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RESTING-SPORE GERMINATION IN *SYNCHYTRIUM ENDOBIOTICUM*[1]

Het kiemen van de rustspore by Synchytrium endobioticum

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The resting spores of *S. endobioticum* function as a prosorus in germination. The contents of the resting spore are extruded as a vesicle. Within this vesicle one single sporangium develops. Germinating resting spores resemble resting spores which have been parasitized by certain fungi, but it is obvious from several facts presented that no parasites were present. On the basis of the mode of germination described, *S. endobioticum* should be transferred from the subgenus *Mesochytrium* to the subgenus *Microsynchytrium*.

INTRODUCTION

In fungi of the genus *Synchytrium* two types of resting-spore germination have been reported, and these types, among other characteristics, have been used as taxonomic criteria in distinguishing subgenera and species. In one type of germination the resting spore functions as a sporangium and forms zoospores directly; in the other type it functions as a prosorus, the contents emerging to form a thin-walled vesicle which differentiates into sporangia. Also, the initial cell or thallus of summer sporangia may function directly as a sorus of sporangia, or as a prosorus whose content emerges to form a superficial sorus of sporangia. On the basis of these differences in resting-spore germination and behaviour of the initial cell which gives rise to the summer sporangia KARLING (1964) recognized six subgenera of *Synchytrium*. Long-cycled species whose initial summer cell and resting spores both function as prosori were included in the subgenus *Microsynchytrium*. Species whose initial summer cell functions as a prosorus but whose resting spores function as a sporangium and give rise directly to zoospores were placed in *Mesochytrium*. In other long-cycled species the initial summer cell functions directly as a sorus of sporangia and the resting spores function as sporangia in germination. Such species were placed in *Synchytrium (Eusynchytrium)*. In similar long-cycled species, however, the resting spore functions as a prosorus in germination, and these were assigned to the subgenus *Exosynchytrium*. The short-cycled species which develop only sporangial sori, or only resting spores, were included, respectively, in the subgenera *Woroninella* and *Pycnochytrium*, as shown in Fig. 1. According to this classification and the reports of its life cycle in the literature *S. endobioticum* (Schilb.) Perc. belongs to the subgenus *Mesochytrium*.

Resting-spore germination in *S. endobioticum* has been described by several workers in the past and they have reported that the spore functions as a sporangium and gives rise directly to zoospores. According to CURTIS (1921) the resting spore or resting sporangium, as she calls it, enlarges as much as 50 per cent shortly before the maturation of the zoospores within it. As they mature

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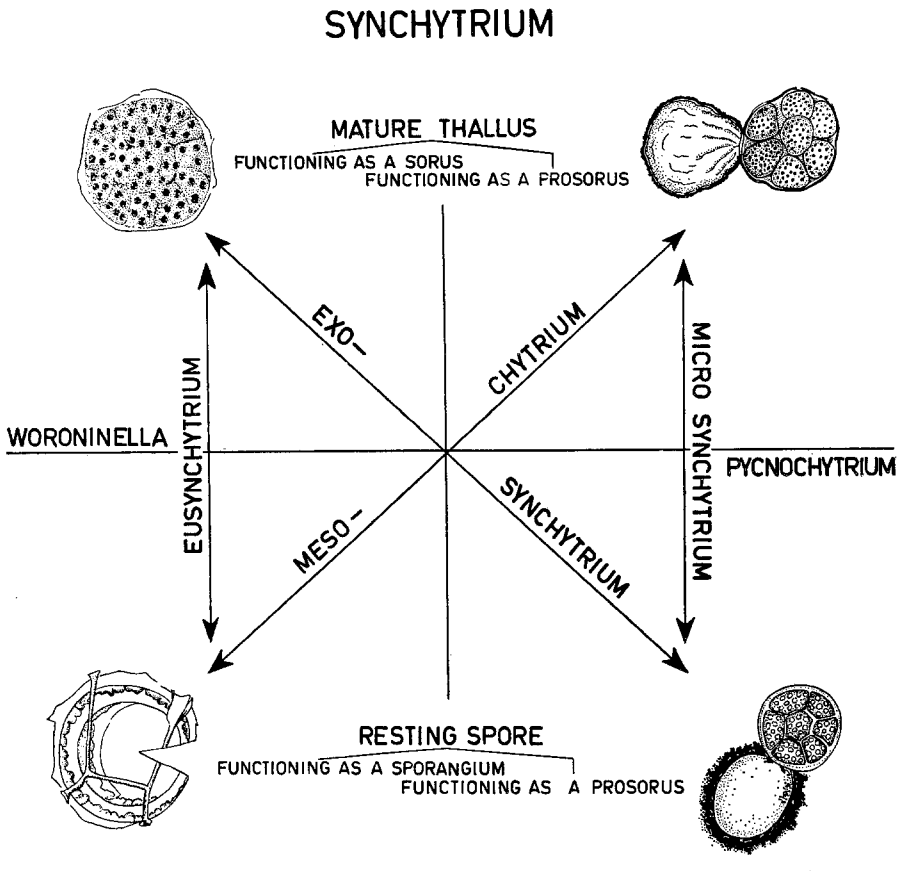


FIG. 1. Schematic representation of the classification of the subgenera in *Synchytrium*. Explanation in the text. Drawings after CURTIS and KARLING.
Schematische weergave van de classificatie van de subgenera in Synchytrium. *Verklaring in de tekst. Tekeningen naar CURTIS en KARLING.*

"one portion of the innermost membrane of the sporangium swells up, forming a conical projection, which extends inwards, sometimes as far as the centre of the sporangium. Eventually the sporangium bursts, and the slit formed may extend almost round its periphery."

However, in experiments designed to study the influence of external conditions on resting-spore germination the process and method of germination were found by the author to be quite different from those described by CURTIS and other workers, and the present publication concerns this mode of germination as well as the reclassification of *S. endobioticum*.

MATERIALS AND METHODS

The resting spores used in all experiments but one, were isolated from compost which is used by the Netherlands Plant Protection Service (P.D.) as

inoculation material for testing new potato varieties for wart-disease resistance[3]. This compost is prepared by mixing warts of *S. endobioticum* with fine-grained sand and keeping it in the open for one winter. The mixture is then spread out in order to dry and finally it is sieved. The compost is kept in store in the laboratory in dry condition.

Following THIEDE & WIERLING's (1960) methods, the resting spores were activated by keeping the compost, prior to the experiments, in a wetted condition for two to three weeks at a temperature of 18–20 °C. Then the compost was diluted with water and pulverised in a mixer. The coarse parts were removed from the suspension by means of a 100 μ sieve, and then most of the resting spores were collected with a 30 μ sieve. The spore material also contained organic debris of the same size, but these did not impede the further experiments.

For one experiment the spores were obtained from fresh warts which had been dried and kept in store in the laboratory for 20 months. The warts were first soaked in a petri dish with moist filter paper for two days, after which the resting spores were isolated in the same way as has been described for the compost.

All resting spores were kept in store on the 30 μ sieves in petri dishes with moist filter paper.

RESULTS

1. Under a dissecting microscope, × 100, separate spores were transferred with a fine needle to water agar in four petri dishes ⌀ 5 cm. In order to locate each individual spore later, they were arranged in the form of a cross. Removal of the dish covers for a short while to dry out the agar slightly made the spores stick to the agar surface and retain their original position in the cross. Then the agar was covered with a thin layer of tap water which was replaced every two days. The spores were assessed one by one with the aid of a compound microscope; all spores whose viability was doubted were removed. The exact position of all the remaining spores, totalling 64, was annotated. The dishes were kept at a temperature of 18 °C, and from then on the resting spores were inspected daily with a compound microscope.

Eleven observations on these spores from the 7th to the 16th day after the experiments were started revealed that resting spores, which had shown nothing particularly different on the previous day, were empty, but attached to each of them was a superficial vesicle of protoplasmic content. A few days after the appearance of the vesicle a second wall was formed on its insides, creating the appearance of an enveloped globular body inside which almost filled the entire contents. The contents of this vesicle did not differentiate further in the course of 18 days, and the experiment was terminated. The experiment was repeated with 26 spores, but after seven days only two of the spores germinated.

Nevertheless, the phenomena observed showed a sharp resemblance to the descriptions and figures in the literature of resting spores which function as prosori in germination.

[3] The author is indebted to Mr. H. VAN LOOKEREN CAMPAGNE, former scientific officer at the Netherlands Plant Protection Service, for giving information on the subject and providing compost and wart material.

2. Germination also occurred in the resting spores which had been kept in storage on the sieves in petri dishes with moist filter paper. Germinating resting spores could easily be recognized by the presence of a small white globular body attached to the resting spore. Considering the great number of resting spores that were kept under observation, the percentage of germinating spores was very small. The results of three experiments in which the resting spores from compost were brought to germination, can be summarized as follows.

Number of days the compost has been kept wet (pretreatment)	Period, after the pretreatment, during which germination was observed (germination period)
16 days	6th – 40th day
17 days	3rd – 24th day
23 days	4th – 11th day

A single experiment with resting spores which had been isolated from dried warts, gave the following data.

Pretreatment	Germination period
2 days	19th – 43rd day

As compared with the already small percentage of germination in the resting spores from the compost, the resting spores from the dry warts only germinated sporadically.

3. For microscopical examination, germinating spores were picked up from the sieve with a fine needle and mounted in water. The attached vesicle is almost globular with a diameter of 40–60 μ, and its contents consist of finely dispersed protoplasm (Fig. 2). Germinating resting spores always have an irregular aperature of 20 to 30 μ diameter in the epispore (Fig. 3) and a small circular aperature in the endospore (Fig. 4). The base of the vesicle protrudes into the aperature of the endospore as a plug, which anchors it to the empty hull of the resting spore. Such aperatures and plugs have been found by KARLING (1964) and others to be persistently present in all germinated resting spores of *Microsynchytrium*, *Exosynchytrium* and *Pycnochytrium* which have functioned as prosori, as well as in the wall of initial summer cells which function in the same manner. He reported that they persist and are visible long after the initial prosori and germinated spores become empty and collapsed. Thus, their presence is an effective key in determining whether or not the initial summer cell and resting spore have functioned as prosori.

After some time a second wall is formed within the vesicle which delimits a single sporangium (Fig. 5). Thus, from the material studied, the sorus of *S. endobioticum* appears to be monosporangiate, and the single sporangium is liberated from the vesicle or sorus at the slightest touch (Fig. 6). The remainder of the vesicle or sorus wall remains attached to the resting spore (Fig. 7). After three days in tap water which was replaced repeatedly, the contents of the sporangia differentiated into globular bodies, but these never developed into motile zoospores. However, when such sporangia were crushed and their contents examined by phase-contrast microscopy it was found that these bodies were immature or incipient zoospores with a single flagellum, but they never showed any movement.

DISCUSSION

Contrary to the numerous reports in the literature, these observations show that the resting spore of *S. endobioticum* functions as a prosorus in germination instead of as a sporangium. They also indicate that PERCIVAL (1910) may have seen the same thing when he said ".... in some instances the inner sacs with their contents were observed free from the outer thick walls having apparently come out through gaping slits in the latter." It is obvious from these observations that *S. endobioticum* does not belong in *Mesochytrium* as this subgenus is defined and must be transferred to the subgenus *Microsynchytrium*.

When the first indications were obtained that the resting spore might function as a prosorus in germination, the possibility had to be taken in account that the globular body on the resting spore might be a parasite of the resting spores, as has been described by KÖHLER (1924). Already KUSANO (1930) had remarked that resting spores of *S. fulgens* functioning as a prosorus in germination, closely resemble resting spores of *S. endobioticum* which were parasitized by *Phlyctochytrium synchytrii*, the parasite described by KÖHLER.

In this study confusion with fungi parasitizing the resting spores can be excluded on the following grounds:

1. A number of spores which were kept under daily observation showed the normal contents of the resting spores prior to germination, while they were invariably empty after germination. In case of parasitism the contents would have desintegrated gradually.
2. Germinating resting spores always showed one vesicle only, while parasitized resting spores may bear more than one sporangium of a parasite.
3. The absence of a subsporangial vesicle within the resting spore which occurs in resting spores having been parasitized by *Phlyctochytrium synchytrii*.
4. The presence of a separate sporangium within the vesicle of germinating resting spores, while *Phlyctochytrium* sporangia have a single membrane only.
5. The absence of preformed germination pores, which are present in *Phlyctochytrium* sporangia.

Although LINGAPPA (1955) has succeeded in bringing the zoospores in germinating resting spores to a complete development in all the 24 species he examined, by supplying fresh water, this procedure had no result in *S. endobioticum*. Apparently *S. endobioticum* requires special conditions in this respect.

ACKNOWLEDGEMENT

The author is indebted to Prof. J. S. KARLING, Purdue University, Lafayette, Indiana, U.S.A., for his assistance in preparing the text of the manuscript and for adding the following note (December 12, 1964).

"I am very grateful to Dr. KOLE for the opportunity of reading this article in manuscript form, and my only regret is that it was not published before I completed my monograph on *Synchytrium*. This is a notable contribution in that it disproves the views of hundreds of other workers on *S. endobioticum* that the resting spores function as sporangia and give rise directly to zoospores. It emphasizes anew the fact that the life cycles of all *Synchytrium* species must be

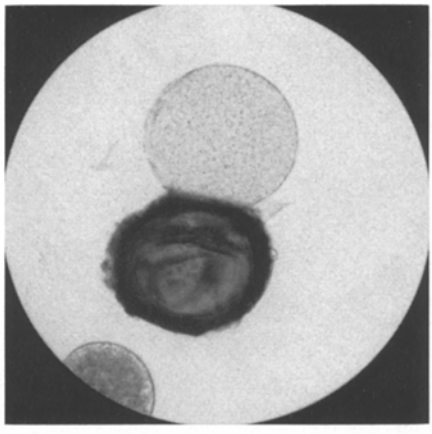
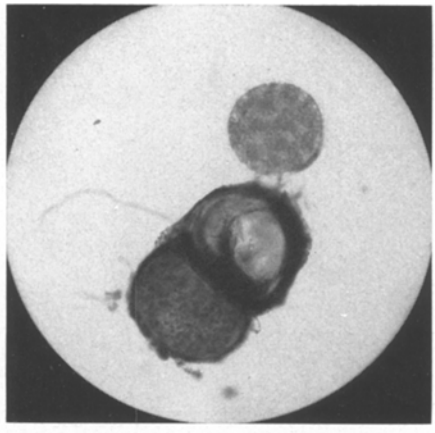


FIG. 2



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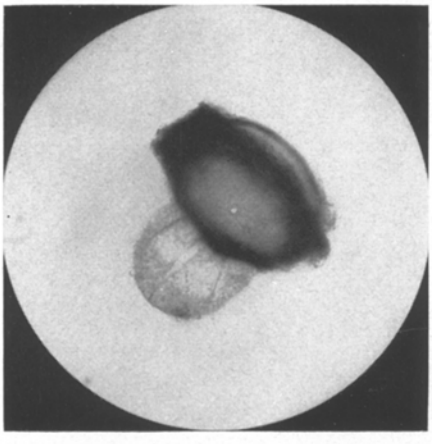

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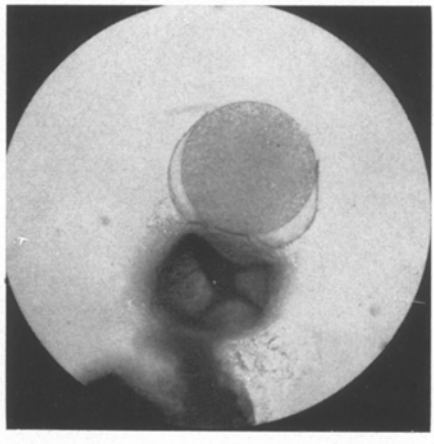

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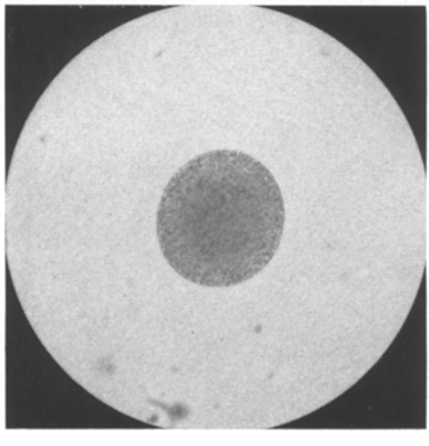

FIG. 6

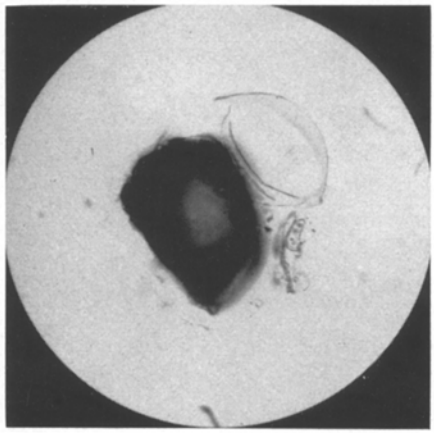

FIG. 7

100 μ

studied intensively and become fully known before the species can be properly classified. In my monograph of *Synchytrium* only two species were included in the subgenus *Mesochytrium* on the basis of the information available, and the removal of *S. endobioticum* leaves only one, *S. desmodiae* Munasinghe, incompletely known species in this subgenus. MUNASINGHE (1955) reported that the resting spores function as sporangia in germination, but he gave no figures to show this. It is not at all unlikely that the resting spores of this species, also, function as prosori in germination. At least several years ago Dr. KOLE sent me photographs of this species which showed a sorus of sporangia lying above an empty prosorus, but I could not determine with certainty whether the prosorus relates to an initial summer cell or a resting spore. In the event it relates to the latter, *S. desmodiae*, also, should be transferred to *Microsynchytrium*, eliminating thus the necessity for the subgenus *Mesochytrium*.

It is to be hoped that Dr. KOLE will carefully restudy his sections of this species to solve this problem."

SAMENVATTING

De taxonomie van het geslacht *Synchytrium* is hoofdzakelijk gebaseerd op de wijze waarop de rustsporen kiemen en de zomersporangia tot ontwikkeling komen. In fig. 1 wordt daarvan een schematisch overzicht gegeven.

In tegenstelling met hetgeen uit de literatuur bekend is, blijken de rustsporen van *S. endobioticum* bij het kiemen als een prosorus te fungeren. De inhoud treedt daarbij als een melkwit blaasje naar buiten (fig. 2). Bij de kieming ontstaat een relatief grote opening in de epispore en een kleine opening in de endospore (fig. 3 en 4). Binnen het blaasje ontwikkelt zich één sporangium (fig. 5 en 6), dat bij de geringste aanraking vrijkomt. Daarna blijft alleen het omhulsel op de rustspore achter (fig. 7).

FIG. 2. Germinating resting spore showing a vesicle with undifferentiated finely dispersed protoplasmic contents.
Kiemende rustspore, gekenmerkt door een blaasje met ongedifferentieerde, fijn verdeelde protoplasmatische inhoud.

FIG. 3. A double resting spore, one of which has germinated. The vesicle has been pushed away. The empty resting spore shows a hole in the epispore and a small opening in the endospore.
Een dubbele rustspore, waarvan er één is gekiemd. Het blaasje is opzij gedrukt. De lege rustspore heeft een relatief grote opening in de epispore en een kleine opening in de endospore.

FIG. 4. Germinating resting spore, showing germ pore in the endospore.
Kiemende rustspore met een kiemopening in de endospore.

FIG. 5. Germinating resting spore, showing a partly ruptured vesicle and one sporangium.
Kiemende rustspore met een opengesprongen blaasje en één sporangium.

FIG.6. A single sporangium.
Eén afzonderlijk sporangium.

FIG. 7. Empty hull of vesicle on a germinated resting spore.
Resten van een blaasje op een gekiemde rustspore.

Kiemende rustsporen vertonen veel gelijkenis met rustsporen die door bepaalde schimmels zijn beparasiteerd, maar zij kunnen daarvan worden onderscheiden.

Op grond van deze nieuwe gegevens over de kieming van de rustsporen dient *S. endobioticum* van het subgenus *Mesochytrium* naar het subgenus *Microsynchytrium* te worden overgebracht.

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