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THE SIGNIFICANCE OF THE ZOOSPORANGIAL STAGE IN THE LIFE CYCLE OF THE PLASMODIOPHORALES[1]

De betekenis van het zoösporangiumstadium in de levenscyclus van de Plasmodiophorales

BY

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INTRODUCTION

Since the discovery of the zoosporangial stage in the life cycle of the Plasmodiophorales (COOK, 1926) it has never been demonstrated whether the zoosporangial zoospores, after re-infecting the plant, again produce zoosporangia. Alternatively, the zoosporangial zoospores might only be able to initiate the next stage of the life cycle, leading to the development of resting spores.

With regard to the work by LEDINGHAM (1935) on the occurrence of zoosporangia in *Spongospora subterranea* KARLING (1942) remarks: "The zoosporangia and zoospores found by Ledingham are apparently a means of rapid vegetative multiplication and doubtless relate to the haploid phase, ...". Referring to COOK's (1926, 1933) investigations of *Ligniera* he writes: "It is not improbable that the zoosporangia and zoospores are merely means of vegetative multiplication without sexual significance and relate to the haploid generation, ...".

From these suggestions it is not certain whether the zoosporangial stage can recur or not. The way in which KARLING (1942, text fig. 10) has included the zoosporangial stage discovered by LEDINGHAM within the life cycle of *Spongospora subterranea* according to COOK (1933) suggests that he thought that it does.

Our aim was to get more information about this aspect of the life cycle of the Plasmodiophorales and it seemed that it could be accomplished by the following simple experiment. An attempt could be made to inoculate plants by zoospores from infected plants in which the fungus was present only in the zoosporangial stage. It would then have to be shown that plants exposed to such inoculum became infected and that zoosporangia developed in the roots. If this happened the newly infected plants could then serve to inoculate yet other healthy plants. An important condition for success would be to ensure that the infected roots, used as infection material at the beginning, were entirely free from resting spores. *Plasmodiophora brassicae* is not very suitable in this respect because its resting spores are so small that it is impossible to determine with certainty that none are attached to the roots of the starting material. Because of this we used *Spongospora subterranea*, the resting spores of which are in aggregates (spore balls), easily recognizable on microscopical examination of the roots.

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Method and material

Glass pots were used. These were prepared by cutting pieces of 5 cm length from a glass tube (diameter 4 cm). The glass rings obtained in this way were painted black and later white on the outside. The bottom was formed by a nylon gauze held by a rubber band. According to the stage of the experiment the pots were filled with infested or non infested steam sterilized soil. The inoculum consisted of spore balls of *Spongospora subterranea* which had been collected in 1958, dried and sieved and since then stored in the laboratory. Infested soil was obtained by mixing 1.5 gr of spore balls with 500 gr moist steam sterilized soil. As test plants we used small tomato seedlings of the variety 'Tuckqueen'. The pots were placed in an aquarium tank closed by plastic foil and kept in a glasshouse, at a temperature of 18–20° C.

Infection experiment

Six pots were filled with infested soil, and six small tomato seedlings planted in each. On the 17th day after starting the experiment one seedling was removed from one of the pots. After rinsing the roots were examined microscopically for the presence of *Spongospora* (zoosporangia) and were found to be heavily infected. However, after placing the roots in water only a few zoospores emerged. Subsequently we waited till the 27th day of the experiment when many zoospores soon escaped from the zoosporangia in the seedling examined. All the other plantlets were then removed from the pots, cleaned thoroughly by rinsing with a fine jet of tap water and examined microscopically for the absence of spore balls. At the same time attention was paid to the rate of sporangial discharge. The heavily infected plants from which zoospores were being liberated profusely were directly transplanted into pots with non infested soil in which 6 tomato seedlings had been raised during the previous 14, or so, days. In each pot 4–6, labelled, infected plants were planted amidst the healthy plants. After 15 days the roots of some plants which had been healthy at first were, locally, heavily infected. Zoospores were not liberated then, but after 30 days many were released. In their turn, newly infected plants with many mature zoosporangia were used to inoculate more healthy plants in the above manner. After 23 days their roots were, locally, heavily infected and zoospores were liberated almost immediately. At this stage the experiment was terminated. The whole experiment was then repeated, with the same result.

From this experiment it is clear that after infection by zoosporangial zoospores new zoosporangia can indeed be obtained. Thus the zoosporangial stage can recur an unlimited number of times and the fungus is able to maintain itself and multiply in the zoosporangial stage when young roots of the host plant are available and when conditions are favourable.

For the experiment to be successful two conditions are essential.

1. Transfer of the fungus succeeds only when the infection material produces zoospores at the time of transplanting. Infection experiments with material in which the zoosporangia are not yet mature do not succeed. Presumably this is because the further development of the zoosporangia is unfavourably influenced by the transplantation.
2. The plants to be inoculated should be transferred to the experimental pots

some time before the experiment to allow their roots to become established again after being disturbed.

Discussion

Now that it has been shown, at least for *Spongospora subterranea*, that zoospores from zoosporangia, after re-infecting the root, again produce zoosporangia, the question arises how the resting spores are formed. In other words, how should the zoosporangial stage be fitted into the life cycle of the fungus. This can be explained by the behaviour of the zoopores. There are data in the literature (cf. KARLING, 1942) on the fusion of zoospores in the Plasmodiophorales, but the possibility that the compound zoospores observed were not the result of fusion but of incomplete cleavage during their origin had not been excluded in this earlier work. KOLE (1954) established by observation of living zoospores with a phase-contrast microscope that, in *Spongospora subterranea*, the compound zoospores do, in fact, originate by fusion of separate zoospores. Although this strongly suggests a sexual stage in the life cycle definite proof is lacking because karyogamy has never been shown to follow fusion of zoospores. The many attempts, described in the literature, to detect sexuality on the basis of chromosome numbers in dividing nuclei at different developmental stages of these fungi are really inconclusive, probably because the nuclei are so small. In 1958 MILLER wrote about his investigation on *Sorosphaera veronicae:* "Because no nuclear fusions have ever been found to occur in the Plasmodiophorales and reports of meiosis are strictly interpretive, it is suggested here that most data do not convincingly support the contentions that nuclear fusions and meiosis occur in the life cycle of these parasites".

The facts on which a life cycle schema can be based therefore remain restricted to the indefinite repetition of the zoosporangial stage and the fusion of zoosporangial zoospores. It is still uncertain whether, after fusion of zoospores, a zygote is formed. Whether the zoospores from resting spores can also fuse is equally unknown. Analogies may perhaps be made with the Synchytriaceae. CURTIS (1921) found that single zoosporangial zoospores of *Synchytrium endobioticum* reproduce the zoosporangial stage while fused zoospores formed a zygote which developed to a resting spore. KUSANO (1930) found for *S. fulgens* that zoospores from resting spores may fuse and produce a zygote. From the data on the Plasmodiophorales and by analogy with the Synchytriaceae a diagram is given in fig. 1 of the life cycle of the Plasmodiophorales. Uncertain stages are enclosed by dotted lines.

Summary

An infection experiment with *Spongospora subterranea* in tomato plants has shown that zoosporangial zoospores, after re-infection, may again produce zoosporangia. This affords the fungus an opportunity for vegetative multiplication. It is assumed that the resting-spore stage is brought about after infection by a zygote which has been produced by fusion of two zoospores acting as gametes. In fig. 1 the probable life cycle of the Plasmodiophorales has been presented schematically.

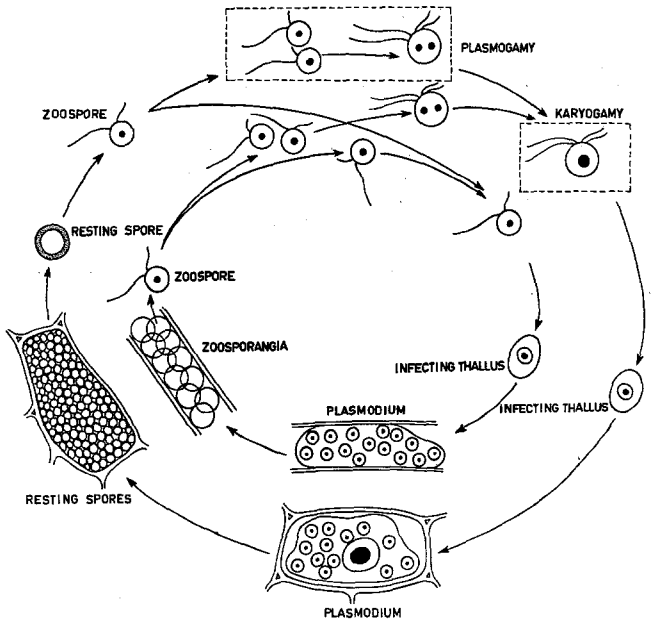

FIG. 1. Probable life cycle of the Plasmodiophorales. The encircled parts are still uncertain. *Vermoedelijke levenscyclus van de de Plasmodiophorales. Over de omlijnde gedeelten bestaat nog geen zekerheid.*

SAMENVATTING

Bij de Plasmodiophorales onderscheidt men het zoösporangium- en het rustsporenstadium. Het zoösporangiumstadium wordt gekenmerkt door de aanwezigheid van zoösporangia, vaak in wortelharen en andere schorsepidermiscellen; het rustsporenstadium door het tot ontwikkeling komen van rustsporen, dikwijls in gehypertrofieerde delen van de waardplant. Sinds de ontdekking van het zoösporangiumstadium is nimmer zekerheid verkregen over de vraag of zoösporen uit de zoösporangia bij herinfectie wederom zoösporangia kunnen geven. Het alternatief zou zijn dat de zoösporen uit de zoösporangia de levenscyclus vervolgen en tot de ontwikkeling van rustsporen leiden.

Een infectieproef met *Spongospora subterranea* bij tomateplanten heeft nu uitgewezen dat de eerste veronderstelling juist is; zoösporen uit de zoösporangia geven bij herinfectie inderdaad weer zoösporangia. Het rustsporenstadium komt waarschijnlijk tot ontwikkeling na herinfectie door een zygote, welke ontstaat indien twee als gameten fungerende zoösporen versmelten. Deze opbouw van de levenscyclus (fig. 1) geeft de schimmel de mogelijkheid tot een sterke vegetatieve vermeerdering in het zoösporangiumstadium.

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