HISTOLOGICAL STUDY OF PLASMODIOPHORA BRASSICAE.

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MMETHODS. Cabbage seedlings showing the disease in the early and in the later stages were procured and killed by giving a bath of 24 hours in chrom-acetic acid composed of 1% of each ingredient. After dehydrating by the usual method, the material was imbedded in paraffin, using xylol as a solvent. This material was sectioned from 3-8 mi. thick on a rotary microtome. For studying the general distribution of the parasite, in the host cells , I found sections cut 8mi. thick and stained with Delafield's Haematoxylon and a counter stain of eosin gave best results. Sections cut from 3 to 5 mi. thick and stained in Flemming's triple stain proved the most satisfactory for a critical study of the myxomycete and its effect on the protoplasm and cytoplasm of the host cells. Since safrønin readily stains the host cells and the nucleus of the myxomycete, the sections need not remain in the safrønin more than twelve hours. A fixative containing 0.5% gelatin water and 3% phenol proved very satisfactory.*

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* Bacteria in Relation to Plant Diseases, p. 119, .Erwin F. Smith.

GENERAL EFFECT ON THE HOST. This parasite attacks many members of the Cruciferae, some of the more common of which are cabbage, cauliflower , Brussels sprouts , turnips, rutibaga, radishes, mustard etc. Seedling plants are frequently attacked while yet in the seed bed and quite young. The infectd plants soon show a decided "flagging" and after a weak, puny growth die. Infected cabbage plants often live sufficiently long to form heads, but so much of the plant's vitality seems to be utilized in forming the abnormally large and clubby root that the head cannot develop.

MANNER OF INVASION. Since this organism lives in a single cell of the host, we naturally inquire whether or not the myxomycete proceeds from cell to cell or whether the cells are infected in some other manner. In order to ascertain the effect of the myxomycete on the cell wall, some sections were stained with Bismark brown and a counter stain of Eosin, the former of which is especially good for staining cell-walls.

The walls of the diseased cells,however, showed no disintegration that would indicate a migration of the amoebae from diseased to healthy cells. Nawaschin S.*, in his cytological study of this organism, found that diseased host cells divide in the normal manner, and hence the myxomycete of the mother cell is transmitted to the two daughter cells, till a group of cells have originated from the division of a single diseased cell. This is the explanation of the fact that the diseased cells lie in groups composed of a number of diseased cells that are contiguous. Indeed, the groups are so arranged in place and shape that one can readily see that they may have originated from the division of a single diseased cell, and the disease therein from the division of a single amoeba.

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* Beobactungen über den feinen Bau. u. Umwandlungen von Plasmodiophora. Flora 86 , 404-427; 1899.

Nawaschin,who did considerable work on this organism,found that when the amoeba has reached a certain stage in its development it divides by number mytotic divisions into a number of spores. These divisions occur simultaneously and are very similar to the mytotic divisions taking place in the higher plants. Subsequent to this division a cell-wall is formed about the spore. Perhaps it would be more exact to say instead of a cell-wall, a film of dense protoplasm surrounds the spores; for this film does not act as a cell-wall towards stains.

DISTRIBUTION IN THE HOST PLANT Theae groups of diseased cells described above are most abundant in the parenchymatous cells,especially those in the vicinity of the cambium. The phloem is abnormally developed untill it shows considerable hypertrophy. The xylemm portions of the affected roots are relatively incospicuous. My observation verifies previous statements to the effect that the organism is sometimes found in the fibrovascular bundles; yet in my experience it occurs there very rarely.

EFFECT ON THE HOST CELL.

Effect on Protoplasm. The normal healthy cell of the host has a protoplasmic film surrounding the nucleus. The nucleolus is composed of very dense protoplasm

that readily absorbs safranin . Surrounding the nucleolus is a clear hyaline ring that does not take any of the stains, hence we infer it is an oily film surrounding the nucleolus. (See fig. 1.) The cytplasm of the nucleus is permeated by a number of protoplasmic threads or strands at intervals in which there are knots of densed protoplasm which is the chromatin.

On examining a section of a diseased cabbage root, one observes that the diseased cells are much larger than the normal cells, and that the increased size is not due entirely to the mass of the myxomycete, for the swelling is largely composed of parenchymatous tissue of the host. The presence of the myxomycete seems to act as a stimulous to the invaded cells, causing excessive devlopment. The fact is very striking that the uninfected cells in the vicinity of the diseased cells are also frequently hypertrophied just as the infected cells. Nawaschin attributes this to a tendency that extends beyond the immediate diseased cells; or he thinks perhaps the impact of the diseased cell may act as a stimulous. It occurs to me that perhaps the myxomycete secretes some enzyme that dialyzes through the infected cell-wall into an uninfected cell and thus produces hypertrophy just as it does in the infected cell.

Nawaschin in his analyses found an excess of starch in the diseased roots, while Dr. Ellett in his analyses found an abnormal amount of potassium in the diseased roots. The excess of potassium perhaps accounts for the excess of starch, for potassium is necessary for starch formation.

While the myxomycete is in the viscid or amoeboid stage it is not surrounded by a cell-wall , and during this stage a symbiotic relation exists between the host cell and the myxomycete ; but after a time a protoplasmic film, which stops all symbiotic relations, is formed around the amoeba. Hypertrophy of the host cell develops simultaneously with the development of the myxomycete, untill the latter reaches a certain point and then its development, and hence the symbiotic relation, ceases. From this point the protoplasmic mass of the host begins to disappear and spore formation soon takes place. After the the formation of spores , the considerable shrinking of the protoplasm from the walls of the diseased cells may be due to the fact that the

myxomycete in its metabolism has used up the protoplasm faster than the host can replace it,even under this stimulation. Or what seems more probable to me, the parasite in its metabolism secretes some enzyme that is deleterious to the protoplasm of the host cell and thus causes a sort of plasmolysis.

Effect on Cytoplasm. Where only a few spores were found in the cell , they were generally found in the vicinity of the nucleus; we , therefore, infer that the nucleus is attacked soon after the cell is attacked. Even when the cell is not entirely filled with spores the nucleus is distorted. The nucleus of a normal cell is surrounded by a protoplasmic film which is disolved soon after the cell is invaded by the parasite. Then the nucleus enlarges and the chromatic threads disappear , but densely stained masses which are perhaps the chromatin may be seen in the cytoplasm.(Seed drawing 2) The nucleolus is somewhat enlarged, but in other respects it appears to be perfectly normal. (See drawing 2) The oily layer surrounding the nucleolus is broken at places (See drawing 2) The myxomycete lives in the cell some time before the nucleus is absorbed, but finally one is not able to distinguish any definite nuclear structure in the diseased cell.

Recapitulation

In view of the fact that this disease has considerable economic importance, an histological study of its effect on the host seemed entirely justifiable.

Infection by this parasite may be detected by weak growth of the plant and hypertrophied roots.

Some investigators believe this parasite inhabits the whole plant- both root and stem, but the majority believe it is confined exclusively to the roots.

The following are the chief facts resulting from this study:

(1) The myxomycete lives in a single cell and does not migrate from diseased to healthy cells; but its only mode of invasionm is by the division of infected cells. Diseased cells divide in the normal manner and thus the two daughter cells are also infected.

(2) The diseased cells are in groups that are most abundant in the vicinity of the cambium.

(3) The phloem tissue is much hypertrophied and the xylem is atrophied.

(4) Not only are the infected cells but also many of the uninfected cells of the host ~~are~~ hypertrophied.

(5) The protoplasmic film surrounding the nucleus and the chromatic threads are destroyed. The nucleus is distended soon after the cell is attacked.

(6) The nucleolus retains its form and characteristics for some time after the nucleus has been attacked.

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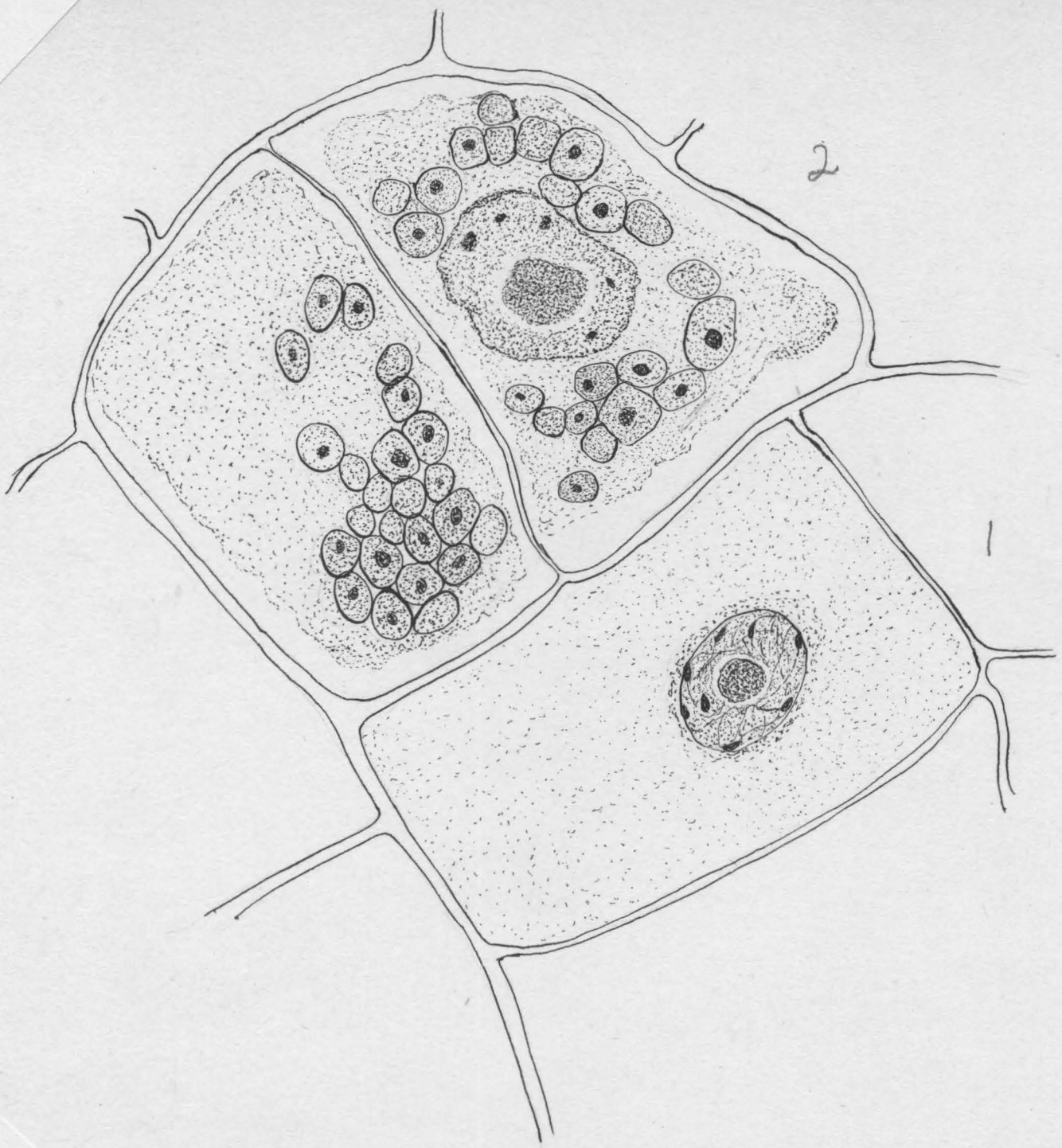
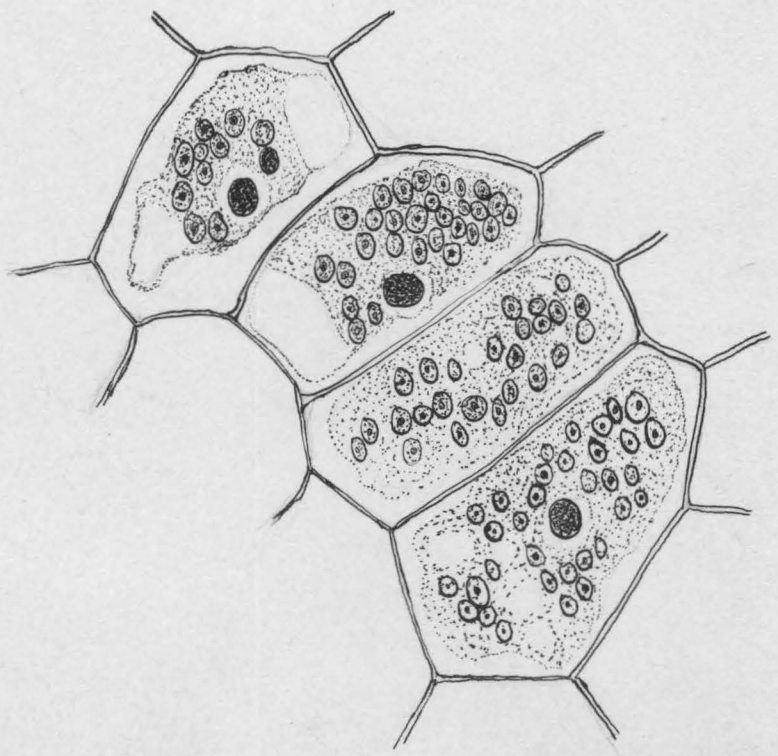
EXPLANATION OF PLATES.

1. A normal cabbage cell showing the film surrounding the nucleus; also the chromatic threads and chromatin . 1-16x 1.

2. A Diseased cell showing the nucleur film and chromatic threads destroyed,; also the nucleus and nucleolus much distended. 1-6 x 1.

3. Several contiguous cells infected showing the shrinking of the protoplasm and of the nucleus. 1-6 x 1.





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