Parasite *Calkinsi* on *Plasmodiophora tabaci* and its possible etiological rôle in tobacco mosaic.

By

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(With Plates 11—14.)

Introduction.

The occurrence of both *Plasmodiophora tabaci* and a parasite in the cells of tobacco plants affected with mosaic symptoms raises the question whether *P. tabaci* or the parasite or both are causally ralated to the disease. This parasite, I have named *Calkinsi*, after Dr. Calkins who first recognized it as a parasite on *P. tabaci*. Since the morphology of the parasite is not known, I can not give it a generic name, but will call it parasite *Calkinsi* for clearness.

The fact that *P. tabaci* and parasite *Calkinsi* have not been found in plants free from mosaic symptoms is significant although it does not prove that the parasite *Calkinsi* is the pathogen, in the case of plants showing mosaic symptoms. If a statistical study of the diseased and healthy plants should reveal that the organism is constantly absent in healthy ones, the possibility would be very strong that there is a causal relation. So far only three hundred healthy and four hundred and fifty diseased plants have been studied. In none of the healthy ones was the organism found and only twice was it obtained in culture after washing in $HgCl_2$ 1—1000. It was found in all of the diseased plants and obtained from them in culture.

Methods.

Since I have described the method of cultivating and fixation in my previous papers (lit. c't'd.—1, 2), I will not repeat those descriptions here.

Observations.

Parasite *Calkinsi* in the papers mentioned above, has been described as forming a sporoblast within the encysted amoeba of *P. tabaci*. Due to its size and rapid motion it has been almost impossible to make out its structure. The parasite is about one and one half microns long and one half a micron wide, tapering at both ends. Just before the sporoblast is broken (Figs. 2, 4, 5, 7, 8), one can observe hundreds of them lashing about with a flagellate movement. Due to their very rapid contraction into round balls, I have never been able to fix them in extended form. The diameter of these balls is one micron.

In the host tissue, parasite *Calkinsi* may leave *P. tabaci* and attack the surrounding tissue or work its way into the meristematic tissue. Fig. 9 shows parasite *Calkinsi* in the spongy parenchyma cells of a tobacco leaf, two of them having just entered a new cell. In Fig. 10 they are attacking the host nucleus. Often the host nucleus undergoes division when attacked. If Fig. 10 is examined closely one can observe them penetrating the host nucleus.

The chlorophasts are also attacked and they decompose very rapidly as one may observe (Figs. 9, 10). The dark stained ones are healthy, and light ones are decomposing. Parasite *Calkinsi* is found in greater abundance in distorted areas of the leaf before the light and green ones are found. Parasite *Calkinsi* may be the same organism described by Miss ECKERSON in tomato plants, although I have not been able to make out the details she described.

In paper No. 2 (lit. c't'd.—2) we described inoculation with the mycetozoan containing parasite *Calkinsi*. Out of fifty plants inoculated, forty developed mosaic disease showing the distorted areas but not the light and green areas, while all the controls remained healthy. As the plants became older the light and green areas appeared. From my observations on the inoculated plants I believe the parasite caused the distorted areas and the large light areas are due to the presence of *P. tabaci*.

The vaculated bodies in Figs. 13 and 16 resemble the bodies described by KUNKEL and GOLDSTEIN as X-bodies. I have often observed these bodies in diseased tissue but I believe they are the results of disturbances of parasite *Calkinsi* in the host tissue and their movement is due to cytoplasmic flow within the host cell.

Plasmodiophora tabaci in culture and in the host plant.

From my studies on mosaic tobacco plants I am convinced that *P. tabaci* is one host and the tobacco plant the other for parasite *Calkinsi*, and that *P. tabaci* and parasite *Calkinsi* are symbiotic. Parasite *Calkinsi* opens the way into the plant for *P. tabaci* and *P. tabaci* in return protects it during unfavorable conditions by forming a cyst wall around it. *P. tabaci* does not appear to have the power to penetrate the host cell wall until the parasite *Calkinsi* has gone through first, but once it enters the cell wall both the cell and plasmodium will increase in size and the host cell wall will thicken while the cytoplasm is being used up (Figs. 27, 28). When the plasmodium has used up all the food and can not get out it will form cyst spores. When *P. tabaci* is in favorable condition in culture or plant, the spores are left behind as it moves (Figs. 12, 20), but in unfavorable conditions the spores are formed within the plasmodium. Figs. 10 and 27 were made from plants and Figs. 31 and 34 were made from cultures.

When *P. tabaci* once gets into the plant it enters the sieve tubes and goes to all parts of the plant. In Figs. 19 and 20 the cyst spores were left by the plasmodium, some being caught in the sieve as they were passing through the sieve tubes. This shows that the plasmodium can pass at will through the plant.

When the nuclei of *P. tabaci* nuclei undergo division by promitosis they become very transparent and the nuclei will not take the stain (Figs. 10, 32). This makes the plasmodium very hard to identify in the young plants, while in the older tissue it can be recognized by its nuclei.

Since the plasmodia fuse when they come in contact, all sizes may be present depending on the number which may have united (Fig. 33).

Discussion.

The work done so far in culturing the organism and inoculating plants with these cultures is of a preliminary nature and is not critical enough to establish the causal relation of the parasite. Inoculation of healthy plants with the cultures of parasite *Calkinsi* as well as *P. tabaci* has produced the disease and the organisms have been demonstrated in the tissue of the inoculated plants and subsequently obtained from them in culture. The difficulty however, is that these cultures were not single pure cultures and the possibility of contamination with virus has not been eliminated. However, in our experiments in a previous paper (lit. c't'd. — 2) it was shown that *P. tabaci* alone can not cause mosaic disease. Parasite *Calkinsi* may be the initial cause of mosaic disease resulting in the distorted areas and *P. tabaci* may follow it into the tobacco plant and helps cause the light and green areas.

In our second paper mentioned above we separated by filtration the bacteria and *P. tabaci,* then inoculated some healthy plants with the filtrate and produced the distortion of the leaves. Seven days after filtration was made I removed the cover glasses from the first for observation. These cover glasses had been sterilized in the flask previous to setting up the filter.

I then fixed and stained the small particles on these cover glasses and found the small round balls which resemble in every respect the round balls in the sporoblast of a fixed amoeba cyst of *P. tabaci* (Figs. 2, 4, 5, 7, 8). It may be possible that the observation made by Miss ECKERSON on „An organism of tomato mosaic" (lit. c't'd. — 3); those of Madame SOROKIN on „The phenomena associated with the destruction of the chloroplast in tomato mosaic" (lit. c't'd. — 4); and those of KUNKEL and GOLDSTEIN on X-bodies (lit. c't'd. — 5, 6) may be descriptions of the same thing and caused by the action of parasite *Calkinsi* on the chloroplast of the host plant.

Summary.

1. The parasite *Calkinsi* of *Plasmodiophora tabaci* may be the initial cause of mosaic disease in tobacco.

2. Parasite *Calkinsi* has two hosts (a) *Plasmodiophora tabaci* and (b) tobacco plant.

3. *Plasmodiophora tabaci* does not alone cause the disease.

4. Parasite *Calkinsi* attacks the nucleus of the host plant.

5. Parasite *Calkinsi* can be seen in the living condition only when there are hundreds moving violently inside the sporoblast.

6. Parasite *Calkinsi* is about one and one-half micorons in length and one half micron in width. It has the power to contract into a ball.

7. The host plant can thicken the cell wall around the plasmodium.

8. Both young and old plasmodia as well as the spore cysts have been found in the stem of tobacco plants principally in the sieve tubes.

9. The plasmodium is found in the light areas of the leaf of mosaic plants.

10. Parasites *Calkinsi* are found most abundantly in the meristematic tissue, and *Plasmodiophora tabaci* is found most abundantly in the older tissue.

11. The nuclei of young a plasmodium, when undergoing division, are almost impossible to stain.

Literature cited.

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3) Eckerson, S. H. (1926): An organism of tomato mosaic. Bot. Gaz. Vol. 81.

4) Sorokin, H. (1927): Phenomena associated with the destruction of the chloroplast in tomato mosaic. Phytopathology Vol. 17.

5) Kunkel, L. O. (1924): Further Studies of the intracellular bodies with certain mosaic diseases. Bull. Exp. Sta. Hawaiian Sugar Planters. Assoc. Bot. Ser. Vol. 3 p. 118—144.

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Explanation of Plates.

|Plate 11.

Fig. 1. Limax type of Amoebae stage of *P. tabaci.* 1000 ×.

Fig. 2. Sporoblast, consisting of parasites *Calkinsi,* which has pushed the nucleus of Amoebae cyst of *P. tabaci* to one side. 1500 ×.

Figs. 3—6. Amoebae stages of *P. tabaci.* 1500 and 1000 ×.

Figs. 4, 5, 8. Amoebae *P. tabaci* rounded up preparing to form a sporoblast. They are filled with parasites. 1500 and 1000 ×.

Fig. 7. Two sporoblasts found in one amoebae cyst. 1000 ×.

Fig. 9. Parasite *Calkinsi* entering and causing necrosis in the spongy parenchyma cell of tobacco leaf. 1000 ×.

Fig. 10. Parasite *Calkinsi* attacking the nucleus of a mesophyll cell of tobacco leaf. Some have already entered while others are entering. *P. tabaci* surround this nucleus. The chloroplasts are disintergrating. 1500 ×.

Plate 12.

Longitudinal section of naturally infected plant three months old. 1500 $\times$.

Figs. 11, 17, 18. Host nucleus of stem.

Fig. 12. Cyst spores left by plasmodium in sieve tube.

Figs. 13, 16. Bodies resembling the X-bodies described by KUNKEL and GOLDSTEIN.

Figs. 14, 15. Chloroplast.

Fig. 19. Some spore cysts caught in sieve of sieve tube as plasmodium passed through.

Plate 13.

Longitudinal section of naturally infected plant three months old. 1500 $\times$.

Fig. 20. Spore cyst in sieve tube.

Figs. 21, 29. Young plasmodia.

Figs. 22, 24. Chloroplasts.

Figs. 23, 25, 30. Host nucleus.

Fig. 26. Diffraction.

Fig. 27. Plasmodium filled with spore cysts. The host cell wall has enlarged and thickened in order to keep the plasmodium within it.

Fig. 28. Young plasmodium with one nucleus, the ectosarc quite prominent, the host cell wall thickened.

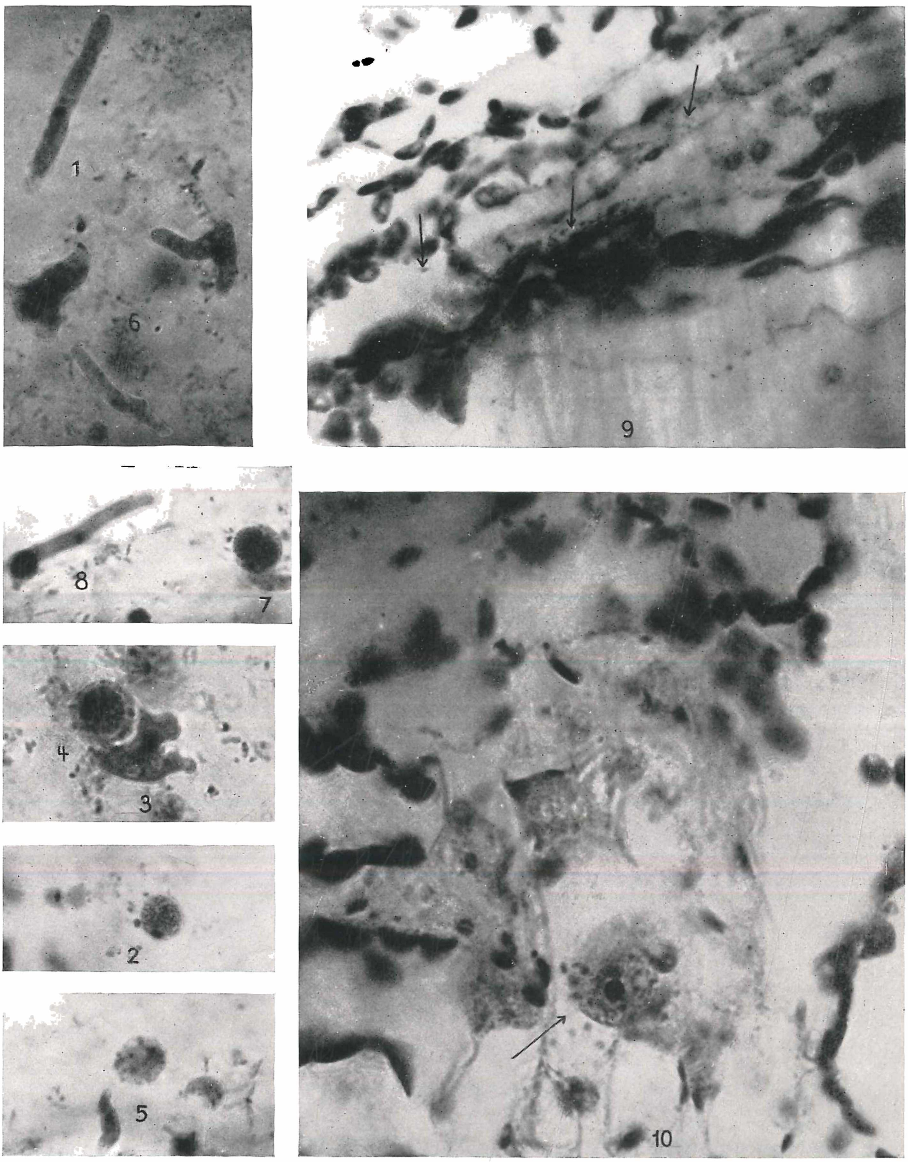
Plate 14.

All from culture.

Figs. 31, 34. Spore cysts found in plasmodium when the culture began to dry up. 1000 $\times$.

Fig. 32. Plasmodium *P. tabaci* nuclei undergoing division by promitosis. 1500 $\times$.

Fig. 33. Plasmodia fusing. 750 $\times$.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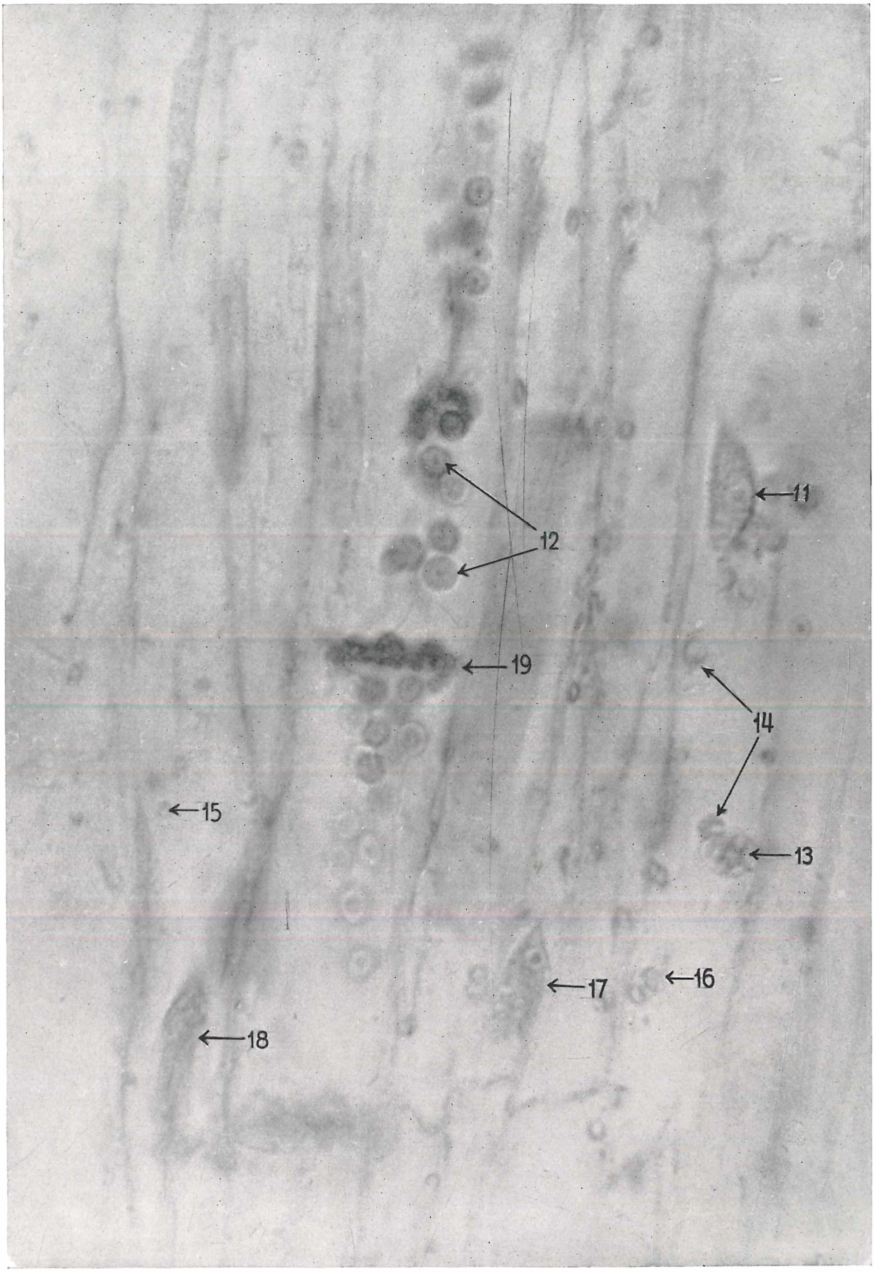

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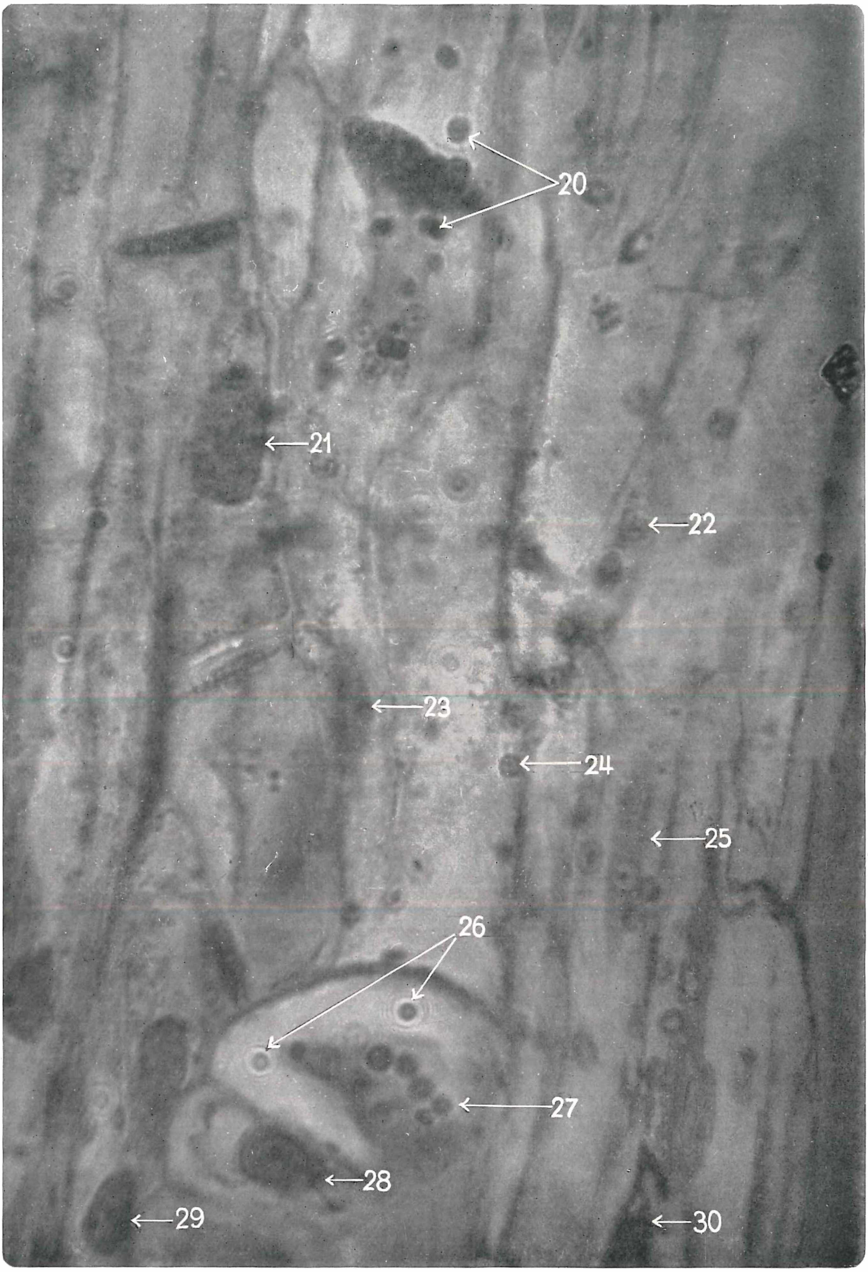
VERLAG VON GUSTAV FISCHER IN JENA.



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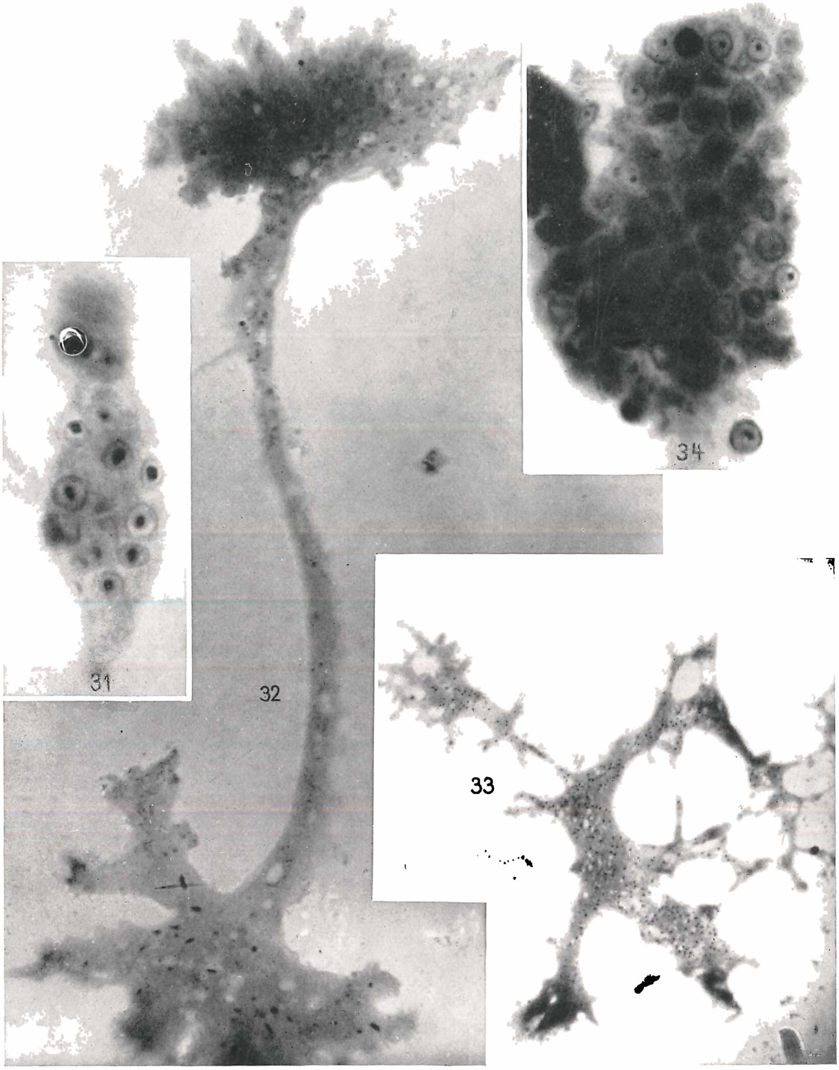

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