Spore Germination

THE FUNGAL SPORES AND THEIR GERMINATION:

The fungal spore is a part of the fungus highly specialized for reproduction, survival and dispersal and with a minimal metabolic turnover, low water content and lack of cytoplasmic movements with thick walls and heavy pigmentation (Gregory, 1966). All the fungal spores are considered to show some degree of dormancy because they are not involved in active synthesis or have much reduced metabolic activity.

Basically, during spore germination of smut fungi or also in others, there are two types of spore dormancies: Constitutional and exogenous. To overcome or break the dormancies, it is necessary to fulfil some requirments to germinate the spores (stimulation or to activate the spores, to provide suitable environmental conditions, to break the nutritional block or metabolic blocks or to remove self inhibitors etc.)

When all dormancy barriers have been removed spores normally require a favourable moisture level and temperature and substances to begin the germination process. Aerobic respiration appears to be normal requirement for most spores to initiate germination while some requires CO_2 and external source of organic substances or sometimes need only

water.

Germination basically comprises of many processes and changes that occur during the resumption of development of growth of the resting structure and its subsequent transformation to a morphological different structure.

In higher fungi, generally filamentous, this involves the change from a non-polar spore to a polar germ-tube which will continuously grow by extension at the tip.

Spores can be considered as the beginning and the end of the developmental cycle of a fungus.

Generally, smut spores (teleutospores) remain dormant for a short to long time and this period of dormancy varies from species to species and correlated to its viability depending on many factors, mainly the environmental, and internally related to a spore endoplasmic reticulum which rapidly increases during germination. Mitochondrial number is also claimed to be increased alongwith the size of spore In Rusts and Smut, the teleutospores which do not often germinate but undergo a period of dormancy. The breaking of dormancy for spore germination, generally, has been achieved by chilling or by alternately wetting and drying the spores. In common practice , generally, treating the spores with other chemicals has also proved effective (Cochrana).

Germination of spore (resting) may be stimulated by root excretions or the specific or appoapriate host, but in some cases there is no such host specificity e.g. Noble, R.J. (1924) has shown that spore germination of *Urocystis tritici (U. agropyri)* stimulated by the roots of a number of plants which are not hosts for this smut.

Environmental conditions (factors) act differently on different fungi and thus, operate in selection of species available for community evolution. Germination is stimulated by extreme environment which is more harmful to promycelium/germtube and mycelium. Spores often fail to germinate in the indentical conditions under which they are produced because each phase in life cycle of an organism is enfluenced by different conditions of the environment. Self inhibition of spore germination has more frequently been observed in dense population of spores in two different ways:

(1) It reduces germination frequency (%) and

(2) Rate of germination of spores.

More commonly observed in rust spores (Uredinospores-repeating spores), the factor i.e., spore germination inhibition, is one of the ecological function to extend the availibility of spores for a long duration to extend the value of survival of the taxa.

Many workers in smut systematics have realized the importance of spore germination and Ruben Duran (1971) commented that the collection of smut species has proved much difficult than to germinate.

Study of spore germination is very important because:

1. Spore germination a direct proof of the generic indenti ty of the smut genera to their respective families.
2. Spore germination studies provides the additional features of the taxa, its physiological status, nuclear cycle, nature of spore dormancy, role of environmental factors as well as internal conditions, maturity in addition to its taxonomical significance.
3. It also provides the genetical nature of the taxa whether the basidiospores-sporidia show their fusion *in situ* or not i.e. compatible or incompatible nature. Because natural hybridization is unquestionably a source of varition within the same species, as well as between species.
4. Fungal spores generally considered as a dormant stage in the life of the fungus and not only contain enzymes necessary for their metaboloic activities but also possessed machinery for transformation of substrates during metabolism, which do not contradict the concept

of dormancy as any rest period or reversible interruption of the phenotypic development of organism (Sussman,1966).

The capcity of the fungi to produce spores (several types) is a hereditary attribute, and its phenotypic expression qualitatively (Hauker, 1966 a).

GERMINATION

The germination and production of sporidia is the main criterion of the classification of the smut fungi into their respective families (Tulasne and Tulasne, 1847). On the basis of that, the smut fungi have been divided into two families viz. Ustilaginaceae and Tilletiaceae. All the members of the family Ustilaginaceae produced septate promycelium which develops lateral and terminal sporidia. On the other hand the genera of the family Tilletiaceae produced only terminal sporidia on aseptate promycelium. These sporidia may or may not conjugate *in situ* or after their separation from the promycelium. The secondary sporidia are produced from the primary sporidia which do not fuse. e.g. in *Tilletia* and *Neovossia*. In *Tilletia* and *Neovossia* the number of sporidia may increase. Cunningham (1924) in expressing his opposition to the two-family system of classification, stated that if the two methods of germination warrnat two families, then, logically a third family should be proposed to include the species which produce an infection mycelium

without ever producing a promycelium and sporidia (= basidiospore) (Fischer and Holton, 1957). Mundkur and Thirumalachar (1952) have separated *Tilletia* and *Neovossia* on the basis of formation of sporidia and their fusion.

The teliospores germination in case of *Entyloma mundkuri* Patil and Gandhe was studied by Gandhe (1994). Teliospores on germination give a cluster of 4-8 subcylindrical, uninucleate primary sporidia.

Teliospore germination in smuts is a difficult task but has great taxonomical significance not only at family level which was recognized by Tulasne brothers as early as 1847, but even at generic and species level. Because in systematics, smut fungi provided a limited number of characters based on their morphology which are indeed not sufficient to separate the species of an individual genus or even many genera having similar morphological characters e.g. *Tilletia* and *Neovossia*, *Melanotaenium* and *Entyloma*, *Cintractia* and *Anthracoidea*, *Thecophora*, *Glomosporium* and *Tubarcinia*, *Ustilago* and *Sphacelotheca*, *Urocystis* and *Ustacystis*, *Entyloma* and *Ustientyloma* etc.

Eventhough, it is not definite and also not known whether each species of a genus will show a same mode of germination and such variations are attributed to various factors as commented by R. Duran (1973) and he further emphasized

that difficulties in demonstrating spore germination in some species is no way reduce the need to do so. Moreover, in these species of smuts which did not know the smut fully but is not impossible to germinate in future and that does not reduce the value of germination of the spores.

Thus, there are numerous examples which clearly showed the importance of demonstrating spore germination not only from the taxonomical point of view but also others e.g. homothalic and heterothalic nature; formation of secondary, tertiary sporidia, nuclear (meiosis) division, number of nuclei and septa in promycelium, monopolar, bipolar or multipolar germination, disease cycle of the pathogen, behaviour of sporidia during germination, mode of infection, nature of spore dormancy, rate of growth of promycelium, effects of various environmental factors and internal characters, value of survival, viability of spores, nature of inhibitors of the germination, effects of different stimulants, formation of number of promycelium/sporidia, position of sporidia on promycelium, morphology of promycelium (short, long, thick, stought, single, branched, septate, nonseptate, determinate/indeterminate etc.), nutrition status (requirement) in culture media, both liquid and solid, non-sporidial production behaviour of promycelium, infective hyphae, their behaviour *in situ* to form fusion bridges (homothalic) or other genetical and cytological features can be studied if proper germination of the spore

is studied. This unique and fundamental feature helped a lot in smut systematics. And on these characteres many Ustilaginologists came to the conclusion that, there should be only one family recognized for all smuts and i.e. Ustilaginaceae (Vanky, 1985).

METHODS OF SPORE GERMINATION

Generally, teleutospores of smuts are rather resistant to germinate except a few species in which they germinate readily, otherwise different stimulents (physical and chemical) are used to stimulate germination. Therefore, different methods have been used by different workers, and by experience it is said that fresh collections show the germination within the six hour in sterile tap water without any treatment and without any period of dormancy (Patil 1956). Duran was able to germinate old spore on 2% PDA (Potato Dextrose Agar); soil extract agar (SEA), Corn meal agar (CMA); water agar (WA) by sowing spores in it. Thirumalachar (1940) used many different techniques; different chemicals may be used to break their dormancy e.g. dilute Hydrochloric acid (HCL), Hydrogen peroxide (H_2O_2), bleaching powder, potassium permanganate ($KMnO_4$) etc. which are used as stimulaters at lower concentrations.

MATERIALS AND METHODS

Here, an attempt has been made to germinate the spores of a few species collected. Spore suspension from unopened but matured sorus was prepeared in sterile distilled water after its surface sterilization. This suspension was then spread on a clean glass slides with sterile scalpel or clean blade. These slides were then allowed to dry and were immersed in 0.4% solution of $KMnO_4$ for 4 to 10 minutes. In addition to that sugar solution (Dextrose) of 5% concentration was also used for the germination.

The slides with plated spores were kept inverted (facing down) for germination on thick glass rods in petri dishes, which were kept moist with lining filter paper or cotton which affords an ideal condition for germination in the laboratary at room temperature (29°C). Periodically the slides were observed to ensure the germination by taking proper care to avoid contamination. Those slides on which spores showed germination sufficiently were made semipermanent by mounting in lactophenol and stained with cotton blue. These slides were sealed by paraffin wax, for further observations. The material which was used for spore germination studied as follows.

1. *Tilletia transvaalensis* Zundel

The infected but matured unburst ovaries of *Eragrostis*

sp. (Family - Poaceae) collected at S.U.C., Kolhapur (M.S.).

Date of collection: 19.10.92

Date of germination: 28.10.92

Methods used: Suspension made in distilled water and pre treatment of 0.4% $KMnO_4$ for 5-10 minutes.

Temperature : At room temperature,
day temperature 29-30°C
night temperature 25°C.

Whether germination of this species is known: not known.

The matured unopened sorus (infected) was surface sterilized and washed with distilled water and then sterilized needle (with flame) was pierced in the sorus to get dusty mass of spores (teliospores) along with the sterile cells, to avoid contamination and were plated as usual for germination and incubated in petri dishes (moistened) in the laboratory at 29°C in duplicate/triplicate under normal day light.

It was observed that the teliospores showed their germination from 4th day onwards but a few in number, but maximum number of spore germination was found on 8th day. The slides of germinated teliospores were very carefully dried, stained with cotton blue in lactophenol as mounting medium. So that

germinated spores, their promycelium/promycelia and sporidia distributed minimally and long coverslip was used and then sealed with sealant. Sealed slides were observed very carefully and critically with compound microscope and the following observations were made.

1. Most of the germinated spores (teleutospores) showed only one promycelium, i.e. monopolar germination.

2. Comparatively, frequency of teliospore germination was found to be low (as many spores remained ungerminated).

3. The promycelium being stout and of different length of diffrent spores were plated for germination of the same sorus on the same slide under same conditions.

4. The promycelium without any septum but with dense cytoplasm, slightly tapering towards the base, variable in length (25-380um long) and also breadth (3-14.5 um) with large oil globules, thin-walled, simple, hyaline and produce cluster of sporidia (=basidiospores) mostly from the dichotomously or irregularly divided, digited tips at the end as crown.

5. Sporidia: Mostly 4-16 per promycelium, each with a slender, stout tapering, simple basal part with upper body branched or Y-shaped or forked, one (main) arm mostly staight, pointed tips and hyaline, sporadia did not show further germination, or fusion to form 'H'-

shaped bodies, formation of secondary sporadia. Basidiospores mostly deciduous.

Discussion

In many species of the genus *Tilletia* such germination patterns have been reported. Branched promycelium or dicotomously branched tips of promycelium or development of lateral branch to function as promycelium are the additional evidences indicate that the species may be heterotrophic in nature and no *in situ* fusion of sporadia, also supports its heterothallic nature.

2. ***Sporisorium holci-sorghi*** (R. Volta) Vanky

In the infected ovaries of *Sorghum* species (Family Poaceae). Material was collected from Ambap (Dist. Kolhapur).

Date of collection: 19.8.92

Date of germination : 26.8.92

Any pretreatment : Nil

Whether genmination known of this species: Yes

The teliospores of *Sporisorium holci-sorghi* (R. Volta) Vanky of *Sorghum* sp. also showed germination within 4-8 days

in distilled water on glass slide. The spores showed germination with septate promycelium which produced terminal and lateral sporidia, singly on sterigmata. The length of promycelium varied from 50-230 um. Sometimes more than one promycelium also produced from a single spore. The promycelium produced 1-4 sporidia. Sometimes thread like infection hyphae also developed insted of sporidia but it requires confirmation. Sporidia one-celled, simple, ellipsoid, thin-walled, hyaline and 3-3.5(-4)um long.

All species of smuts of *Sorghum* have been cultured and studied (vide T.S. Ramakrishnan, 1963). The spores of this species showed a low frequency of spore germination in water at a time and produced a stout 4-celled promycelium bearing sporidia typically like *Ustilago* type. Rate of germination varies as per medium. In nutrient solution, sporidia formed profusely while on the solid agar medium, forming colonies with copious sporidial formation at 28°-30°C.

The present spore germination in this species of smut also resembles to the observations made previously.

EXPLANATION OF TEXT FIGURES: 36-42

Germination of teliospores of *Tilletia transvaalensis* Zun.

(36) Teliospores with shortest promycelium

(37) Teliospores with shortest promycelium showing digited dicotomy

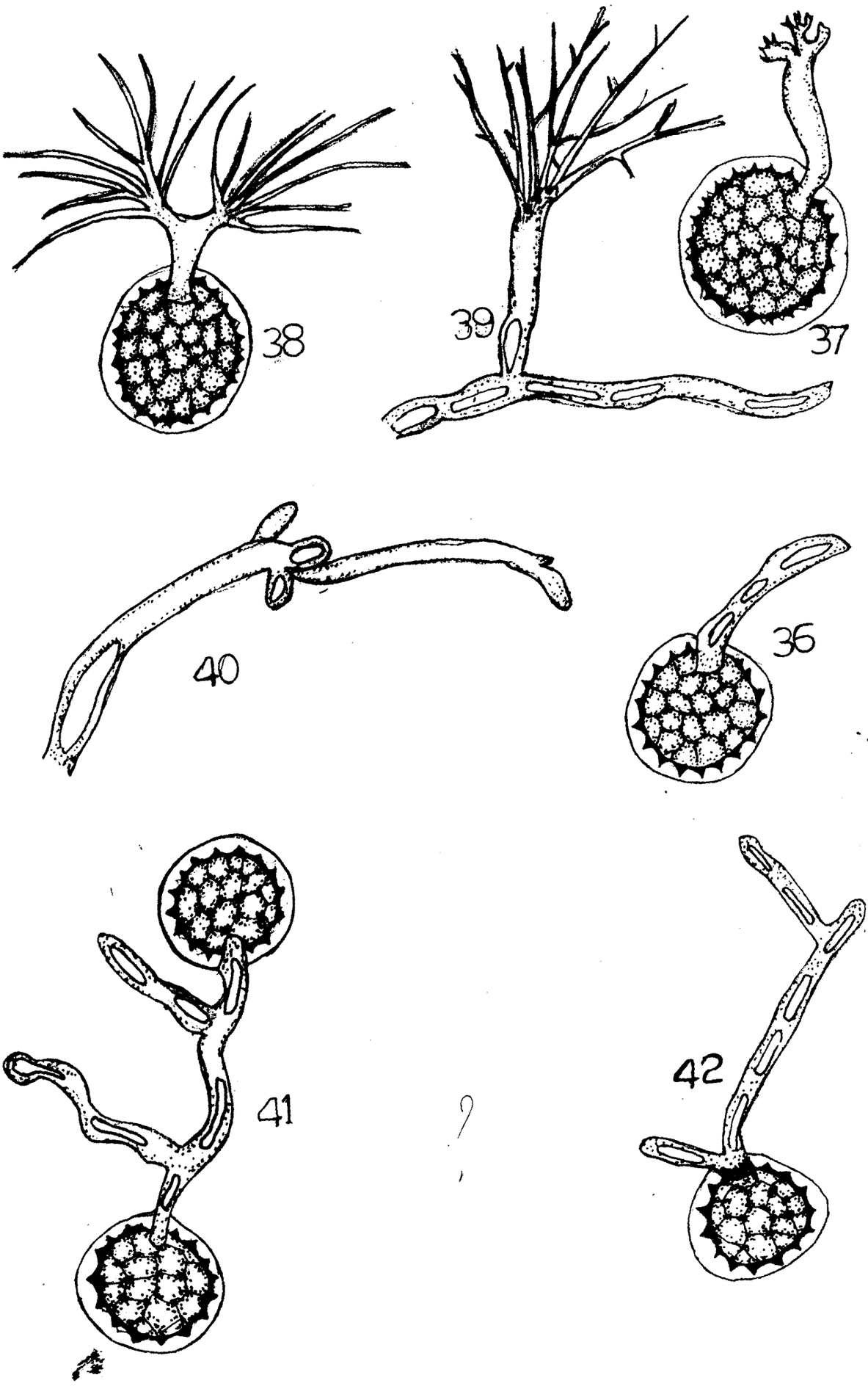
(38) Teliospores showing dicotomously branched promycelium with terminal sporidia

(39) Promycelium with Y-shaed sporidia

(40) Promycelium with terminal extention

(41) Two promycelium of two teliospores fuse to from secondary mycelium

(42) Teliospore shows aseptate promycelium with vacuole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EXPLANATION OF TEXT FIGURES: 48-55

Germination of spores of *Sporsorium holci-sorghi* (Rerolta) Vanky

(48) Teliospore which have short septate promycelium

(49) Teliospore with septate & branched promycelium

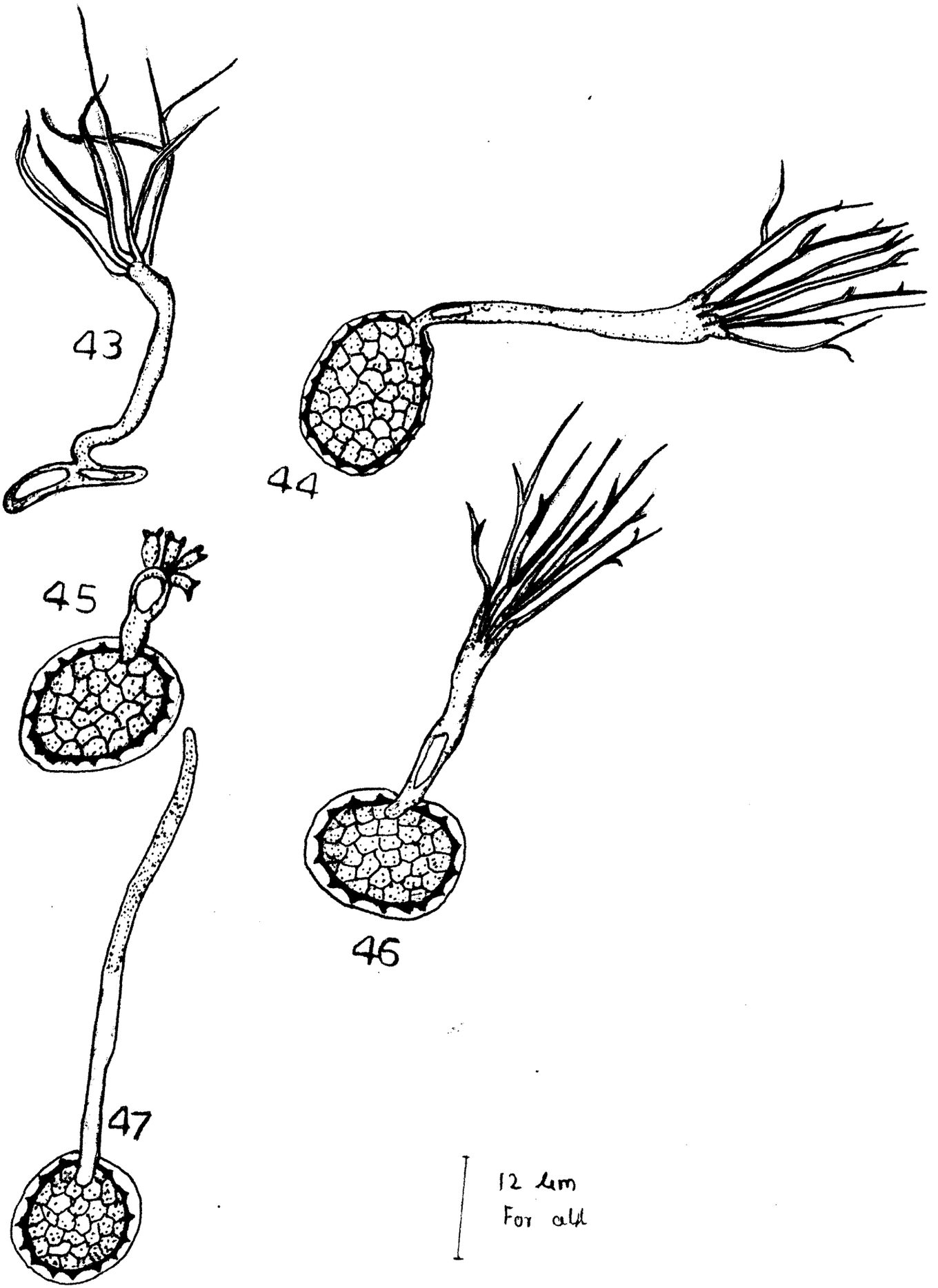
(50) Promycelium with lateral and terminal sporidia

(51) Teliospores having long promycelium

(52) Teliospore with more than one promycelium

(53) Septate promycelium have terminal dicotomy with minute sporidia

(54-55) Branched promycelium with infectious hyphae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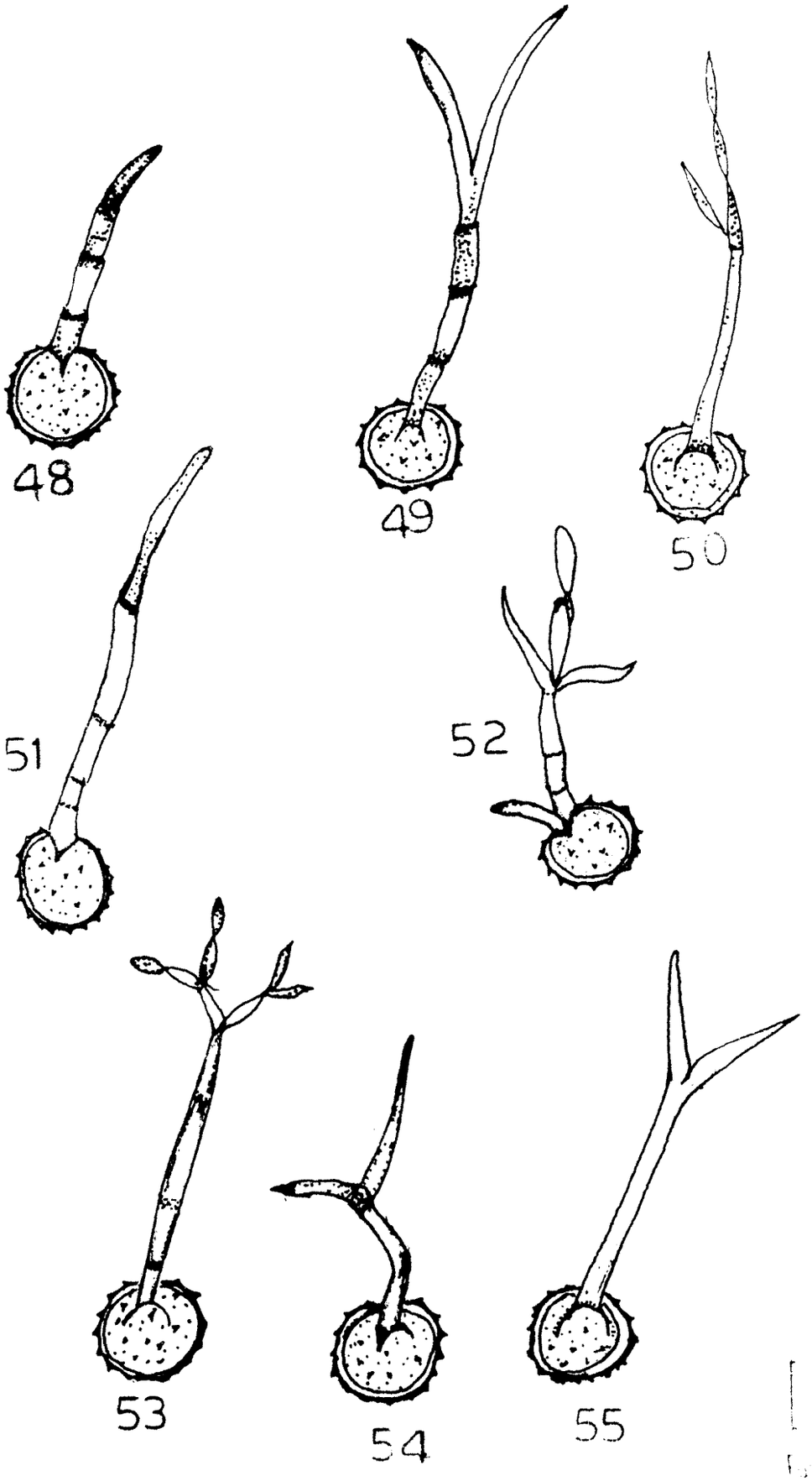
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EXPLANATION OF TEXT FIGURES: 43-47

Germination of Teliospores of *T. transvaalensis* Zon.

(43) Promycelium with terminal sporidia

(44) Teliospore with promycelium which is narrow at base & broader at apex with terminal sporidia

(45) Teliospores with shortest promycelium with terminal digits

(46) Teliospore having promycelium with terminal sporidia

(47) Teliospores with long aseptate promycelium.