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GERMINATION OF RESTING SPORES IN SYNCHYTRIUM SPECIES PARASITIC ON CUCURBITACEAE

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Abstract

Germination of resting spores is described in 2 *Synchytrium* species viz. *Synchytrium lagenariae* Mhatre and Mundkur and *S. trichosanthidis* Mhatre and Mundkur, parasitic on cucurbitaceous hosts. The resting spores of both species behave as prosorus in germination giving rise to an attached superficial sorus of sporangia. Several anomalies observed in germination are briefly described. Efficacy of methods inducing germination is discussed relative to the field conditions.

Introduction

Resting spores in the genus *Synchytrium* De Bary et Woronin are important structures in perpetuating the species and have been reported to be zygotic or nonzygotic in origin. Differences in their germination are important to elucidate placement of a species in its taxonomic position relative to the subgenus recognized by Karling (4, 7). Based on the resting spore germination and their other characters, *Synchytrium* species are classified as long-cycled and short-cycled. Germination of the resting spores has been observed only in a few species of the total of more than 200 species; the species in which the resting spores have been germinated number 42 (11, 12). Therefore, classification of all the remaining species stands tentative and subject to revision.

Synchytrium lagenariae Mhatre and Mundkur and *S. trichosanthidis* Mhatre and Mundkur (15) incite a destructive gall disease of various cucurbitaceous hosts in Varanasi and neighboring regions of eastern Uttar Pradesh. Both species are known to form sporangial and resting spore galls on all the cucurbitaceous host species (17). The sporangial galls develop from August onward, whereas the resting spore galls form toward the end of the crop season during October. The resting spores may even form earlier, if the environment does not favor multiplication of the prosoral stage. Details of resting spore germination in the 2 species are yet unknown making their taxonomic position uncertain. Karling (8) believed the 2 species to be long-cycled and tentatively placed them in the subgenus *Microsynchytrium* Karling. Present contribution relates to details of the process of resting spore germination in the 2 species.

Materials and methods

Infected host material comprising leaves, stems, inflorescence stalks and fruits bearing abundant resting spore galls was collected from different cucurbitaceous hosts [1]) toward the end of crop season from October onward, air-dried and stored in polyethylene bags at room temperature (20–35 °C). Resting spores scraped out of these materials were induced to germinate at periodical intervals following the methods described earlier (6, 7, 12), but went without success until 9 months of storage. Perceptible signs of germination were discerned later (after 9 months) and abundant germination upto 85 % was obtained in spores stored over 12 months. The methods followed for the resting spore germination are briefly described.

1. The resting spores were separated from the gall tissue by finely powdering the crisp-dry infected material in a pestle and mortar or by hand. A small quantity of the powdered material was uniformly sprinkled over a variety of substrates such as 2 % water agar, moist filter paper and cellophane sheet in petri plates etc. (7, 12). The substrates were

[1]) *Benincasa hispida* (Thumb.) Cogn., *Citrullus lanatus* Thumb. & Munsf., *Cucumis melo* L. c. *melo* var. *utilissimus* Duth. & Full., *C. melo* var. *momordica* Duth. & Full., *C. sativus* L., *Cucurbita moschata* Poir, *Coccinia cordifolia* W. & A., *Luffa cylindrica* Roem., *L. acutangula* Roxb., *Lagenaria siceraria* Mol. & Standl., *Momordica charantia* L. *Trichosanthes anguina* L. and *T. dioica* Roxb..

air-dried and flooded with distilled water to remove the host debris leaving the spores adhering to the substrate. Alternately, dry spores were also fixed onto microslides smeared with mucilage of *Tradescantia* sp. and air-dried at room temperature (24–26 °C). The resting spores on these substrates except water agar were inverted on slide racks lined with moist cotton toweling and incubated at 30–35 °C (18, 19). Frequent wetting of the substrate with charcoal-adsorbed water restrained the growth of saprophytic fungi and other microorganisms (12).

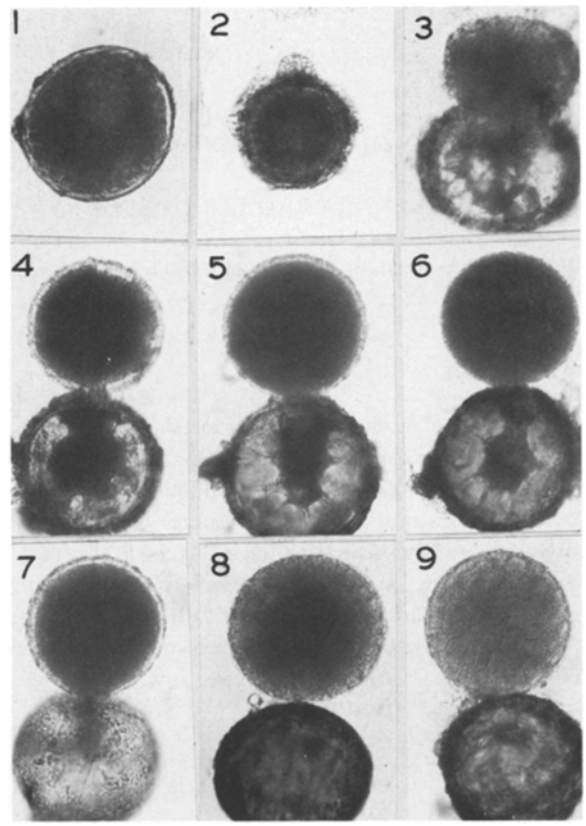
2. Dry infected material containing the resting spores was left out-of-doors and exposed to varying atmospheric conditions as described for *Synchytrium australe* Speg. (6). The spores were separated from the gall tissue and incubated under moisture for germination as above.

3. The dry infected material was soaked in tap water for 24–48 hrs until the tissues became soft. The softened material was separately treated with a) a dip in 0.5 % conc. HCl, H_2SO_4, NaOH or KOH for 15–20 min (acid or alkali treatment), or b) exposure to moist heat at 40–45 °C for 1–2 hrs (temperature treatment) or c) exposure of acid-treated material to moist heat at 40–45 °C for 1–2 hrs (acid – temperature treatment).

The treated material was washed under tap water for 30 min, crushed in a mortar and pestle and suspended in water. The host debris separated out after repeated filtration through 2–3-layered cheese cloth. The resting spores suspended in clean water (filtrate) were collected by sedimentation. They were finally washed several times with distilled water and sown on moist filter papers in petri plates or fixed on microslides by alternate wetting and drying method suggested by Thirumalachar et al. (18, 19) and incubated at 30–35 °C. Morphology of zoospores and flagella was studied in stained preparations as described by Couch (1).

Observations

Resting spore germination was observed in most of the cucurbitaceous host collections and the process and sequence of germination were found essentially similar in them. Details of the sequence are illustrated for *Synchytrium lagenariae* on *Luffa cylindrica* Roem. as a representative model. The resting spores from most of the collections started germination after 7–8 days at 30–35 °C and most of them (upto 85 %) germinated after 12–14 days. The spores drawn from 2-year old collections germinated better and earlier than those from fresh or one year old collections. Mature, dormant (resting) spores separated from the gall tissue were spherical to oval, dark reddish brown, measuring 90–177 μm in diam. The exosporium was smooth, 2.5–6.5 μm thick, often encrusted with the gall tissue imparting it a rough, uneven surface. The endosporium was hyaline and the cytoplasm coarse and densely granular. The primary nucleus was deeply stainable with a homogeneous nucleolus and a compact lunate body.

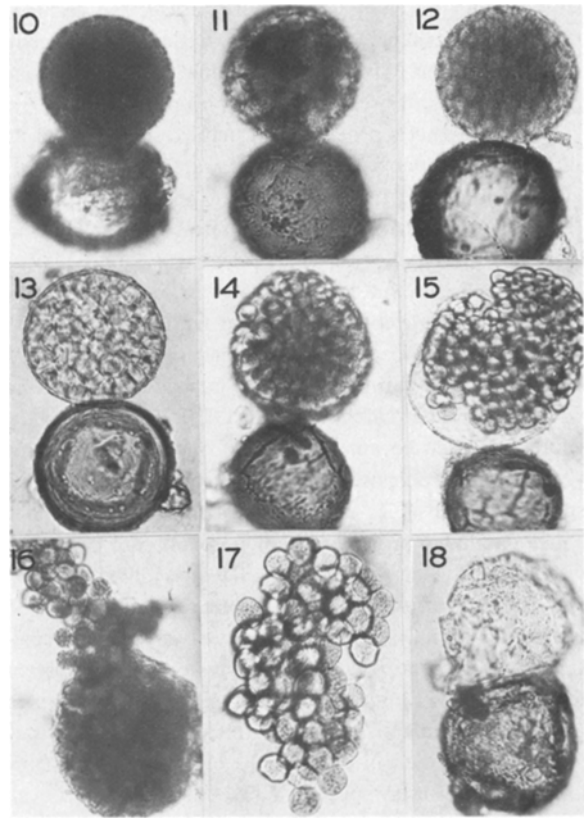


Figs. 1–9. Resting spore germination in *Synchytrium lagenariae*. 1, 2. A mature resting spore initiating a papillar projection in the outer wall and spore contents moving into the papilla. 3. Projection shaping into a vesicle after migration of protoplasmic contents. 4–6. Progressive migration of spore contents into the vesicle developing into an incipient sorus. 7. Fully developed incipient sorus. 8, 9. Initiation of cleavage channels around the periphery and in central portion of the incipient sorus. (All figs. × 175).

The first visible sign was a slight increase in size of the resting spores due to imbibition of water. Coincidently the dense granular cytoplasm became aggregated in the center,

often separated from the exosporium. The cytoplasmic aggregate gradually diminished and the contents became homogeneous, still retaining the original ochre yellow color (Figs 1, 24). A small papillate projection appeared below the endosporium (Figs 1, 25) and extruded piercing the exosporium (Figs. 2, 26). No predetermined site for the papillate projection was discerned. The resting spores became somewhat pear-shaped at this stage (Figs. 2, 25). The papillar projection gradually enlarged and the endosporic contents slowly moved in it (Figs. 3–6, 27–35). Finally, the projection assumed the shape of a thin-walled, spherical vesicle containing the endosporic contents (Figs. 7, 36). The immature young vesicle is termed as an incipient sorus. During the passage of protoplasmic contents, the primary nucleus gradually lost stainability and was presumed to have passed into the vesicle with the contents. Lingappa (13) also could not observe passage of the primary nucleus into the vesicle in *Synchytrium fulgens* Schröter (= *Synchytrium brownii* Karling). However, Kusano (10) showed actual passage of the primary nucleus into the vesicle in the species. The pore through which the protoplasmic contents had moved out became plugged with a hyaline, homogeneous material. The plug could not be clearly discerned in living preparations and probably functioned in preventing the backflow of vesicular contents into the empty spore. The empty spore case still retained its shape, though occasionally crumpled and remained firmly attached to the vesicle. It probably helped in the dissemination of sori. The vesicle or incipient sorus was usually spherical with a tough, hyaline bounding membrane enclosing deep olive brown contents (Figs. 7, 36). The dark brown pigment completely masked the vesicular contents making the nucleus unstainable and all attempts to remove the pigment with various bleaching agents were unsuccessful. The exact chemical nature of the pigment could not be ascertained, which was probably similar to melanin found in the human skin. The nuclear divisions, therefore, could not be observed in their sequence. The primary nucleus in the vesicle apparently divided meiotically first followed by mitotic multiplications of the secondary nuclei in a manner similar to other *Synchytrium* species (10, 13).

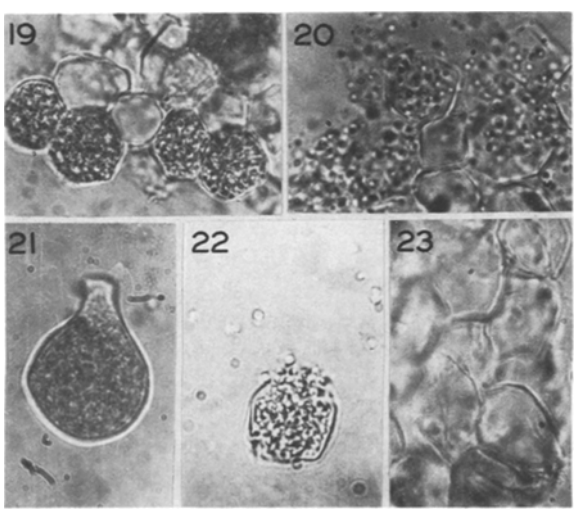
The dark brown pigment in the incipient sorus gradually dissolved making it translucent and optically more homogeneous (Figs. 8–10, 37), as the cleavage planes developed delimiting the protoplasm in the sorus. The cleavage was gradual and progressive, being first noticed along the periphery of the vesicle, centripetally progressing to the center (Figs. 11, 12, 38, 39). Finally, it cleaved into numerous polyhedral segments or sporangia. The delimited segments now comprised the mature sorus (Figs. 13, 40). A mature sorus was bright orange yellow under transmitted light with all the sporangia closely held together (Figs. 13, 40), while the vesicular wall functioned as a common soral membrane (Fig. 15). The sporangia escaped from the sorus by an irregular rupture (Figs. 14, 15, 41) or through a well-defined apical suture in the soral membrane (Fig. 16). Individual sporangia were orange yellow, predominantly polyhedral and bounded by a thin, hyaline wall (Figs. 17, 41), their number varying from 50–200 per sorus. The sporangial contents became coarsely granular as they attained physiological maturity. The sori at this stage could be stored at low temperature (8–10 °C) in a viable stage for 1–2 weeks under minimum moisture. During



Figs. 10–18. Resting spore germination in *S. lagenariae* (contd.). 10–12. Progressive stages in cleavage channels. 13. A mature sorus after cleavage completed. 14. Mature sorus before sporangial dissemination. 15, 16. Rupture of soral wall releasing the sporangia. 17. Sporangia liberated (enlarged view). 18. Empty sorus with soral membrane after sporangial dissemination. (Figs. 10–13 × 175; 14, 17, 18 × 165; 15, 16 × 125).

storage, they became slightly subspherical, but regained their original shape after flooding with fresh charcoal-adsorbed water. The tough, hyaline soral membrane persisted even after release of the sporangia (Fig. 18).

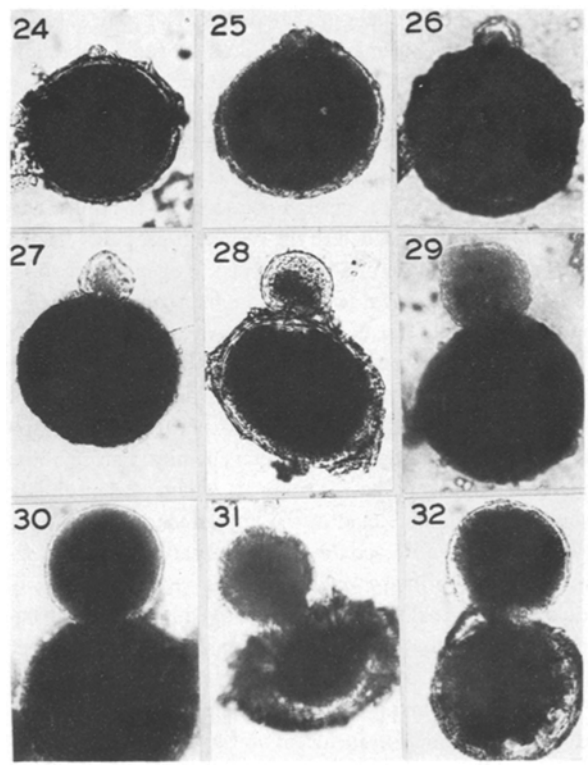
Zoosporogenesis proceeded in mature sporangia in an intact sorus under a thick film of water, but frequently after their release from the sorus. The process was accelerated when mature sporangia released from the sori were transferred to fresh charcoal-treated water. During zoosporogenesis the homogeneous protoplasm of the sporangia became coarsely granular with the preliminary organization of zoospore initials in the cytoplasm (Fig. 19). Zoospores were formed within 20 min and started rapid whirling movement in the sporangia. During this stage, the sporangia often became globose to obpyriform in shape (Fig. 20). The zoospores escaped either by a rupture in the sporangial wall (Fig. 21) or more commonly through a small papillate (projection-like) pore on the sporangial wall at a point where the polyhedral planes joined (Fig. 22). The entire process of zoosporogenesis was completed within 45–60 min after transfer of sori to fresh charcoal-treated water. The released zoospores remained quiscent for a few seconds, but regained rapid motility and remained so for 40–50 min in abundant fresh water. Later, they collapsed and died in the absence of a suitable host for infection. The empty sporangial case remained intact even after complete evacuation, presenting the appearance of a honeycomb (Fig. 23).



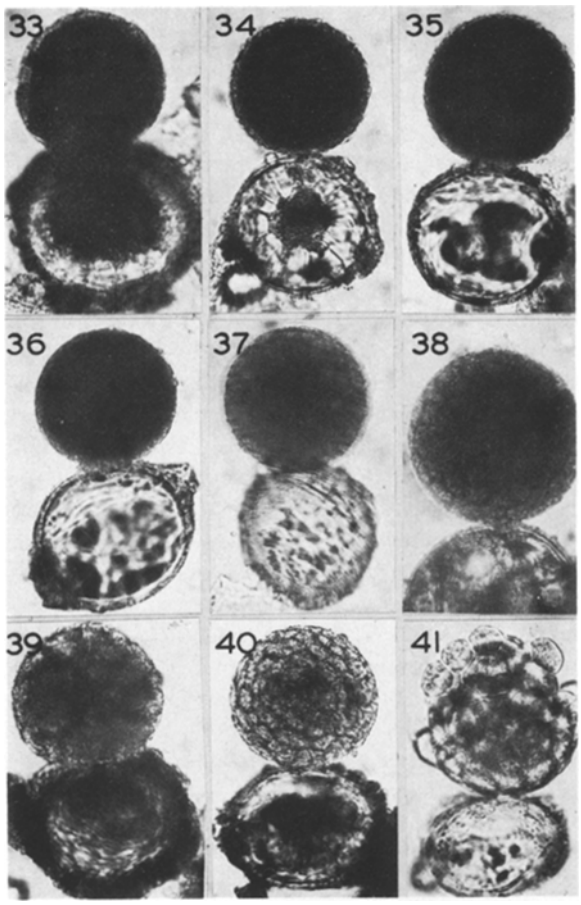
Figs. 19–23. Zoosporogenesis in the sporangia of *S. lagenariae*. 19. Densely granulated protoplasm in the sporangia. 20. Few sporangia releasing zoospores. 21. A papillate sporangium. 22. Zoospores released through papillate opening on a sporangium. 23. Empty sporangia. (Figs. 19, 21, 23 ×400; 20 ×700; 22 ×500).

Morphology of zoospores in the 2 species was similar. They were mostly spherical, occasionally subspherical or oval and possessed a single, posterior whiplash flagellum. The flagellum was long, slender, bearing a heterochromatic tip (Figs. 51–53, 57, 58). In *S. lagenariae*, the zoospores measured 2.5–5 μm in diam. and the flagellum 10–13 μm in length and in *S. trichosanthidis* 2–4 μm, the flagellum being 12–15 μm long. In both species, bi-, tri- and multiflagellate giant zoospores were occasionally encountered, which measured 7–10 μm in diam. with flagella measuring the same as in the uniflagellate zoospores (Figs. 54, 59). Occurrence of multiflagellate giant zoospores has also been reported in *Synchytrium fulgens* (9, 14). They are believed to arise by unequal cleavage of the sporangial protoplasm or by failure in the development of cleavage planes.

The zoospores were occasionally observed to function as gametes and fuse to form diploid zygotes. Both iso- and



Figs. 24–32. Resting spore germination in *Synchytrium trichosanthidis*. 24. A mature resting spore. 25, 26. Papillate projection developing on the outer wall. 27–32. Migration of spore contents into the superficial vesicle and development of incipient sorus in progressive stages. (All figs. ×165).



Figs. 33–41. Resting spore germination in *S. trichosanthidis* (contd.). 33–35. Further stages in the migration of spore contents into the superficial vesicle and development of an incipient sorus. 36. An incipient sorus. 37–39. Progressive development of cleavage channels. 40. A mature sorus. 41. Release of sporangia from a sorus. (Figs. 33–37, 39, 40 ×165; 38 ×225; 41 ×175).

anisomorphic planogametes participated in the fusion, but in the latter case one of the fusing planospores was smaller (Fig. 55). The diploid zygote was ellipsoidal to cylindrical and usually biflagellate (Figs. 56, 60). The species of *Synchytrium* so far investigated for determination of sexuality, have exhibited fusion of only isomorphic planogametes, but Percival (16) observed in *S. endobioticum* that the zoospores (planogametes) were anisomorphic indicating conjugation between them, but failed to observe any evidence of their fusion later.

Aberrations in the resting spore germination

Infrequently some anomalous but interesting types of resting spore germination were observed representing 5 catagories as below:

1. Variation in the emergence of vesicle: The endosporic contents emerged through a longitudinal slit or circumscissile rupture in the spore wall instead of a regular papillate pore. However, the vesicle developed and normal sporangia were delimited (Figs. 42, 43, 48).

2. The papillate pore, usually very small, extended into a tubular process through which the endosporic contents rapidly migrated into the vesicle (Fig. 44).

3. Development of more than one vesicle: In this interesting anomaly, the resting spore instead of giving rise to a single vesicle, occasionally produced 2–5 (or more) vesicles. There was a corresponding decrease in size with the increase in their number. Normal sporangial delimitation and zoosporogenesis were observed in cases upto 2 vesicles, but with more than 2, the vesicles failed to proceed in the normal development and usually aborted (Figs. 45–47).

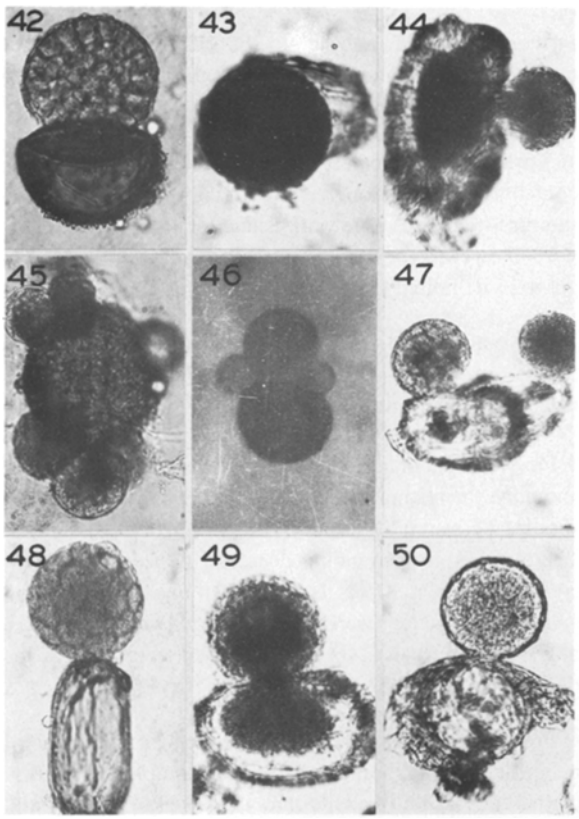
4. Precocious zoosporogenesis: The incipient sorus started delimiting sporangia much before the complete migration of the endosporic contents into the vesicle. The size and shape of the sporangia departed from the normal (Fig. 49).

5. This anomaly was manifested by failure of the incipient sorus or vesicle to differentiate sporangia within. Suppression was partial or complete. In a partial suppression, few cleavage planes were laid down dividing the contents into only 3–4 segments, each forming a giant sporangium. The zoosporogenesis proceeded as in the regular sequence. In complete suppression, the dark brown vesicular contents gradually became translucent and disintegrated (Fig. 50).

The process of resting spore germination from other host collections was essentially similar in manner and sequence described above. Biometry of the resting spores, sori and sporangia from some collections is given below:

Discussion

Presence of a thick exosporium, lack of knowledge concerning the physiology and relatively a difficult task of germinating the resting spores may be some of the factors for not determining the mode of resting spore germination in a majority of *Synchytrium* species. Initially difficulties

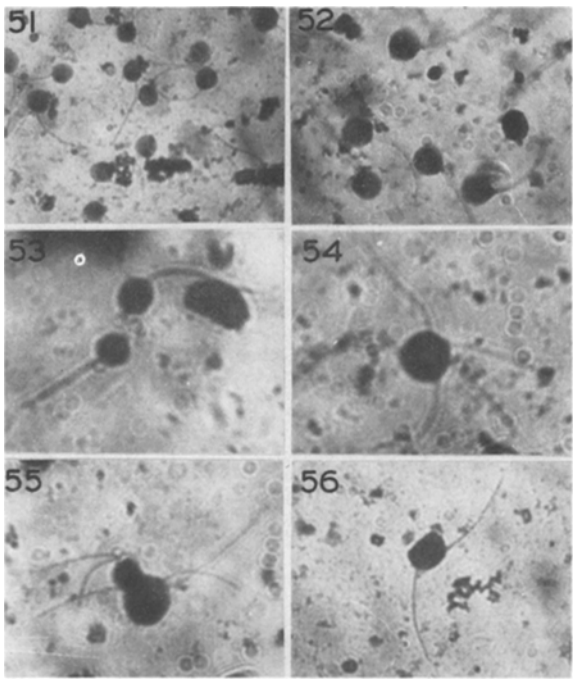


Figs. 42–50. Anomalies in resting spore germination of *S. lagenariae* and *S. trichosanthidis*. 42, 43, 48. Emergence of endosporic contents through a longitudinal slit or circumscissile rupture in the spore wall. 44. Papillate pore extending into a tubular process and migration of endosporic contents. 45, 46. Development of 6 or 3 vesicles over a resting spore with abnormal sporangiogenesis. 47. 2 vesicles developing on a spore with normal sporangial formation. 49. Precocious sporangial delimitation before complete migration of the spore contents. 50. Suppression of sporangial formation and disintegration of contents of the incipient sorus. (Figs. 42, 43, 45, 48 ×175; 44 ×165; 46, 47 ×100; 49, 50 ×150).

were encountered in securing success; however, it soon became evident that permeability of the thick exosporium through corrosion was an essential step in initiating the spore germination. Among the acids and alkalies tested, a dilute strength of HCl was found best in corroding the thick exosporium and making it permeable. The alkalies, on the other hand tended to cause damage/injury to the resting spore, thereby making them nongerminable, although the results with dil. HCl did not yield a very high count (60–65 %). A higher percent germination (upto 85 %) was obtained by subjecting HCl-treated spores to moist heat at 40–45 °C for 1–2 hrs (17). This temperature shock-triggerred germination could be compared with the environmental conditions prior to initiation of the disease in the field. Its incidence in the field usually appears in early August after the first monsoon showers. The soil becomes heated to 45–50 °C due to the high summer temperature during May – June and with the onset of the monsoon rains the (pre-heated) soil emits steam or moist heat, providing the necessary trigger for germination of the resting spores (17). The spores in the soil are known to resist high temperature; a treatment preceded by a dip in acidulated water proved very effective in inducing their germination in dormant stage scoring a high count of 85–90 %, compared to other treatments, wherein the percentage scarcely exceeded 30–35 %. This treatment also appears to work successfully for other tropical species of *Synchytrium* (17).

The resting spores from all the cucurbitaceous collections functioned as a prosorus in germination, giving rise to an attached superficial sorus of sporangia in which the zoospores were subsequently delimited. Karling (4, 5) has recognized differences in the resting spore germination as a distinctive taxonomic criterion in the identification of *Synchytrium* species. Mhatre and Mundkur (15) and Gupta and Sinha (2, 3), who had described different *Synchytrium* species on cucurbits, ignored the occurrence of prosori and germination pattern of resting spores in these species. Karling (8), however, indicated the oc-

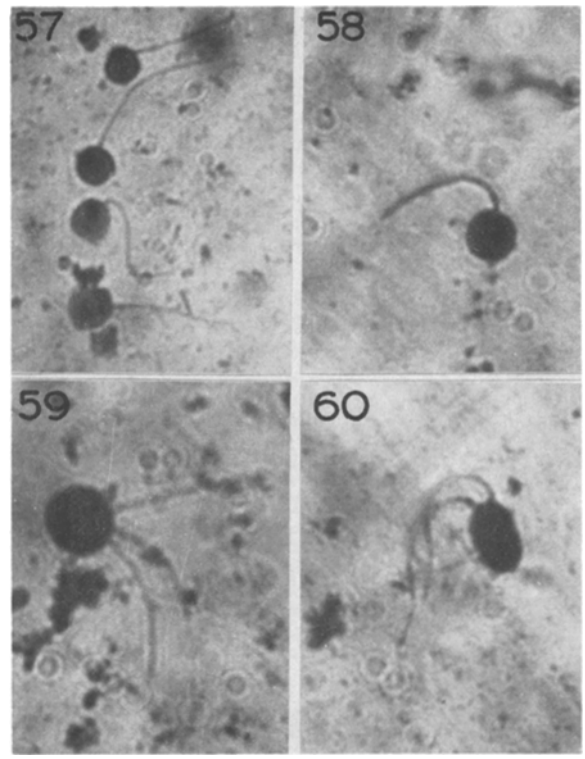
Host species	Diameter range of Resting spore	Sorus	Sporangium
Benincasa hispida (Thunb.) Cogn.	70 –148 μm	75–156 μm	15 –27 μm
Cucumis sativus L.	100 –145 μm	112–168 μm	18 –27 μm
Cucurbita moschata Poir.	110 –145 μm	88–142 μm	16 –21 μm
Lagenaria sicoraria Mol. & Standl.	95 –150 μm	90–128 μm	15 –20 μm
Luffa cylindrica Roem.	90 –177 μm	100–133 μm	17.5–22.5 μm
Momordica charanthia L.	105 –145 μm	110–151 μm	16 –21 μm
Trichosanthes anguina L.	66.5–133 μm	61–110 μm	15.5–25 μm
Trichosanthes dioica Roxb.	98 –145 μm	66–155 μm	19 –27.5 μm



Figs. 51–56. Morphology of zoospores and fusion of planospores in *S. lagenariae*. 51–53. Typical posteriorly uniflagellate zoospores showing heterochromatic flagellar tips. 54. A multiflagellate giant zoospore. 55. Fusion of anisomorphic planospores. 56. Biflagellate zygote. (Figs. 51 ×500; 52, 56 ×625; 53, 54, 55 ×900).

currence of prosori and germination of resting spores in these species, but emphasized the need for a thorough examination of the species to justify their inclusion in the subgenus *Microsynchytrium* Karling. The subgenus *Microsynchytrium* includes species, where the resting spore functions as a prosorus in germination and the initial cell also as a prosorus. This subgenus originally included 3 aquatic species of algal parasites earlier included under the genus *Micromyces* Dangeard. Now the subgenus is known to represent 30 species, most of them terrestrial in habitat and all long-cycled, as both mature thallus and resting spore function as the prosorus in germination with subsequent formation of an attached sorus of sporangia. Present observations firmly corroborate Karling's observations and justify inclusion of the species parasitizing cucurbitaceous hosts under the subgenus *Microsynchytrium*.

The zoospores in the 2 species were morphologically similar except minor variations in dimensions. Occurrence of giant zoospores was observed in both the species, which



Figs. 57–60. Morphology of zoospores and zygote in *S. trichosanthidis*. 57, 58. Typical posteriorly uniflagellate zoospores with heterochromatic flagellar tips. 59. A multiflagellate giant zoospore. 60. Biflagellate zygote. (Figs. 57 ×625; 58, 60 ×875; 59 ×900).

probably arose by failure in cleavage partitions in the sporangium. In both species, the zoospores functioned as planogametes and fused in pairs to form diploid zygotes. Occasional fusion between anisomorphic zoospores was a common feature in both the species.

Acknowledgements

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