chlamydospores was prepared in sterile water under asceptic conditions. The extraneous leaf material was removed by filtration using muslin cloth. The suspension was diluted to approximately 10 to 20 spores per field under low power of the microscope (x 100). One drop of chlamydospore suspension was placed on the glass slide and allowed to dry. The glass slide was later incubated in saturated atmosphere in a sterilized petriplate humid chamber.

RESULTS AND DISCUSSION

The chlamydospores were germinated under room temperature 23 ± 3°C and within 72 hours produced nonseptate promycelia of variable length (Fig. 1 A, B). Few chlamydospores, however, produced long septate mycelium. The distal end of the promycelium was characteristically branched and seldom in a dichotomous fashion (Fig. 1 C-E). The sickle-shaped primary sporidia were produced at the distal end of the promycelium in clusters of

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FIG. 1. Photomicrographs showing chlamydospore germination of *Entyloma vignae*.

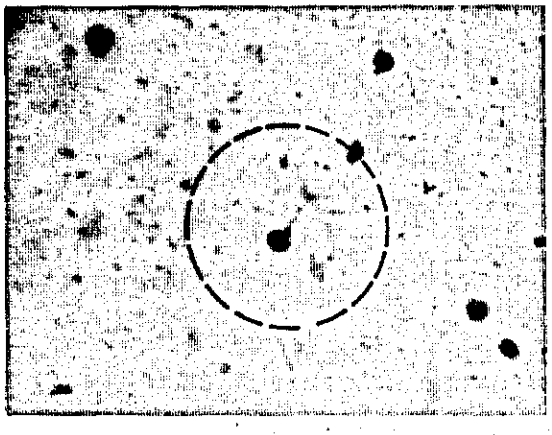

FIG. A. A germinating chlamydospore with promycelium and attached primary sporidia (x 25).

FIG. B. A magnified view of the thick walled chlamydospore and a part of the promycelium (x 400).

FIG. C. Distal end of promycelium showing branching and terminal primary sporidia (x 400).

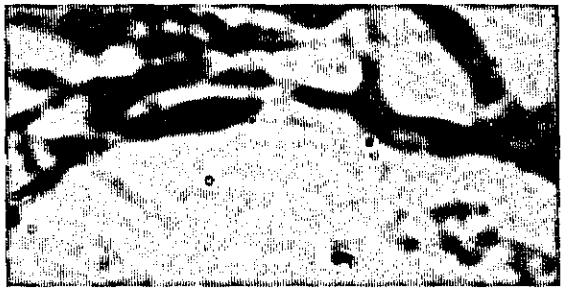

FIG. D. Distal end of promycelium showing branching and primary sporidia (x 400).

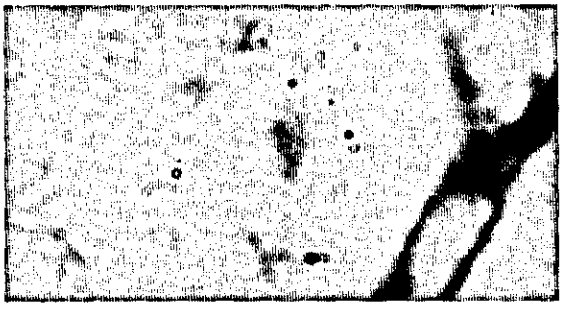

FIG. E. Distal end of promycelium showing branching and terminal primary sporidia (x 400).

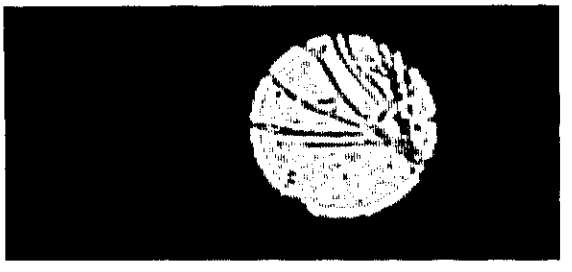

FIG. F. Fusion of primary sporidia at the distal end of the promycelium (x 160).

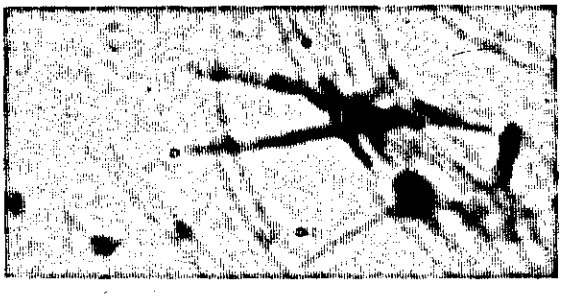

FIG. G. Typical H - shaped structures indicating the fusion in the detached sickle - shaped primary sporidia and initiation of secondary sporidia (x 400).

10 to 20 (Fig. 1 C-F). Typical H-shaped structures indicating the fusion of compatible primary sporidia in pairs were observed in the clusters of sporidia attached to the distal end of the promycelium as well as in the detached sporidia (Fig. 1 F-G). The secondary infective mycelium had originated from fused primary sporidia (Fig. 2).

Repeated isolations of the fungus from the infected host tissue were easily made and the fungus grew well on PDA medium. The mycelium in culture produced clustures of chlamydospores which were mostly intercalary and nonpathogenic as was evident from the inoculation tests. Culturing appeared to have brought major change in nuclear condition. It is probable that the binucleate condition of the parasitic mycelium in host tissue had changed to mononucleate nonparasitic mycelium in culture forming asexual haploid chlamydospores. The formation of sexual, diploid chlamydospores which upon germination capable of producing primary sporidia and infective mycelium had not been obtained in culture.

On the surface of the leaf lesions a long promycelium resembling white mold emerging from the stomata had been observed. The primary sporidia were often found associated with the host lesions. The successful infection of the inoculated plants under humid conditions with the chlamydospores collected from the host lesions and the rapid spread of the disease in the field indicated the ease with which the secondary parasitic mycelium is formed under natural conditions.

Further studies, however, are needed to induce the formation of sexual diploid chlamydospores and infective mycelium or secondary sporidia in culture.

The "smut" of cowpea in Brazil is called as "false leaf smut" in Nigeria (Williams & Allen 1976). The causal organism has been identified as

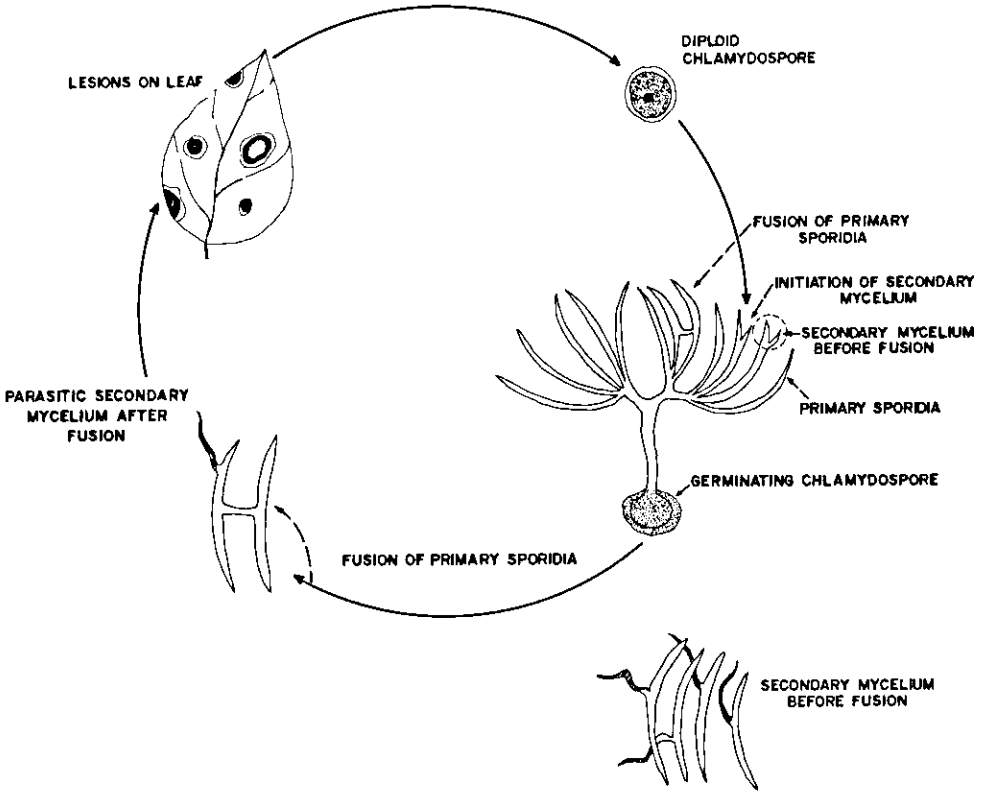

FIG. 2. Life cycle of *Entyloma vignae* (Diagramatic).

Protomycopsis phaseoli Ramakrishnan and Subraman belonging to the class Hemiascomycetes. The identification may have been erroneously made considering the presence of chlamydospores of smut within the host tissue as resting sporangia of *P. phaseoli*. The results herein reported conclusively proved that the fungus in all its respects is a smut belonging to Basidiomycetes confirming the original description (Batista et al. 1966).

CONCLUSIONS

The chlamydospores of *Entyloma vignae* could be germinated in saturated atmosphere under room temperature condition. Promycelia were long and often branched at the distal end bearing clusture of primary sporidia. The existence of secondary sporidia, if any, and their further role in the perpetuation of the fungus has to be determined.

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