# MORPHOLOGY AND CYTOLOGY OF THE ZOOSPORANGIA AND CYSTOSORI OF *SOROSPHAERA VERONICAE*

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The cystosori of *Sorosphaera veronicae* which occur so commonly in enlargements of the shoot system of *Veronica* have been known since their discovery by Schroeter in 1897. In the mycological laboratory at Chapel Hill this parasite, which occurs abundantly close to the botany building, has been used for many years in the mycology classes. In this laboratory thorough searches were made for zoosporangial thalli, but without success, until in February, 1956, for the first time entire host plants were brought into the laboratory and, after the root systems had been washed, they were examined for zoosporangial thalli. In these roots zoosporangial thalli of a plasmodiophoraceous organism as well as zoosporangia of a chytrid were found. Could these zoosporangial thalli be the long sought sporangial stage of *Sorosphaera*? It was in the hope of throwing some light on this problem that this study was undertaken.

Zoosporangia have been found in all true members of the Plasmodiophoraceae except *Tetramyxa* and *Sorosphaera*, in which only the cystosori are known. This paper contains an announcement of the discovery of the zoosporangia of *Sorosphaera veronicae*, with a description of their morphology and cytology, and some new observations on the cystosori.

## Morphology and Life History Studies on Living Materal

*Initial observations, experiments and results.*— The initial stages of this study were confused by the several apparently different stages in parasite development which were found. Plasmodia and cystosori typical of *Sorosphaera veronicae* were present in the galls produced on the shoots of the host. Cystosori and sporangiosori which seemed to belong to the genus *Ligniera* or possibly *Polymyxa* were found in the roots. In addition, relatively giant sporangia-like structures with large discharge apertures were present as well as small star-shaped resting thalli and spherical, thin-walled sporangia each with or without a single tube-like discharge pore. With further study it became clear that there were two plasmodiophoraceous parasites present in the roots of *Veronica persica* and *V. hederaefolia: Ligniera verrucosa*, including both the cystosori and the previously unknown sporangial stage which is to be described in a later paper; and the giant sporangia mentioned above. The small star-shaped resting thalli and spherical thin-walled sporangia were found to be *Asterocystis radicis*, an olpidiaceous parasite of the roots of some crop plants. The large plasmodiophoraceous sporangia were assumed to be the heretofore unknown sporangia of *Sorosphaera veronicae*.

*Veronica persica*, because of the very large number of seeds which it produces, was used exclusively as the host in the host-parasite cultural studies. Seeds and galls from the aerial portions of this plant, which begins growth in October and November, flowers during December through April, and fruits in April and May, were collected in large quantities. During April, 1956, attempts to germinate freshly collected seeds of the host plant, so that cultural studies could be started, were unsuccessful. This was not surprising, for as Crocker and Barton (1953) point out, weed seeds remain dormant and viable for long periods of time in the soil, until physical and chemical changes have taken place. Attempts were made to break this dormancy. Scarification, sulfuric acid and boiling water treatments of seeds did not prevail, for no seeds germinated. Seeds were washed with slowly running water to remove possible germination inhibiting substances. Experiments whereby after-ripening changes in these seeds could be sped up were started. Some seeds were placed in a moist condition at a temperature of 2°–4°C.; alternating temperatures of 15°–30°C., sixteen hours in the

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former and eight hours in the latter, were tried. Under none of the conditions described above did germination occur, and the seeds were then stored in the refrigator at 2°–4°C., dry, in hopes that low temperature and time might aid in breaking dormancy.

Six months later some of these seeds were taken out of the refrigerator and placed in petri dishes on moist filter paper at room temperature. After one week only four of fifty seeds had germinated. These same dishes containing the ungerminated seeds on moist filter paper were now returned to the refrigerator. Nine days later, ninety per cent of these seeds germinated when they were returned to room temperature. Repeated experiments indicated that generally six days in a moist condition at 2°–4°C. after the initial wetting would give high percentages of germination.

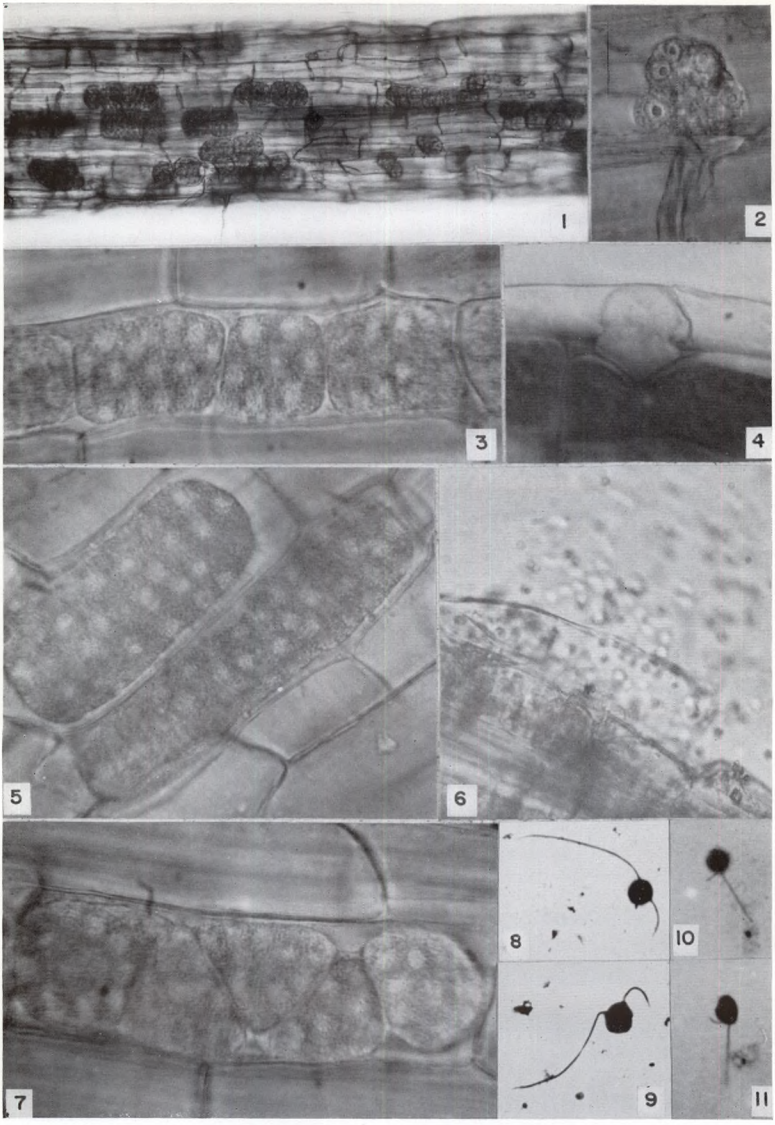
Now that the seeds would germinate, plans to grow and infect the seedlings with the parasite could be realized. Soil was obtained from one of the areas in which the host plant and the parasite were found. This soil was spread out in large stainless steel trays and autoclaved for thirty minutes at fifteen pounds pressure on three successive days. The sterile soil was then placed in new clay pots, and these were autoclaved at fifteen pounds pressure for fifteen minutes. Into each of the six resulting pots were planted several young seedlings of *V. persica*. These plants were grown in the algal culture room at temperatures and periods and intensities of light which simulated the external environmental conditions under which the host plant, a winter annual in this part of the United States, thrives.

The zoosporangia of this parasite were collected by first searching for host plants bearing galls on their shoot systems. When a desired specimen was found, an area of soil surrounding the plant, deep enough to include the terminal portions of the roots, was dug up. After this material had been brought back to the laboratory, it was washed with cold tap water to remove all the soil; at the same time other roots and debris present were removed. The root system thus freed from external foreign matter was then placed in ice water so that sporangial discharge, which occurred best in cold water, was promoted. Unlike the cystosori, which produce distinct galls on the shoot, the zoosporangial thalli produce no external symptoms such as hypertrophy or discoloration by which their presence may be detected with the unaided eye. It was necessary therefore to cut small bits off the ends of the root system which were then mounted on slides for viewing with the compound microscope, or a search was made with a dissecting microscope for heavily infested roots and these were cut from the plant. Zoosporangia thus collected were placed in small dishes of cold water and allowed to discharge their spores. This water containing swimming zoospores was used in the experiments described below.

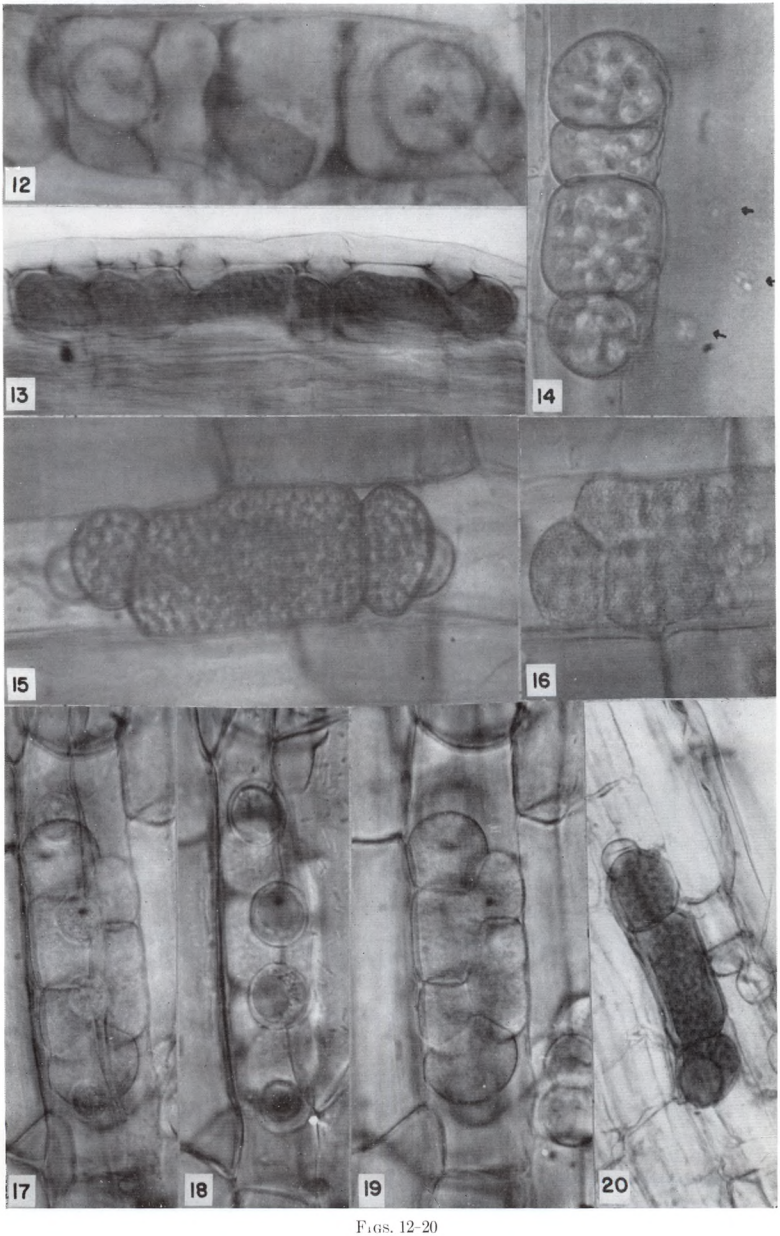
*Ligniera verrucosa* was occasionally found parasitizing the same host plant. This parasite completes its life cycle in the epidermal and subepidermal cells of the roots of the host. Unlike *Sorosphaera veronicae*, which forms zoosporangia in the roots and cystosori in the shoot system, *L. verrucosa* forms both structures in the roots. Also the zoosporangia of *S. veronicae* have never been found in root hairs, but both zoosporangia and cystosori of *L. verrucosa* occur in root hairs as well as in unmodified epidermal cells. In selecting host roots infested with zoosporangia to be used for the collection of zoospores of *S. veronicae*, efforts were made to exclude those roots which were found to contain zoosporangia and/or cystosori of *L. verrucosa*. Because of this, probably, zoosporangia or cystosori of *L. verrucosa* were never found in any of the cells of the experimental plants.

Two of three pots with host plant seedlings as much alike as possible growing in them were flooded several times with water containing swimming zoospores. As a result of this, the seedlings were often covered with water for several minutes. The third pot, the control, was watered with distilled water. One week later another experiment exactly as above was set up, making a total of four pots which had zoospores added to them. Approximately two months later, the roots of the plants in three of the four pots were found to be heavily parasitized by the plasmodia and zoosporangia of *S. veronicae*. In addition one plant produced a relatively large gall in the axil of the cotyledon. This gall contained typical cystosori of *S. veronicae*. The plants in the two control pots showed no evidence of plasmodia, zoosporangia or cystosori. All the plants in the above experiments were flooded with distilled water every day, and they were watered with a complete nutrient solution (Greulach, 1952) once a week.

Because the soil as used in the above two experiments contained large amounts of organic matter which tended to support contaminating




FIGS. 1–11. *Sorosphaera veronicae* in root cells of *Veronica persica*. Figs. 1–7 from living material. Fig. 1. Host cells infected with plasmodia and zoosporangia. ×176. Fig. 2. Young plasmodium showing nuclei in interphase. ×819. Fig. 3. Young plasmodia. ×819. Fig. 4. Enlargement of a single discharge pore of sporangia pictured in fig. 13. ×1088. Fig. 5. Large multinucleate plasmodia. ×819. Fig. 6. Discharge of zoospores. ×819. Fig. 7. Five plasmodia in a single cell. ×1229. Figs. 8, 9. Biflagellate heterocont zoospores from zoosporangia killed and fixed over 2 per cent osmic acid fumes and stained using Löffler technique. Note whip-lashes on both flagella. ×1088. Figs. 10, 11. Biflagellate heterocont zoospores from resting spores of cystosori stained with crystal violet. ×1135.




FIGS. 12-20

fungi such as *Penicillium* and *Rhizopus*, it was decided to use sand and a complete nutrient solution as a substrate in future repeat experiments. Therefore, 30 mesh pure quartz sand was washed for 24 hours in running tap water and sterilized in an oven at 270°C. for 24 hours. This sand was then placed in new clay pots, and two of these pots were then placed in a plastic container which held the supply of nutrient solution.

*Veronica persica* seedlings were planted in pots as prepared above. Into two of these pots, cold water containing swimming zoospores of the parasite was poured. Finely ground galls of the host were sprinkled on the surface of the sand of two other pots. Approximately every five days the remaining nutrient solution was poured off and a fresh supply added to each container. At the end of one month the plants were removed from the pots and examined. In the experiment where zoospores were added to the young seedlings, almost all of the plants were found to have large numbers of plasmodia and zoosporangia in their roots. In addition, two plants, one in each of the experimental pots, produced severely hypertrophied shoot systems which were found to be galls containing typical cystosori of *Sorosphaera veronicae*. The roots of the plants in the other two pots to which had been added finely ground galls were found to contain large numbers of plasmodia and zoosporangia of the parasite. The experiment described above where finely ground galls containing vast numbers of *S. veronicae* cystosori were sprinkled on young host plant seedlings was repreated two more times. The gall material used in all experiments of this type had been stored dry for ten to twelve months before it was ground. Every experimental plant in all four pots of both experiments was found to have abundant plasmodia and zoosporangia in its root system. In all experiments reported above the control plants never showed any evidence of parasite infection.

Additional evidence which supports the premise that the sporangia and plasmodia found only in the roots of *V. persica* and the cystosori found only on the shoot system are part of the life cycle of *S. veronicae* was obtained from a survey of host plants from widely separated areas on the campus. Whenever galls were found on the shoot systems of the host plants, plasmodia and zoosporangia were found in the root systems. Also, the zoospores of cystosori and zoosporangia are biflagellate and heterocont, typical of those found in the Plasmodiophoraceae (Figs. 8–11), and cytological studies reported below indicate that both phases are plasmodiophoraceous parasites.

*Sorosphaera veronicae* was described as a parasite on *Veronica hederaefolia*, *V. chamaedrys* and *V. triphyllos* by Schroeter. Since that time it has been found parasitizing ten other species of this genus by various workers. On the campus of the University of North Carolina, *S. veronicae* has been observed parasitizing *V. hederaefolia*, *V. persica* and *V. peregrina*, the latter two being newly reported hosts.

*Morphology and Development of Sporangia.*—The zoosporangium of *Sorosphaera veronicae* begins its development in the epidermal cells, root hairs excepted, and cortical cells of the roots of *Veronica* as a uninucleate, naked thallus (Fig. 85). This thallus grows and eventually becomes a naked multinucleate protoplast usually referred to as a plasmodium (Figs. 1–3, 5, 7, 16, 86–89, 92). The heavily granular cytoplasm of the living plasmodium surrounds relatively clear areas, which are the nuclei of the thalus. Eventually the plasmodium ceases growth, and it may become lobed or segmented into segments of various sizes (Fig. 1). Exactly how this process occurs has not been determined. Walls are eventually formed around these multinucleate segments (Figs. 13, 15, 19, 20, 90, 91). However, each segment apparently remains connected with adjacent ones, for the zoospores seem to be able to swim without hindrance from one segment to

FIGS. 12–20. *Sorosphaera veronicae* in root cells of *Veronica persica*. All Figs. except 13 and 20 from lining material. Fig. 12. Empty zoosporangia. ×1088. Fig. 13. Zoosporangia with discharge pores seen in side view. ×503. Fig. 14. Discharging zoosporangia. Zoospores swarming in sporangia; some have escaped (see to right of sporangia). ×819. Fig. 15. Zoosporangia with zoospore initials. ×754. Fig. 16. Young plasmodia. ×819. Figs. 17–19. The same sporangia photographed at different focal levels of the microscope. ×754. Fig. 17. Double exposed photograph showing the focal level as seen in fig. 18 superimposed on the focal level as seen in fig. 19. Fig. 18. Top view of discharge pores, taken at higher focal level. Fig. 19. Photograph taken at a lower focal level so that discharge pores are not seen. Fig. 20. Undischarged zoosporangia on left; empty zoosporangia to the right. ×503.

another. Also several segments may share a single discharge pore (Figs. 17, 18, 19, 20).

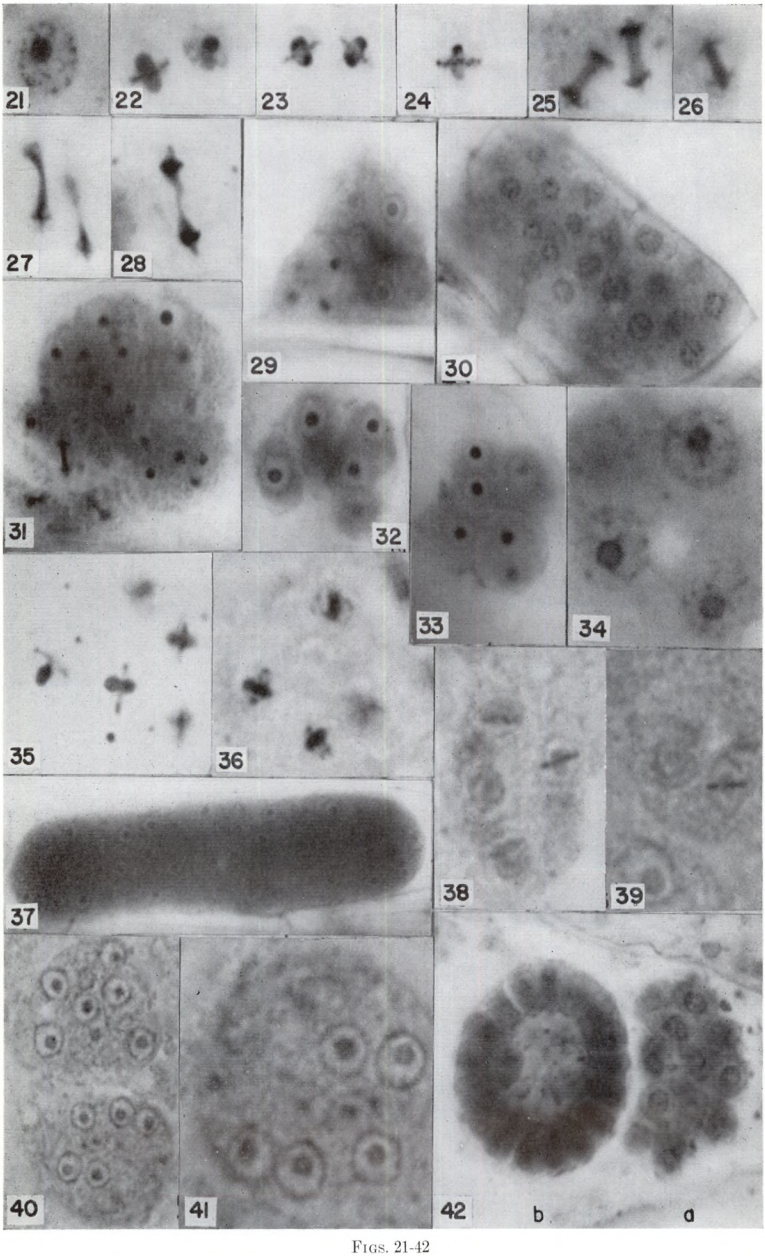
Actual penetration of the host cells by the infecting zoospore has not been seen, although many zoospores which had encysted on epidermal cells of fresh, young *V. persica* seedlings were watched for long periods of time. Empty cysts were not observed on the surface of host cells. Possibly these could have been washed away during the preparation of the material for study. On the other hand, Ledingham (1939) also found no empty cysts on the surface of root hairs after penetration by the zoospore of *Polymyxa graminis*. He suggested that possibly, as was found by Curtis (1921) for *Synchytrium endobioticum*, the entire amoeba passes into the cell.

The terms sporangiosorus and sporangium are useful in designating zoospore-producing structures in fungi. Usually the sporangiosorus is an aggregation or sorus of sporangia, each sporangium producing its own discharge pore as found for example in the Synchytriaceae. However, in some of the Plasmodiophoraceae several zoosporangia may be connected by narrow isthmuses as was reported by Couch, Leitner and Whiffen (1939) in *Octomyxa achlyae*. Goldie-Smith (1951, 1954) reports for *Sorodiscus cokeri* and *Woronina polycystis* and Couch, Leitner and Whiffen (1939) for *Octomyxa achlyae* that sporangia in the interior of the sorus do not produce exit tubes but that their zoospores escape by passing from one sporangium to another "through narrow pores that develop in contiguous walls" (Goldie-Smith, 1951). In *Polymyxa graminis*, a genus erected by Ledingham (1939) because the zoosporangial characteristics of this organism differed from those of other genera of the Plasmodiophoraceae, large septate zoosporangia are found. Each multinucleate segment or lobe of these unusually large sporangia has one or more discharge tubes. The zoospores are not able to pass from one segment to another. Thus in this group of organisms it is sometimes not easy to determine the limits of a sporangium.

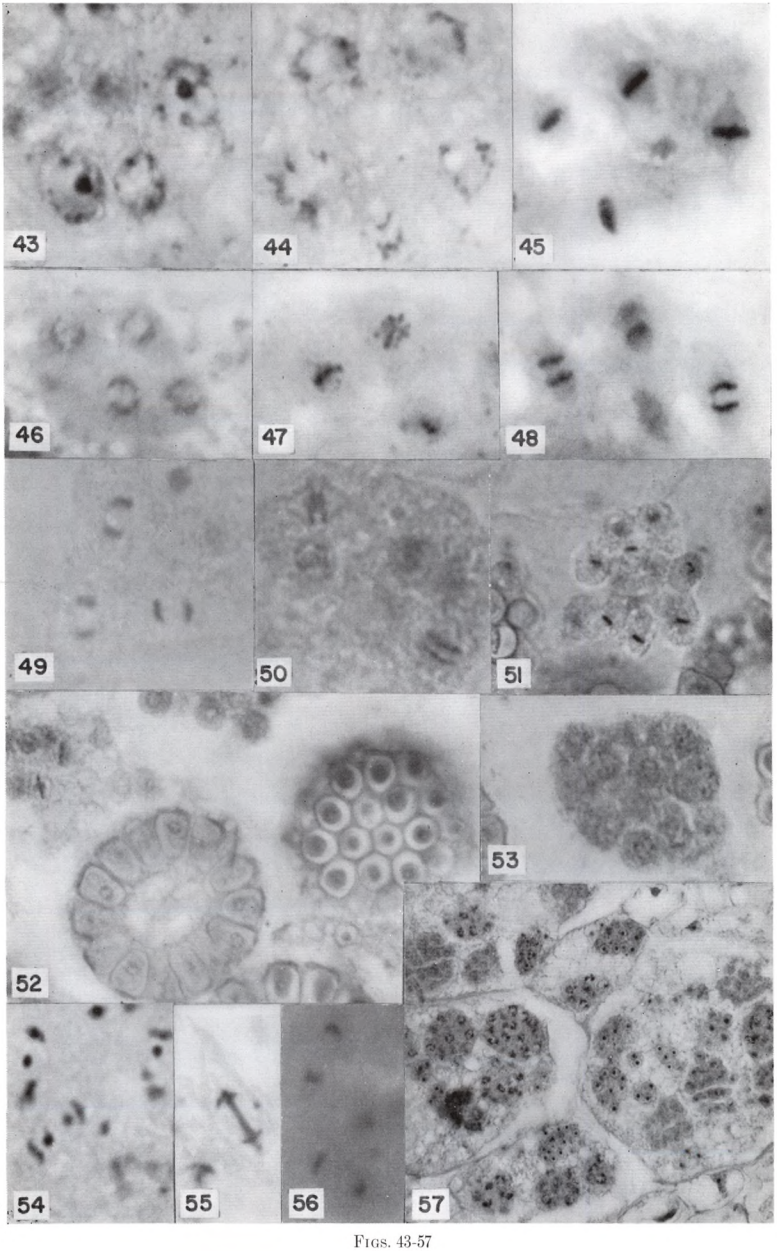
In *Sorosphaera veronicae* this problem seems especially perplexing. Here the compound structure (sporangiosorus ?) appears to be composed of several distinct units or segments (sporangia ?) (Figs. 12, 14, 15, 17–20, 90, 91). However, the septa of these apparently distinct segments do not prevent zoospores from swimming to adjacent units. Often these units share a common discharge pore, and they have always been observed to be in the same stage of development (Figs. 12, 14, 15, 19, 20). It seems necessary to consider if a compound structure composed of units connected by isthmuses or pores is truly a sorus of sporangia. Should it be considered a single lobed or catenulate sporangium? It appears then that there may be a variety of sporangial expressions in the Plasmodiophoraceae, and as Ledingham points out, in this respect this group of organisms is not unique.

The discharge papilla is large, having generally a bulbous shape (Figs. 4, 13, 18, 90, 91). The very large aperture provides in most cases a passage for escape of zoospores from different units of the same sporangium. The escape of zoospores may be made possible by dissolution of the host cell wall at points where the discharge papilla comes in contact with it, or as Ledingham (1935) observed in *Spongospora subterranea*, the pressure on the host cell wall which results from these bulbous swellings may rupture it. The size and

FIGS. 21–28. Stages in cruciform division in zoosporangial plasmodia. Stained with crystal violet. Fig. 21. Interphase nucleus. ×2425. Fig. 22. Cruciform stage, one figure in polar view. ×2910. Fig. 23. Cruciform stage. ×2790. Fig. 24. Cruciform stage. Note chromosomes in ring across the nucleolus. ×2700. Figs. 25, 26. Dumbbell or double anchor stages. ×2910. Fig. 27. Telophase stage. Nucleolus has begun to constrict. ×2910. Fig. 28. Final constriction of nucleolus. ×2910. Figs. 29–36 stained with crystal violet. Fig. 29. Young zoosporangial plasmodia. ×1395. Fig. 30. Developing zoosporangium. ×1455. Fig. 31. Multinucleate zoosporangial plasmodia. One in double anchor stage of division. ×1395. Fig. 32. Young plasmodial thalli in root cells. ×1455. Fig. 33. Young zoosporangial plasmodia. ×1455. Fig. 34. Interphase nuclei of zoosporangial plasmodium. ×2910. Fig. 35. Cruciform stage of cruciform division in cystosoral plasmodia. ×2910. Fig. 36. Cruciform stage of division in cystosoral plasmodia viewed obliquely. Note discontinuous ring of chromosomes. ×2910. Fig. 37. Large multinucleate plasmodium in epidermal cell of the roots. Mounted in Amann's Lacto-phenol plus acid fuchsin. ×970. Figs. 38, 39. Cruciform stages in cruciform division in cystosoral plasmodia. Stained using Feulgen technique and light green. ×2910. Fig. 40. Multinucleate cystosoral plasmodia. Stained using Feulgen technique and light green. ×1455. Fig. 41. Interphase nuclei of cystosoral plasmodium. Stained using Feulgen technique and light green. ×2910. Fig. 42a. Incipient resting spores. Stained with crystal violet. ×1455. Fig. 42b. Young cystosorus. Stained with crystal violet. ×1455.




FIGS. 21-42




FIGS. 43-57

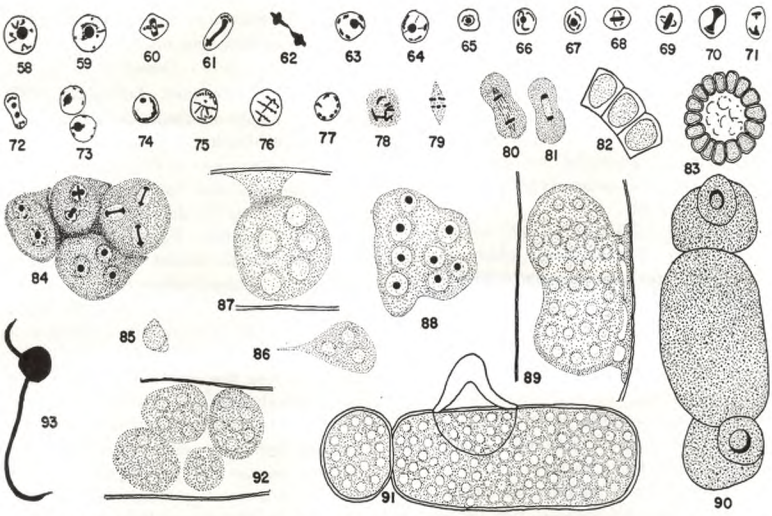
shape of the zoosporangia vary considerably. Often the final size is determined by the host cell, but just as frequently the plasmodium stops growing before filling the host cell. Hypertrophy of the cells of the roots caused by the plasmodia or zoosporangia has never been observed, and this lack of hypertrophy may be one of the reasons why these structures have not been discovered previously.

*Sporangial Discharge, Structure and Behavior of Zoospores.*—The zoospores, which are fully developed in the zoosporangium, exhibit a gentle rocking motion just prior to discharge. The first spore to escape seems to be forced out under pressure while the ones later escaping actually swim out (Figs. 6, 14). The method of discharge is much like that found in *Saprolegnia*, i.e. the first zoospores, expelled under pressure, one by one, remain quiescent, just barely rocking, for approximately thirty seconds, and then with a slight jerk, swim off clumsily and erratically. If conditions are kept suitable, cool temperature being the most important, the swimming becomes quite rapid and more graceful. At discharge and as long as it swims, the zoospore is bulged in the center with pointed ends. Possibly because conditions become unsuitable, the zoospore begins to encyst. As this happens the spore body becomes spherical, and the long posterior flagellum is dragged along behind as the anterior flagellum lashes slowly back and forth. Swimming in this relatively inactive manner, the zoospore may rotate in a circle. Eventually it encysts. No certain examples of amoeboid zoospores were observed.

Prior to the work of Ledingham (1934, 1935, 1939) the zoospores of the Plasmodiophoraceae were reported by all observers as posteriorly uniflagellate. Since that time biflagellate heterocont zoospores have been reported in *Plasmodiophora*, *Spongospora*, *Octomyxa*, *Polymyxa*, *Sorodiscus* and *Woronina*. Ledingham (1935) figured the flagella of *Spongospora subterranea* as thick and blunt-ended. In 1939 he reported the flagella of the zoospores of *Polymyxa graminis* as being blunt-ended also, but close observation of his figure 25, Plate III, shows a whip-lash on the end of each flagellum. Ellison (1945) reported blunt-ended flagella without whip-lashes on the zoospores of *Plasmodiophora brassicae*. Couch, Leitner and Whiffen (1939, plate 48, fig. 3) include a photomicrograph of a zoospore of *Octomyxa achlyae* which appears to have a whip-lash on the long flagellum while the short flagellum apparently lacks the whip-lash. Pendergrass (1950, fig. 14) includes a figure of a zoospore of *Octomyxa brevileginae* which seems to have a whip-lash on the posterior flagellum, and he reports that no tinsels were observed after using the Löffler flagellar staining technique. Dr. W. J. Koch (unpublished research) found whip-lashes on both flagella of the zoospores of *Woronina glomerata*, which he found parasitizing the alga *Vaucheria*. Goldie-Smith (1951) reports biflagellate heterocont zoospores in *Sorodiscus cokeri*, but her figures do not indicate, nor does she report, that whip-lashes are present on the flagella. In 1954 Goldie-Smith reported receiving a communication from Dr. J. T. Barrett which included a photomicrograph showing that each flagellum of the zoospores of *Woronina polycystis* ended in a whip-lash, and she further included in this paper photomicrographs which show the zoospores of her isolate of *W. polycystis* as having a whip-lash on each flagellum.

In the light of the above discussion, and since

FIG. 43. Early prophase of first sporogenic division of cystosoral plasmodia. Stained with crystal violet. ×2910. Fig. 44. Late prophase of same; nucleolus has disappeared. Stained with crystal violet. ×2910. Fig. 45. Metaphase of same. Stained with crystal violet. ×2910. Fig. 46. Prophase (?) of same. Stained with crystal violet. ×1940. Fig. 47. Metaphase of same. Polar view. Stained using Feulgen technique and light green. ×2910. Fig. 48. Anaphase of same. Stained with crystal violet. ×2910. Fig. 49. Telophase of second sporogenic division. Stained using Feulgen technique and light green. ×2910. Fig. 50. Beginning anaphase or so-called double anchor or dumbbell stage of cruciform division in cystosoral plasmodia. Stained using Feulgen technique and light green. ×2910. Fig. 51. Metaphase of second sporogenic division. Stained using Feulgen technique and light green. ×1455. Fig. 52. Incipient resting spores and cystosori. Stained using Feulgen technique and light green. ×1455. Fig. 53. Incipient resting spores. Stained using Feulgen technique and light green. ×1455. Fig. 54. Stage of cruciform division in cystosoral plasmodia just prior to division of nuclear membrane. Stained with crystal violet. ×2910. Fig. 55. Double anchor stage of cruciform division in cystosoral plasmodia. Stained with crystal violet. ×2910. Fig. 56. Telophase of second sporogenic division. Stained with crystal violet. ×2910. Fig. 57. Cross section view of gall of *V. persica* showing host cells containing cystosoral plasmodia. Stained with crystal violet. ×450.




FIGS. 58–62. Stages in vegetative nuclear division in zoosporangial plasmodia. Stained with crystal violet. Fig. 58. Interphase nucleus. ×1250. Fig. 59. Interphase nucleus. ×1200. Fig. 60. Cruciform stage. ×1200. Fig. 61. Final division stage. ×1250. Fig. 62. Final division stage. ×1200. Figs. 63–81. Stages in nuclear divisions in vegetative and sporogenic divisions of cystosoral plasmodia. Stained with crystal violet. ×1250. Figs. 63, 64. Interphase nuclei of vegetative divisions. Figs. 65–67. Cruciform stages of vegetative divisions in polar view. Figs. 68, 69. Cruciform stages of vegetative divisions in oblique view. Fig. 70. Dumbbell stage of cruciform division. Fig. 71. Final division of nucleolus in cruciform division. Fig. 72. Division of nucleus. Note four chromosomes present. Fig. 73. Ring of Saturn stage of cruciform division. Figs. 74–76. Zygotene (?), pachytene (?) and diplotene (?) respectively of the sporogenic division. See text. Figs. 77, 78. Late prophases of sporogenic divisions. Fig. 79. Anaphase of sporogenic division. Fig. 80. Anaphase of second sporogenic division in uninucleate thallus. Fig. 81. Telophase of second sporogenic division in uninucleate thallus. Fig. 82. Resting spores. ×1200. Fig. 83. Cystosorus. ×540. Fig. 84. Young zoosporangial plasmodia. One plasmodium with nuclei in cruciform stage, another with nuclei in dumbbell stage. Stained with crystal violet. ×1250. Fig. 85. Young infecting thallus in roots of host. ×880. Fig. 86. Young zoosporangial plasmodium. ×880. Fig. 87. Developing zoosporangial plasmodium. ×880. Fig. 88. Living multinucleate zoosporangial plasmodium with distinct nuclei. ×880. Fig. 89. Multinucleate zoosporangial plasmodium. ×605. Fig. 90. Young zoosporangia. ×605. Fig. 91. Zoosporangia with bulbous discharge pore. ×880. Fig. 92. Young zoosporangial plasmodia. ×605. Fig. 93. Biflagellate heterocont zoospore from zoosporangium. Stained using Löffler technique. ×1200.

the occurrence of a whip-lash on both flagella of a heterocont zoospore is unique in the fungi, it seemed that an investigation of the zoospores of the sporangia of *S. veronicae* would be interesting and informative. Zoospores were stained with Löffler's flagellar stain or gentian violet. These techniques have been used to demonstrate tinsels on the anterior flagellum and the whip-lash on the posterior flagellum of the zoospores of various aquatic fungi by Couch (1941). No tinsels were found on either flagellum, but a whip-lash was found at the tip of each flagellum, the anterior one being shorter than the posterior (Figs. 8, 9, 93).

Zoospores treated as above are spherical in shape and measure 5.6–7.0$\mu$, with most 6.3$\mu$, in diameter. The nucleus is in the center or slightly to one side of the spore body. The nucleolus is not conspicuous. Because the nucleus stains so densely, it was not possible, with the techniques used, to determine the point of attachment of the flagella, although these could be traced to the surface of the nucleus. There can be seen in some zoospores, in the finely granular cytoplasm,

darkly staining "lines" which appear to radiate from the nucleus and reach almost to the periphery of the cell. The short flagellum is 5.6–7.7$\mu$ long, and the long flagellum is 16.8–22.4 $\mu$ in length.

*Morphology and Development of Cystosori.*— Galls produced by *S. veronicae* in the shoot of *Veronica persica*, and to a lesser extent on *V. hederaefolia*, range in size from minute, almost microscopic swellings to tumors which may reach 3–4 cm. in length and 1 cm. in diameter. Usually they are 1–2 cm. in length and 0.25–0.5 cm. in diameter. In some cases infection is so severe that the entire shoot system appears to be one huge gall. If a cross section of gall material is made, it can be seen that each gall is generally composed of healthy cells, usually at the periphery of the gall, and masses of infected and modified cells with a few vessels showing here and there. The infected cells are extremely enlarged and may be multinucleate; they contain various stages in the development of the parasite (Fig. 57). Up to ten mature cystosori have been counted in one host cell. More detailed descriptions of the action of the parasite on the host cells of four other species of *Veronica* can be found in works by Maire and Tison (1909) and Blomfield and Schwartz (1910).

There is general agreement in the literature that the parasite invades the host plant in the apical meristem. Because of this the galls may involve almost the entire stem. In agreement with the observations of Blomfield and Schwartz (1910), no evidence was found to show that the parasite was able to move from cell to cell. Thus it would seem as they suggest, that the parasite is passively distributed by the repeated division of already infected cells, an opinion also expressed by Nawaschin (1899) in his paper on *Plasmodiophora*. In *V. persica*, infection seems to take place in the following manner. Some of the zoospores, liberated from the zoosporangia at times when soil water is plentiful, reach the surface of the soil. Because the host plants, after they have achieved some size, are recumbent, many apical meristems in the axils of leaves are available to the zoospores, which are able to swim for long periods of time under the cold conditions that are found during the winter months. These zoospores penetrate the host cells of the apical meristem and develop there into multinucleate plasmodia. Further development includes cleavage of plasmodia into incipient resting spores which are arranged in the form of hollow spheres or ellipsoids (Figs. 42, 52). The walls thicken and turn golden-brown upon achieving apparent maturity.

Circumstantial evidence in favor of the above conclusions may be had by observing infected plants, which in almost all cases will show that the lower axillary buds on the plants are infected. In addition, experimental evidence is available to substantiate the above argument, for galls were produced, under laboratory conditions, in the axillary buds of some *V. persica* plants by keeping the plants recumbent and by flooding the soil in the pots with water daily. Previously zoospores from sporangia had been added to the pots, and these were known to have infected the roots and to have produced zoosporangia there.

Maire and Tison (1909) claim that the plasmodium, by the time it has become eight-nucleate, may undergo schizogony and produce uninucleate or multinucleate meronts. Thus vegetative increase in the number of plasmodia takes place. No such happenings can be reported from this study. However, because of the intrinsic difficulty of such determinations on material of this nature, the above results are not considered to be definitive. On the other hand, Karling (1942) states ". . . that these workers [Maire and Tison] have never observed the actual splitting off of meronts, and their reports on the presence of schizogony are based only on the appearance of constricted plasmodia and the great abundance of uninucleate amoeba in infected cells."

Ivimey Cook (1933) states that the cystosori of *S. veronicae* are "enclosed in a universal membrane." Webb (1935) reports that a thin envelope covers the cystosorous after a thick wall is laid down around each spore. Other workers make no mention of such a membrane; no membrane, enveloping the cystosori of this isolate of *S. veronicae*, was found in the present study.

Living cystosori removed from freshly collected galls were found to be generally spherical and ellipsoidal and occasionally pyriform and irregular in shape. Spherical cystosori were 18.2–32.2$\mu$ in diameter, mostly 28.0$\mu$ in diameter; ellipsoidal ones 21.0 × 18.2$\mu$–36.4 × 32.2$\mu$ in diameter. Cystosori were separated into individual spores by mounting them on a slide in concentrated sodium hydroxide and pressing on the cover slip. Individual resting spores were generally pyriform and pyramidal in shape, spherical on the surface forming the outside of the sphere and six-sided at the base which forms the inside of the hollow sphere (Figs. 42, 52, 82, 83). They measured 6.3 × 4.2 × 2.8–7.0 × 4.9 × 3.5$\mu$.

Ledingham (1939) reports that he germinated

the resting spores of *S. veronicae*. According to Karling (1942), Professor J. T. Barrett germinated the resting spores of this parasite; he was unable to determine the number of flagella on the zoospores. After extensive periods of wetting, freezing and thawing, the cystosori of this isolate were germinated several times. It is thought that each resting spore produces one zoospore. The zoospores were found to be biflagellate and heterocont (Figs. 10, 11). The spore body was slightly smaller than the body of the zoospore from the zoosporangium, ranging from 4.0 to 4.8$\mu$ in diameter.

## Cytological Studies

Ever since Nawaschin (1899) first observed that nuclear division during vegetative development of the plasmodium in *Plasmodiophora brassicae* differed from typical mitosis, cytological studies in this group for the past fifty years in the main have dealt with this type of nuclear division. Because of the minuteness of the nuclei, the ephemeral nature of these stages, the unselectivity of nuclear stains used, and the influence of protozoological findings, some conflicting information concerning these parasites can be found in the literature. However, students of this group do agree that during this type of nuclear division the nucleolus and nuclear membrane persist and the chromatin and nucleolus divide simultaneously, producing a cruciform figure during metaphase. It has been suggested by some workers that this cruciform division or "promitosis" or "protomitosis", as it often has been called, might be useful as a diagnostic character of the Plasmodiophorales.

*Cytological Methods.*—Gall and root material was killed and fixed either in Randolph's Modified Nawashin Fluid for twelve to twenty-four hours (Johansen, 1940), or in a modified Carnoy's Fluid for one hour (2 parts absolute alcohol:1 part acetic acid:1 part chloroform). After killing and fixation the material was transferred to 70 per cent alcohol for storage. Infected plant parts were embeded in paraffin after dehydration with tertiary butyl alcohol (Johansen, 1940), or they were dehydrated through 100 per cent ethanol and embedded using an alcohol-chloroform series: 3 parts absolute ethanol–1 part chloroform; 1 part absolute ethanol–1 part chloroform; 1 part absolute ethanol–3 parts chloroform, for 1–2 hours in each solution. The infected parts were then transferred to pure chloroform saturated with paraffin, and the chloroform was evaporated in a paraffin oven; the material was then transferred to fresh paraffin for two hours and then embedded. The paraffin blocks were sectioned at 8–10 microns, and the resulting ribbons were fixed to glass slides for future staining and study.

The paraffin was removed from the ribbons with xylene. The material was then hydrated by passing it through a decreasing ethanol series to pure water and stained in a 1 per cent aqueous solution of crystal violet for 15–30 minutes and then passed through the following series: water, 5 min.; iodine solution (iodine, 1 gm., potassium iodide, 2 gms.; water 300 cc.), 3 min.: water, rinse to indefinite time; 95 per cent ethanol, 5 sec.; absolute ethanol, 30 sec.; clove oil, 20 sec.; xylene, wash thoroughly; mount in balsam.

Semmens and Bhaduri (1939, 41) describe a cytological staining technique combining the Feulgen technique and light green which they found to be very satisfactory in differential staining of the nucleolus and chromosomes in some higher plant material. The schedule which they recommended has been slightly modified in that the Feulgen reagent used was prepared according to the method of Darlington and La Cour (1947), and the hydrolyzing HCl solution used was 1N instead of 12 per cent.

*Cruciform Division in Zoosporangial Plasmodia.*—The zoosporangial plasmodium begins as a naked uninucleate thallus in the roots of *Veronica persica*. By divisions of this infecting nucleus a multinucleate plasmodium is produced (Figs. 29, 31, 33). Because of the visible differences in the nuclear development of the plasmodia illustrated in figures 29, 31–33, and 84, it is concluded that each plasmodium remains an entity, at least during the early stages of development. The sequence of events to be reported here is found only in young plasmodia, and it seems that these stages are very rapidly passed through.

The interphase nucleus of the plasmodium has a very conspicuous nucleolus usually in the center of the nucleus. Chromatic strands and granules are seen within and toward the periphery of the nuclear membrane (Figs. 21, 34, 58, 59). As these nuclei begin to divide, and all nuclei in a single plasmodium divide simultaneously, there is an aggregation of the chromatin in the equatorial plane of the nucleus and around the persistent nucleolus which now begins to elongate in the direction of the poles. Thus the so-called cruci-

form stage is produced when the chromosomes form a discontinous ring around the elongating nucleolus and perpendicular to its long axis (Figs. 22–24, 60). Earlier workers describe this as a solid ring of chromatic material around the nucleolus. However, Horne (1930), Webb (1935), and Whiffen (1939) report the presence of individual chromosomes, and the observations recorded here are in agreement with their findings (Figs. 24, 60). The ring of chromosomes now divides, and the daughter chromosomes pass to the poles around the elongating nucleolus (Figs. 25, 26). This is the so-called dumbbell or double anchor stage. The nucleolus continues to elongate (Figs. 27, 28, 61, 62) finally breaking in the middle, each half taking part in the reorganization of the two daughter nuclei as the nuclear membrane completes its division.

As these vegetative nuclear divisions continue the plasmodium increases in size (Fig. 37), sometimes completely filling the host cell. Cruciform division, which occurs during the growth of the plasmodium, was not found to take place in the ensuing development of the zoosporangium. All divisions were of the typical mitotic type (Fig. 30). It was not possible to ascertain the number of these divisions because intermediate stages were not seen. Whiffen (1939) reports for the zoosporangia of *Octomyxa achlyae* that there may be three, four, or even five such divisions, and the spindles in all divisions are alike in size and form.

*Cruciform Division in Cystosoral Plasmodia.*—The interphase nuclei of the plasmodium are characterized by conspicuous chromatic granules, some of which seem to be connected with each other and are mainly concentrated immediately within the nuclear membrane; the nucleolus occupies usually a central position within the nucleus (Figs. 40, 41, 63, 64). Webb (1935) reports that these chromatic granules become aligned as the chromatic threads contract, and gradually four distinct chromosomes are seen. Similar stages have been seen in this study (Figs. 65–67); there appear to be four chromosomes present. Illustrations of cruciform divisions viewed obliquely are presented in figures 36, 68, 69, and from these it can be seen that the chromosomes do not form a continuous band or ring. This was reported by Milovidov in *Plasmodiophora brassicae* and by Webb in *Sorosphaera veronicae*.

The nucleolus continues to elongate and the chromosomes divide as shown in the photographs of early and late anaphase, often called the dumbbell stage of cruciform division (Figs. 50, 55). During late anaphase and telophase the nucleolus begins to constrict (Fig. 70), finally separating into daughter fragments. (Figs. 54, 71, 72). Lastly the nuclear membrane constricts and divides forming two daughter nuclei in which, at first, the chromosomes mass around the nucleolus. Figure 102 probably represents recent daughter nuclei which are beginning another division. The so-called "ring of Saturn stage" of cruciform division is seen; the nucleolus has not yet begun to elongate.

*Further Development of the Cystosori.*—The so-called transitional phase or akaryote stage of division was not recognized as such. These observations are in agreement with those of Milovidov who reported that in *Plasmodiophora* the chromatin never completely disappears from the nucleus. Webb observed in *Sorosphaera veronicae* that these changes were "of a simple nature, consisting of the degeneration of the large and conspicuous nucleolus . . ."; the chromatin reticulum remained clearly visible. The reports of other workers, especially Osborne (1911), that a fusion takes place during this stage, are based on a few apparently paired nuclei and on the fact that the nuclei appear to undergo reduction at sporogenesis, which presupposes a nuclear fusion. Horne (1930) for *Spongospora* and especially Webb (1935) for *Sorosphaera* found nuclear configurations which indicated to them that an alternation of four and eight chromosomes occurred. The haploid and diploid phases are supposedly separated by a fusion during the arkaryote stage, which is immediately followed by a reduction division. Whiffen (1939) concludes that *Octomyxa achlyae* has a similar life cycle. Although four chromosomes were counted in the vegetative stages of *S. veronicae*, no chromosome configurations were found which might indicate that a fusion and a reduction occur. However, certain configurations which are similar to those figured by Webb as reduction stages are included, and they may be interpreted as pointing up Webb's argument for an alternation of a haploid and a diploid number of chromosomes. The nucleolus becomes flattened against the nuclear membrane and the two groups of chromatin move together (Figs. 46, 74). These configurations are similar to those described by Webb as collapsed zygotenes, while figures 75 and 76 are similar to those described as pachytene and diplotene respectively.

According to Webb the nuclear membrane now disappears, and the chromosomes move to the equatorial plate; this heterotypic division is followed closely by the homeotypic division, interkinesis being of short duration. Metaphase (Figs. 45, 47), anaphase (Figs. 48, 79) and telophase (Figs. 49, 56) configurations found during the present study cannot be determined as belonging to either meiosis I or meiosis II.

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. Thus another interpretation of the illustrations listed above in support of meiosis might be to present them as evidence for typical mitosis such as that which is thought to occur in zoospore formation. Then, after cruciform division has ceased, the so-called akaryote stage, which is really the prophase stage of the mitotic divisions which follow, occurs (Figs. 43, 44, 46, 77, 78). Metaphase (Figs. 45, 47), anaphase (Figs. 48, 79) and telophase (Figs. 49, 56) stages complete the division. Chromosome counts made during late anaphase from material which had been stained with the Feulgen technique show that the chromosome number is four, the same as that reported during cruciform division.

Two divisions occur. One is heterotypic and the other homeotypic, according to Webb. The present author observed nothing but typical mitotic divisions. During the first division the plasmodium cleaves into uninucleate portions (Figs. 42a, 53), in which the second division then takes place (Figs. 51, 80, 81). These incipient resting spores are associated in a hollow sphere (Figs. 42b, 52), and a thick wall is laid down around each resting spore (Fig. 82, 83).

*Additional Observations on Cruciform Division* —Because of the dual nature which has been attributed to the nucleolus of the Plasmodiophorales, i.e., it is said to be composed of two kinds of chromatin or to contain all or part of the chromatin of the nucleus, it seemed worthwhile to reinvestigate this matter. In addition to this is the question of whether or not true chromosomes are present in these organisms. Milovidov[2], using the Feulgen technique, demonstrated the presence of distinct chromosomes in the ring surrounding the nucleolus during vegetative division in *Plasmodiophora brassicae*. He further demonstrated that the nucleolus, unlike the chromosomes, gave no reaction for desoxyribonucleic acid. This indicates then that the nucleolus of this parasite is homologous with the nucleolus of other organisms.

*Sorosphaera veronicae* plasmodia were stained with Feulgen reagent and counterstained with light green according to a method devised by Semmens and Bhaduri (1939, 41). The results give positive indication that the persistent structure during cruciform division, which has been called the nucleolus, is truly this structure. Using this technique the nucleoli are stained a light to dark green and are easily distinguished from the purple chromosomes which respond to the Feulgen reaction. In interphase nuclei the nucleolus stains a bright green; during cruciform nuclear divisions, an elongated green nucleolus and a ring of purple chromosomes form the configurations so characteristic of these divisions.

Under the microscope it is very easy to distinguish between the colors of purple and bright green, but this difference is difficult to render with ordinary black and white photographic materials. However, the following illustrations are offered for what they do show. Figure 41 represents an interphase nucleus with a bright green nucleolus and purple chromatin, for the most part, around the internal periphery of the nuclear membrane. Figures 38 and 39 represent these differential stains much better. The contrast between the Feulgen-positive chromosome ring and the light green stained nucleolus is notable. These may be compared with like configurations shown in figures 35 and 36 which were stained with crystal violet. Figure 50, an anaphase of cruciform division, shows the darker rings of chromosomes surrounding a light green stained nucleolus.

It is very interesting to note in the work of Singh (1951), on nuclear division of nine species of free-living amoebae, that the karyosome "which should be called nucleolus" because it is Feulgen-negative, has been found to be void of true chromatin. Singh further states that the claim, which has been made that "peripheral chromatin" sometimes gives rise to chromosomes and sometimes disappears completely during division, [a phenomenon often claimed to be

[2] *See* J. S. Karling, 1942, The Plasmodiophorales, p. 5; and L. S. Olive, 1953, The structure and behavior of fungus nuclei, Bot. Rev. **19:** 449.

characteristic of certain Plasmodiophorales] is now thought to be caused by, in part, unspecificity of the stains for chromosomal chromatin. Sole reliance on non-specific stains led to the fragmentation theory which offers the origin of chromosomal chromatin in the nucleolus.

## SUMMARY

The discovery of zoosporangia in the life cycle of *Sorosphaera veronicae*, as determined by morphological, cytological and cultural studies, is described for the first time. The zoosporangia are located in the epidermal and sub-epidermal tissues of the roots, root hairs excepted; the cystosori are found only in the shoot system. Zoospores, from resting spores found in the soil, penetrate root cells, developing there into zoosporangial plasmodia. Mature zoosporangia discharge biflagellate heterocont zoospores—with a whip-lash on each flagellum, which may reinfect root cells. It is assumed that these zoospores, at times when soil water is plentiful, reach the surface of the soil. Because of the recumbent nature of the host plants, many apical meristems are available to the zoospores, which may enter them and form cystosoral plasmodia. Mature cystosori are liberated in the soil upon the death and decay of the infected host plants. Cystosori are not produced in the roots.

Cytological investigations indicate that, during the early stages of plasmodial development in *S. veronicae*, a vegetative nuclear division as characterized below is found. Chromosomes are present; they are not derived wholly or in part from the nucleolus. Division of the chromosomes takes place within the nuclear membrane. The nucleolus is persistent within the ring of chromosomes. Gradually it divides into two portions as the chromosomes reach telophase; the nuclear membrane then divides.

*Veronica persica* and *V. perigrina* are reported as new hosts for *Sorosphaera veronicae*.

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