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SOME OBSERVATIONS ON THE ZOOSPORES FROM THE ZOOSPORANGIA OF *PLASMODIOPHORA BRASSICAE* WORON

Met een samenvatting:
Enkele waarnemingen betreffende de zoösporen uit de zoösporangia van Plasmodiophora brassicae WORON

BY

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INTRODUCTION

The object of the present study was to gain better knowledge of the zoospores from the zoosporangia of *P. brassicae*. According to COOK and SCHWARTZ (1930), who discovered the zoosporangial stage, the zoospores are small, spindle-shaped bodies about 1.5 μ in length and 0.5 μ to 0.7 μ in diameter. Their observations were restricted to stained material, and „consequently no information could be obtained as to their method of motility, nor could a flagellum be always made out. In the few instances when one appeared to be present, it was about equal in length to the zoospore". Further, COOK and SCHWARTZ observed „that the zoospores... migrated from the root hairs to the epidermal and cortical cells of the root, and... passed down into the root tip. In a few cases slightly larger objects were found, containing two nuclei, while in a few other cells two zoospores were found lying alongside one another". On the basis of this evidence COOK and SCHWARTZ were confident that the zoospores fuse to form zygotes. AYERS (1944) reported that the zoospores have two unequal flagella and that when newly released are uniform in size and quite small, being 1.9 to 2.3 μ in diameter. He observed that the spores, which were soon discharged when infected roots of cruciferous seedlings were placed in tap water, escaped through openings which develop at the points of attachment of the sporangia to the host-cell wall. Neither discharge into the root hair nor the presence of zoospores within the root hair were seen. About the same time, similar observations were made by SAMUEL and GARRETT (1945) but they found that zoospores are sometimes discharged into the cavity of the root hair. They also noticed refringent bodies in root hairs with empty zoosporangia and suggested that these might possibly be zygotes resulting from the fusion of two zoospores.

MATERIALS AND METHODS

Infected roots with zoosporangia were obtained by growing cabbage seedlings in garden soil heavily infested by mixing with a suspension of *P. brassicae* resting spores. The soil was previously acidified with sulphuric acid to bring the reaction to pH 5.9. To prepare the spore suspension, club root galls were cut to pieces, kept for 1 week in a beaker at a temperature of 23 °C to disintegrate, strained through a 50 μ sieve and washed six times by centrifugation. Each time the

spores were thrown down in the centrifuge tubes the water was poured off and the spores resuspended in fresh water in a Waring blendor. The final suspension containing 888×10^6 resting spores per ml. was stored in a refrigerator. The cabbage seedlings were grown in glass cylinders 4 cm in diameter and 5 cm high closed at one end with nylon gauze held by a rubber band. To prevent the growth of algae the exterior of each cylinder was painted successively with black and white paint. Each cylinder was filled with 50 g. dry soil with which a total of 10^9 resting spores had been mixed. Ten germinating cabbage seeds were sown in each cylinder and the soil moisture content brought to 80% saturation by absorption of the required amount of water from beakers slightly larger than the cylinders. As soon as all the water was absorbed, the cylinders were transferred to 2000 ml beakers, four in each beaker. The beakers were covered with sheets of polyethylene to prevent loss of water and then placed in a greenhouse with a temperature of 18–20 °C. When plants were needed for investigation the gauze was removed from the bottom of a cylinder and the whole contents pushed into a dish. The roots were removed very carefully from the soil and then washed with a fine jet of water. The roots were next transferred to a petri dish containing a shallow layer of water and examined with a hand lens. Very thin rootlets about 2–3 cm long, bearing root hairs were cut off and mounted in water. The cylinders were refilled with the soil and used for another „crop" of seedlings. For microscope observations both direct and phase-contrast illumination were used. Some permanent preparations were made using a modified Löffler's stain (COUCH, 1941).

RESULTS

Generally, root-hair infections had developed sufficiently one week after the beginning of each experiment, to allow successful observations. The intensity of root-hair infection in successive seedling crops did not decrease within two months. Zoospore discharge started about 30 min. after mounting the rootlets in water and continued for several hours. Generally, the zoospores dispersed rapidly in the water, but in one preparation, they stayed near the root hair from which they originated (fig. 1). Twice, a zoospore was observed swimming round the periphery of an otherwise empty zoosporangium. Several times zoospores were seen swimming around in the cavity of a root hair. The zoospores, which have 2 unequal flagella, swam with the short flagellum in front and the long flagellum trailing behind. When swimming, their shape varied, but was generally pyriform. Eventually the zoospores slowed down, became stationary and spherical. The long flagellum sometimes undulated for some time after the zoospore had become motionless. Some of the zoospores were slightly larger and were observed to have two long and two short flagella. Zoospores were occasionally observed swimming around in pairs, connected to each other by a tiny thread which in some instances was so short that the spores touched each other. Usually one of the two zoospores was spherical, the other irregular in shape. The stained zoospores (fig. 2) measured $3.01 \pm 0.07\,\mu$ in diameter. The lengths of the long and short flagella were $11.80 \pm 0.23\,\mu$ and $3.40 \pm 0.15\,\mu$ respectively. Though most of the zoospores are biflagellate, some with four (fig. 3), six (fig. 4) and even eight flagella were observed in the stained preparations.

DISCUSSION

Zoospores from the zoosporangia of both *P. brassicae* and *Spongospora subterranea* behave in a very similar way (KOLE, 1954) except that those of *Spongospora* appear to be discharged more readily. Compound zoospores of *P. brassicae* have now been found. From what is already known about the zoospores of *S. subterranea* the observation of living zoospores of *P. brassicae* connected by a tiny thread is evidence that these compound spores are the result of fusion of two or more biflagellate zoospores. Fusion of zoospores in pairs might indicate a sexual stage in the life history of the fungus but it is not known whether karyogamy occurs at this time. Further investigations of the development of the compound zoospores are needed to show what happens after zoospore fusion and whether the nuclei fuse. Chromosome counts throughout the life cycle would also serve to locate a sexual stage.

SAMENVATTING

Het onderzoek had ten deel het gedrag van de zoösporen uit de zoösporangia van *Plasmodiophora brassicae* na te gaan. Aangetaste worteltjes van jonge koolplanten, opgekweekt in zwaarbesmette grond, werden goed afgespoeld en in stukjes ter lengte van 2–3 cm geknipt. Hiervan werden waterpreparaten gemaakt, waarin na ongeveer 30 min. de zoösporen uit de zoösporangia begonnen vrij te komen. Het merendeel van de zoösporen had één lange en één korte zweepdraad, maar bij sommige zoösporen werden twee lange en twee korte zweepdraden waargenomen. Enkele malen werd waargenomen, dat twee zoösporen, door een draadvormige verbinding aan elkaar gekoppeld, op korte onderlinge afstand gepaard rondzwommen. In preparaten met gekleurde zoösporen werden behalve zoösporen met één lange en één korte zweepdraad, ook zoösporen met twee lange en twee korte, drie lange en drie korte en zelfs vier lange en vier korte zweepdraden aangetroffen. Op grond van deze waarnemingen wordt – in overeenstemming met hetgeen van de nauw aan *P. brassicae* verwante schimmel *Spongospora subterranea* bekend is – geconcludeerd, dat samengestelde zoösporen ontstaan doordat twee of meer enkelvoudige zoösporen versmelten. Het is niet uitgesloten, dat deze versmelting van zoösporen het begin is van een geslachtelijke phase in de levensloop van de schimmel, maar een kernversmelting in de samengestelde zoösporen kon niet worden aangetoond.

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