LIFE HISTORY OF LIGNIERA VASCULARUM (Matz) Cook

(Formerly known as *Plasmodiophora vascularum*).

MELVILLE T. COOK

(WITH PLATES III–VI)

This organism is the cause of a disease of sugar cane in Porto Rico and was described by Matz (12) in 1920 under the name of *Plasmodiophora vascularum*. The organism has not been reported from any other part of the world. This paper is a record of studies on the life history of the organism which have resulted in the author transferring it from the genus *Plasmodiophora* to the genus *Ligniera* Maire and Tison.

HISTORY

The organism was first reported by Matz (12) in 1920 as the cause of a disease of sugar cane commonly known as "dry top rot". He reports his first discovery on Cavengerie cane at Bayamón in the fall of 1919. In this and a later paper (13) he reports the disease on a Porto Rico seedling, Otaheite, Crystalina, Rayada, D–109 and Yellow Caledonia. In the second paper he states—"The disease was found distributed in practically all the principal sugar-cane-growing sections of the Island, on the north coast as well on the irrigated south coast, in isolated areas on the western end, and in the central district around Cayey and Morovis. It was particularly noticeable in fields which showed evident signs of retarded growth and dwarfing especially in ratoon fields".

In his second paper (13) Matz states that "From observations made on sugar cane diseases in Porto Rico it is now certain that in so far as reduction of yield is concerned this dry top rot or vascular disease is the most serious of the three or four major diseases of sugar cane existing at present in Porto Rico".

The writer of this paper has found the disease well distributed over the Island and on several varieties but has found but one severe outbreak which was in the vicinity of Canóvanas and involved about 300 acres of D–109 which was growing on low wet land. The other outbreaks observed by the writer were in some cases severe but over small areas and always in wet land. Numerous small out-

breaks were observed, usually in the coastal plains. Several infected stools were found in a small poorly drained field near Cayey which is about 1,300 feet above sea level. The controlling factors appear to be susceptibility of varieties and amount of moisture.

SYMPTOMS

The disease gets its name "dry top rot" from the dying and drying of the tops but it should be remembered that a dying of the tops may be due to borers and to other causes. This symptom is proceeded by a reduction of the leaves and frequently by a rolling and wilting of the leaves. In severe cases a part or all of the canes in a stool may be dwarfed and die from the tops down. These dead tops are frequently attacked by saprophytes which cause a decay. It is not unusual to find many canes of various ages, especially those under five feet in height, dead from this disease. These dead canes may lose all their leaves and are frequently attacked by the rind fungus (*Melanconium sacchari*) which may mislead the observer as to the true cause of the dying. When infected canes are cut across at the base, some of the fibro-vascular bundles show a lemon or orange yellow color. When the sections of this tissue are examined under the microscope the tracheary tubes of these bundles will be found to be filled with a plasmodium or large spores. It is very evident that this plugging of these water passages will be disastrous to the growth of the cane. However, weak or dead canes may show only a few infected bundles and it is difficult to understand just how such slight infection can kill the canes. The organism can also be found in the roots. After a time the organism may disappear and the bundles show a pronounced red color. Although some of these external symptoms may be common to other diseases of the sugar cane, the presence of the spores in the tracheary tissues is so characteristic that the disease cannot be confused with any other known disease of sugar cane.

Matz described (12) the organism as follows: "The spores in their advanced stage in the interior of the vessels of fibro-vascular bundles are spherical with smooth, somewhat thick hyaline walls, evenly granulated or sometimes coarsely granulated in the interior, orange, yellow, sometimes slightly brown in color, measuring .014–.016 millimeters in diameter. Spores are embedded in a yellowish hyaline, at length hard matrix. Plasma is composed of a mass of granular cytoplasm, later developing into individuals composed of clear cytoplasmic variable bodies having a dense, darker, granular center".

The writer of this paper was induced to take up the study of

this disease and the organism causing it, (1) because it appeared that the disease was likely to prove of major importance and (2) because it appeared to be desirable to make more extensive comparisons of the organism with other genera and species of the Family-Plasmodiophoraceae.

METHODS

The methods of work were as follows: (1) Field observations were made to determine symptoms, severity and resistant varieties. (2) Free hand sections of fresh material were made and studied in the laboratory. Sections were mounted in sterilized water and kept under observation for the purpose of observing germination of spores. Blocks of fresh material were kept in sterilized water for the same purpose. (3) Material was killed and fixed by several methods, embedded in paraffin, sectioned and stained by several methods. Flemming's weaker fluid and Haidenhain's iron-alum haematoxylin stain were most satisfactory.

LIFE HISTORY

The life history resembles that of a number of other species of the family *Plasmodiophoraceae*. The plasmodium is produced in abundance and frequently fills the tracheary tubes (Fig. 1). It is very evident that this is the product of germinating spores; and spores that have failed to germinate are frequently found in this plasmodial mass (Fig. 1). The plasmodium may be uniform in character (Fig. 1) or it may vary in density (Fig. 2). Matz states that it occurs in the annular and spiral tracheids and pitted vessels in the fibro-vascular bundles in the lower nodes. In some cases the writer of this paper found the organism in the tracheary tubes only, while in other cases it was also found in the surrounding cells of the fibro-vascular bundle and rarely in the parenchyma cells (Figs. 3 and 9). Both the plasmodium and the spores were most abundant in the basal part of the plant but were sometimes found at a considerable distance above ground. The older or more mature stages are always below the younger stages, indicating that the organism was moving upward into the growing plant. The stages of development in these adjoining cells are not always the same; some may shown plasmodia while others show spores in various stages of development (Figs. 3 and 9). Nuclei could not be seen in any of the preparations until the spores began to form (Figs. 4 and 5) and sometimes the nuclei were not visible even when spore formation was well advanced (Figs. 3 and 6). In some few cases the nuclei were very prominent at an

early stage of spore development (Fig. 8). The first stages in spore formation were very indistinct (Figs. 4 and 5), but later became more definite (Fig. 6). In some cases the entire mass of plasmodium appeared to have been consumed in spore formation (Figs. 6 and 7), while in other cases only a part had been used (Figs. 3, 8 and 9), the remainder forming a matrix in which the spores were embedded.

The cell wall appears as a single membrane early in the spore formation (Figs. 8, 9, 10, 11 and 12), but thickens with age (Fig. 18). In the early stage of spore development a spore appears as a uniform granular mass, surrounded by a delicate membrane and with a single nucleus which stains deep and uniform (Figs. 9, 10, 11 and 12). In some few cases these developing spores show bodies which turn black under the action of Flemming's fluid (Fig. 13) and resemble nuclei. They are probably fat bodies. These spores vary greatly in size (Figs. 14, 15, 16 and 17), but the writer was unable to determine just how much growth was made after the formation of the cell wall. In its further development numerous vacuoles are formed in the protoplasm, the nucleus becomes large with a well defined nucleolus and the cell wall becomes very thick (Figs. 15, 16, 17 and 18). The cell wall is so thick that the killing fluids do not penetrate readily. Therefore, it is difficult to study the contents of the mature spores but the protoplasm may be dense or vacuolar and fat bodies may be few or abundant. The spores are smooth and when not subject to pressure are spherical in form (Fig. 18). When mature these spores usually fill the large tracheary tubes (Fig. 19), but in some cases excess plasmodium is visible. In small tubes the spores may lie in a single row (Fig. 16), while in large tubes they form a mass (Fig. 19). When mature the spores in any individual tracheary tube are usually quite uniform and of a maximum size, but some variations may occur (Fig. 19). They are usually lemon or orange yellow or slightly brownish in color when old.

The germination of these spores and their behavior after germination is rather difficult to follow. Matz (12) says: "At first an attempt was made to germinate the spores of the organism in water, in sugar water, in cane juice, in fermented but sterilized cane juice and in several agars but no germination was observed to have taken place. Spores were kept in moist cells for over six months and no germination was observed to have taken place. Portions of cane stalks which contained bundles filled with the organism in its several stages were cut and placed in moist chambers together with healthy seed pieces of Rayada cane, and after five months it was found that the roots of the Rayada cane contained many of the

spherical spores of the organism. Apparently a transfer of the organism from its original seat into the healthy cane had taken place. Inoculations with bits of infested bundles into six healthy canes were made in the basal regions of the latter. The six cane stools thus inoculated showed marked stunting in contrast with other uninoculated canes growing alongside of the former".

The writer of this paper found this phase of the problem quite difficult and is not sure that all the observations are absolutely correct. When apparently mature spores were squeezed out into drops of sterilized water on clean slides and kept in moist chambers, short germ tubes were produced on a large percentage of them (Fig. 20). In many cases the points of these tubes were open and the contents gone but the writer never succeeded in seeing one of them open and the contents emerge. A few spores with germ tubes were found in the tracheary tubes. Free swimming zoospores were observed and, although it was difficult to make sure that sterilization processes for the destruction of other organisms were all that was to be desired, it is reasonable to suppose that they came from the spores. The movement of these zoospores was very rapid and it was difficult to make a satisfactory study, but they appeared to be uni-ciliate. This opinion appears to have been confirmed by the findings of two uni-ciliate zoospores (Fig. 21) in the tracheary tubes in prepared sections. Neither union nor division of these free swimming zoospores were observed but numerous cells of various sizes, that appeared to have possessed euglenoid and amoeboid characters were observed in the tracheary tubes in the prepared sections (Figs. 22 and 23). Therefore, it is reasonable to assume that the flagellate zoospores become euglenoid, then amoeboid and that they eventually unite to form the plasmodium, thus completing the life cycle. Actively growing cane would undoubtedly furnish abundant food for a rapid growth of the organism. However, other stages, such as unions and divisions may have occurred and not been observed by the author.

Judging from the preceeding studies it appears probable that spores may germinate in the tracheary tube and unite to form a plasmodium and that the organism travels from the older to the newer parts of the plant either as zoospores or as a plasmodium and may complete the life history without escaping from the host. Since the new shoots of a diseased stool are very generally infected, it is also reasonable to assume that the zoospores or the plasmodium may travel downward and then out into the new shoots in the same manner that they may travel upward into a growing shoot.

Effect on Tissues of the Host

There was no cell destruction or hypertrophy of the host tissues as in the case of other species of the genus *Plasmodiophora* and this is the character on which Maire and Tison (10, 11) erected the genus *Ligniera*. The hypertrophies of the tissues of the hosts which are induced by some species of *Plasmodiophora* may be explained by the fact that these parasites attack active meristomatic tissues, while this organism attacks the tracheary tissues which have passed the meristomatic stage. Vascular tissues occur in potato warts (*Chrysophlyctis endobiotica* Schild) (1, 7, 15) but the warts are apparently the result of a stimulation of meristomatic tissues of the host which have resulted in the formation of both parenchyma and vascular cells. None of the papers which have come to the authors attention indicate any stimulation of vascular tissues. This interpretation is in harmony with studies by the author on plant galls caused by insects and other abnormal plant growths. The infested fibro-vascular bundles eventually become red, but this reddening which is quite common in the lower parts of canes is not necessarily an indication of disease.

TRANSMISSION

The method of transmission of this organism from plant to plant and from place to place has not been thoroughly studied. It appears that the organism can complete its entire life cycle repeatedly in the fibro-vascular bundles of the growing cane, gradually working from the base upward, so long as it does not kill the individual cane in which it is living. It is also evident that slightly infected canes will have a better chance of surviving than severely infected canes. Slightly infected canes which do not show the symptoms of the disease are very likely to be used for seed and become carriers of the organism. There is abundant evidence to show that this is the case. In the 300 acres of severely infected D–109 to which the writer has referred (page 19) the source of the disease was traced with a reasonable degree of certainty to a field growing on a higher elevation, from which the seed cuttings were obtained and in which the symptoms were few and insignificant, but in which slightly infected canes were found without difficulty. The difference in severity of the disease in the parent and daughter fields was no doubt due to the fact that the differences in elevation and drainage in the two fields made the conditions for the growth of the organism more favorable in the daughter than in the parent field.

The formation of free swimming zoospores and the fact that the disease is most severe in wet soils are reasons for believing that the organism can travel from plant to plant. The experimental work on this phase of the problem is insufficient to justify any definite statements at this time (see page 23). However, it is well known that the zoospores of *Plasmodiophora brassicae* (4) and some species of *Ligniera* (10, 11, 16, 17, 18) gain entrance to healthy plants through the roots. There is some difference of opinion as to whether this entrance is directly into the root or first into the root hairs and thence into the roots, but the evidence indicates that it is through the root hairs. This organism is known to exist in the roots of the sugar cane but its method of entrance has not been studied. Chupp's (4) studies on *P. brassicae* indicate that the zoospores do not travel far in the soil. However, organisms of this kind may be carried on farm implements or in drainage or irrigation water for a considerable distance. This phase of the problem should be studied.

COMPARISON WITH RLATED ORGANISMS

The organism was originally placed by Matz in the genus *Plasmodiophora* but it differs from other species of this genus in that it does not produce hypertrophy of the host tissues. According to the present classification it belongs in the genus *Ligniera* which was formed by Maire and Tison (10, 11) to include those species of *Plasmodiophoraceae* which produce little or no hypertrophy of the host tissues. It is interesting to note that this classification is based primarily on the reaction of the host to the parasite and not on morphological characters or life history of the parasite. However, this classification has been recognized by some of the leading students of this order, who are referred to in this paper. Therefore, it is desirable to compare this organism with recogized species in these and other genera of the *Plasmodiophoraceae,* especially belonging to the genera *Plasmodiophora* and *Ligniera.*

One very marked difference between *Plasmodiophora brassicae* and *Ligniera* (*Plasmodiophora*) *vascularum* is that the former attacks the parenchyma tissues while the latter lives primarily in the tracheary tissues. The plasmodium of *P. brassicae* produces resting spores which germinate in a very similar manner to what we find in *L. vascularum.* Chupp (4) was able to observe the germination of the spores of *P. brassicae* and states that the actual germination is proceeded by a swelling of the resting spore which is not true in the case of *L. vascularum.* The zoospore of *P. brassicae* was uniflagellate and pyriform as in *L. vascularum.* However, he did not observe

the various amoeboid forms recorded by Woronin (20) and which the writer believes to be characteristic of *L. vascularum*. Both Lutman (9) and Chupp (4) have demonstrated that the zoospores of *P. brassicae* enter new plants through the root hairs and Chupp figures an amoeba in a swollen root hair. This has not been worked out for *L. vascularum*. Chupp's experiments indicate that the zoospores of *P. brassicae* travel very short distances in undisturbed soils.

In 1911 Maire and Tison (10, 11) erected the new genus *Ligniera* for two new species, *L. verrucosa* and *L. radicalis*, which they described. To this new genus they transferred *Sorosphaera Junci* Schwartz (16). In 1912 Winge (19) reviewed the order *Plasmodiophorales* and placed in the genus *Ligniera* four of his own species which he had previously placed in the genus *Sorosphaera*. In 1914 Schwartz (17) recognized the validity of the genus *Ligniera* and described three new species. In 1925 Fron and Gaillat (6) described *Ligniera pilorus* on *Poa annua* which they separated from the closely related *Ligniera radicalis* because it produced an enlargement of the root hairs. Schwartz was unable to germinate the spores but Maire and Tison found zoospore formation in *Ligniera radicalis*. Fron and Gaillat also found zoospores. It is very generally believed that the zoospores of the species of this genus gain entrance to the host through the root hairs but the evidence is by no means complete. Cook (2) says "When the amoeba is lying in a root hair a swelling is sometimes noticed, though infected root hairs showing no hypertrophy are more common". In 1925 Fron and Gaillat (6) separated *Ligniera pilorum* from *Ligniera radicalis* because it induces hypertrophy in the root hairs of the host. However, there is no record of species of the genus *Ligniera* causing hypertrophy in the roots.

The preceding studies indicate that this organism should be transferred to the genus *Ligniera*. The writer therefore proposes the following transfer and revised description:

Ligniera vascularum. (Matz) comb. nov.

Plasmodiophora vascularum Matz, Jour. Dept. Agr. P. R. 4: 45. 1920.

A yellowish, granular, plasmodium, resting spores or zoospores inhabiting the tracheary tubes and occasionally surrounding cells of sugar cane. The resting spores usually granular, orange, yellowish or slightly brownish in color with thick hyaline walls and measuring about .014–.016 millimeters in diameter. Zoospores pyriform and uniflagelate, becoming euglenoid and amoeboid.

CONTROL

At this time the disease appears to be of minor importance but its presence on the Island is a menace to susceptible varieties especially when grown on wet lands and it may at any time become an important disease on new or introduced varieties. Therefore it is inadvisable to use seed from fields in which the disease is known to occur. Slightly infected seed cuttings may become carriers of the organism from field to field or to distant localities.

SUMMARY

1. The organism previously known as *Plasmodiophora vascularum* Matz is transferred to the genus *Ligniera* Maire and Tison.

2. This organism lives primary in the tracheary tissues of the sugar cane but accasionally spreads to surrounding tissues. It does not cause hypertrophy of the host tissues.

3. It is the cause of a disease of sugar cane known locally as "dry top rot".

4. It is known only in Porto Rico and only in cane.

5. The life history is very similar to that of *Plasmodiophora brassicae* Wor. and to several species which have been placed in the genus *Ligniera* by Maire and Tison, Fron and Gaillat, Guyot, Schwartz and Winge.

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Insular Experiment Station,
Río Piedras, Porto Rico.

LITERATURE CITED

1. **Artschwager, E. F.** Anatomical Studies on Potato Wart. Jour. Agric. Res. 33: 963–967. 1923.
2. **Cook, W. R.** The genus Ligniera Maire and Tisson. Trans. Brit. Micros. Soc. 11: 196–123. 1926.
3. _____ 1928. Quelques observations sur le genre Ligniera. Bull. Soc. Mycol. Fr. 44: 105–108. 1928.
4. **Chupp, C.** Studies on Clubroot of Cruciferous Plants. N. Y. Agric. Exp. Station (Cornell) Bull. 387: 421–452. 1917.
5. **Eycleschymer, A. C.** Club-root in the United States. Jour. Mycol. 7: 79–88. 1891.

6. **Fron, G. et G. Gallat.** Contribution a l'etude du genre Ligniera. Bull. Soc. Mycol. Fr. 41: 388–390. 1925.
7. **Güssow, H. T.** A serious potato disease occurring in Newfoundland. Dept. of Agric. Central Exp. Farm, Ottowa, Bull. 63. 1909.
8. **Guyot, L.** Quelques observations nouvelles sur les Ligniera radicicoles. Rev. Path. Veg. et Entomol. Agric. 4: 76–183. 1927.
9. **Lutman, B. F.** Studies on Clubroot. I. The Relation of *Plasmodiophora brassicae* to its Host and the Structure and Growth of its Plasmodium. Vermont Agr. Exp. Station. Bull. 175: 1–27. 1913.
10. **Maire, R. et A. Tison.** Nouvelles recherches sur les Plasmodiophoracées non hypertrophiantes. Ann. Mycol. 9: 226–246. 1911.
11. ---------- Sur quelques Plasmodiophoracées non hypertrophiantes. Compt. Rend. Acad. Sci. Paris 152: 206–298. 1911.
12. **Matz, J.** A New Vascular Organism in Sugar Cane. Jour. Dept. Agric. P. R. 4: 41–46. 1920.
13. ---------- Dry Top Rot of Sugar Cane. Jour. Dept. Agric. P. R. 6: 28–47. 1922.
14. **Nawaschin, M.** Beobachtugen uber den Feineren bau and Unwandleingen von Plasmodisphora Brassicae Woronin Laufe ihres Intra cellularen Lebens. Flora Jahr. 8: 404–427. 1899.
15. **Orton, C. R. and F. D. Kern.** The Potato Wart Disease. Penn. State Agric. Exp. Station Bull. 156: 1919.
16. **Schwartz, W. J.** Parasitic Root Diseases of the Juncaceae. Ann. Bot. 24: 511–522. 1910.
17. ---------- The Life History and Cytology of Sorosphaera graminis. Ann. Bot. 25: 721–797. 1911.
18. ---------- The Plasmodiophoraceae and their Relationship to the Mycetozoa and the Chytrideae. Ann. Bot. 28: 227–240. 1914.
19. **Winge, O.** Cytological Studies on the Plasmodioraceae. Arkiv. f. Bot. 12: 1–39. 1912.
20. **Woronin, M.** Plasmodiophora brassicae, Uheber der Kohlplanzenhernie. Jahrb. Wiss. Bot. (Pringsheim) 11: 348–574. 1878.

FIGURES

PLATE III

Fig. 1.—Plasmodium in tracheary tube. Also one resting spore which did not germinate.

Fig. 2.—Cross section of tracheary tube showing plasmodium.

Fig. 3.—Cross section of part of fibro-vascular bundle showing plasmodium in tracheary tube and some surrounding cells. Also early stage of spore formation.

Figs. 4 and 5.—Plasmodium showing early stage of spore formation. Note the nuclei.

PLATE IV

Fig. 6.—Early stage of spore formation and one resting spore of preceding generation which has not germinated.

Fig. 7.—Tracheary tube showing slightly advanced stage in spore formation. Note the nuclei.

Fig. 8.—Early stage in spore formation. Note the nuclei and delicate cell wall.

Fig. 9.—Cross section of part of fibro-vascular bundle, showing spore formation in tracheary tube and plasmodium in surrounding cells.

PLATE V

Fig. 10.—Early Stage in spore formation and one resting spore from same tube which has not germinated.

Fig. 11.—Group of forming spores.

Fig. 12.—Group of forming spores in tracheary tube.

Fig. 13.—Young spores showing black spots which are nuclei and fat bodies.

Fig. 14.—Forming spores of various sizes.

Fig. 15.—Spores in advanced stages showing nuclei and vacuoles.

Fig. 16.—Part of small tracheary tube with single row of spores. Slightly shrunken.

Fig. 17.—Oblique section of tracheary tube showing immature spores of several sizes.

PLATE VI

Fig. 18.—Two mature spores.

Fig. 19.—Diagramatic drawing of spores in a tracheary tube.

Fig. 20.—Two spores with germ tubes.

Fig. 21.—Flagellate cells.

Fig. 22.—Euglenoid cells.

Fig. 23.—Amoeboid cells.

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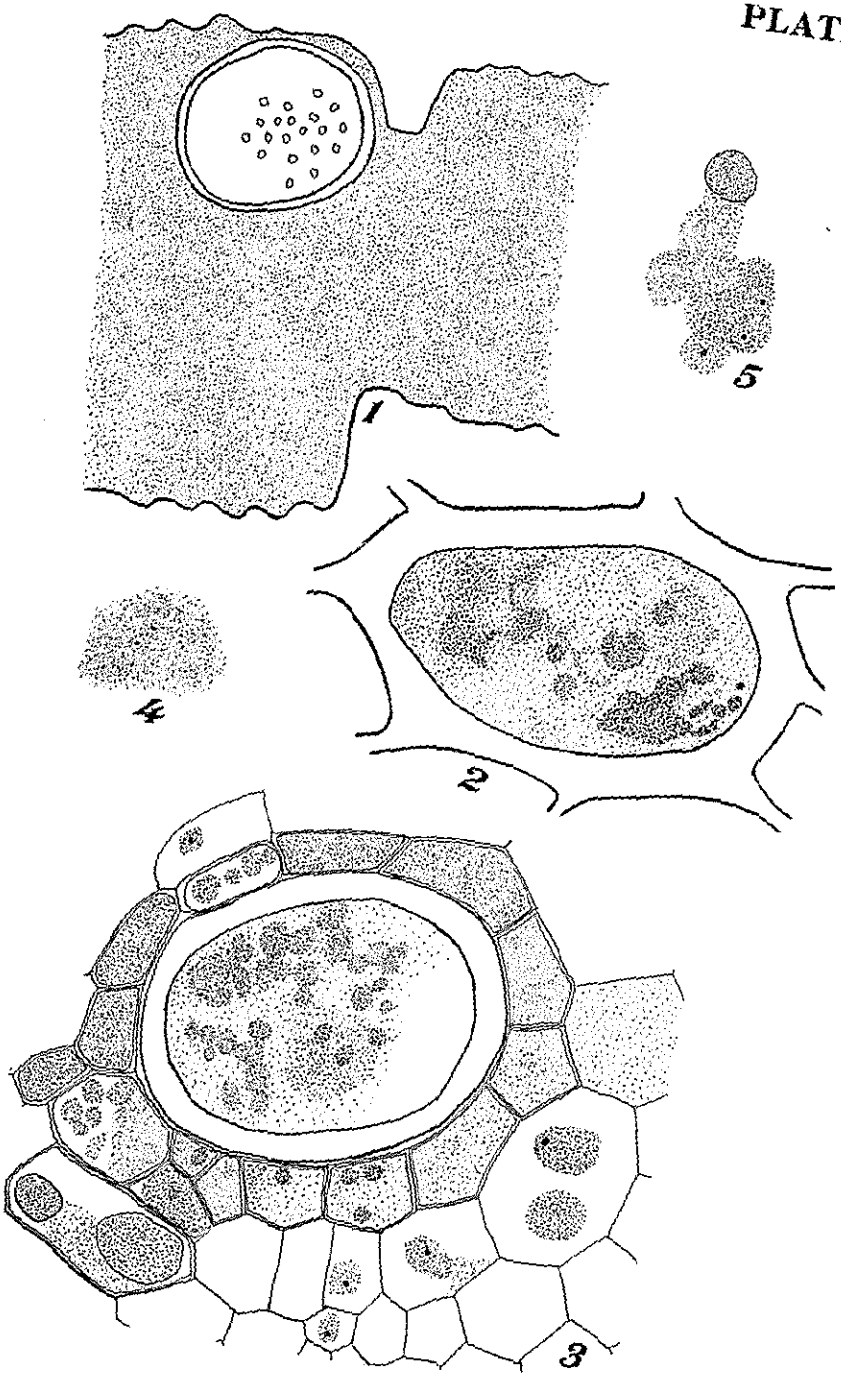
PLATE III.



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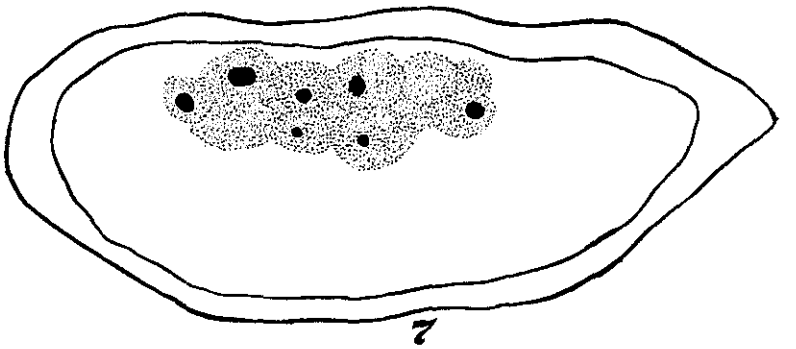

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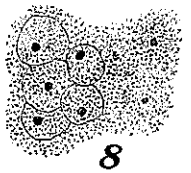

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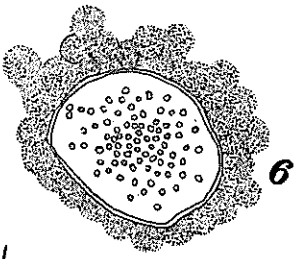

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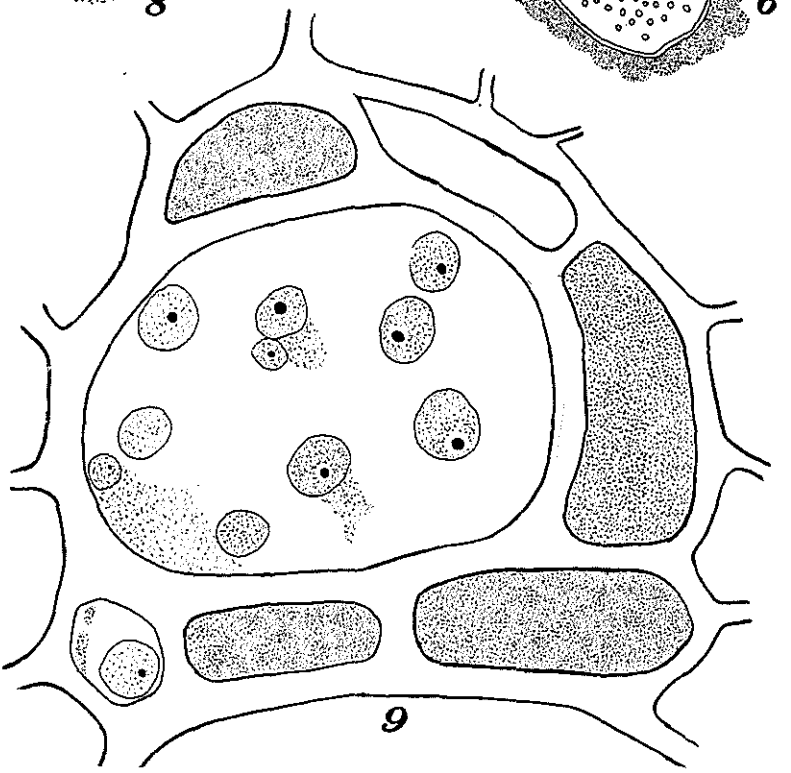

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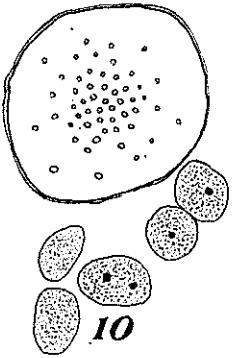
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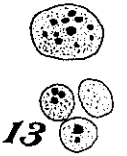
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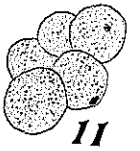
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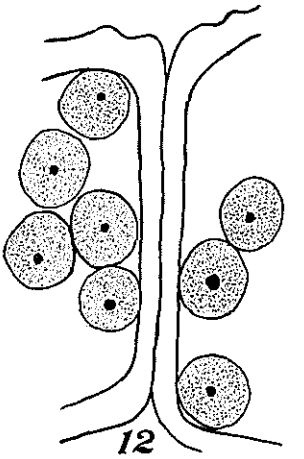
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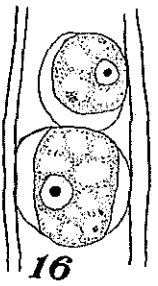
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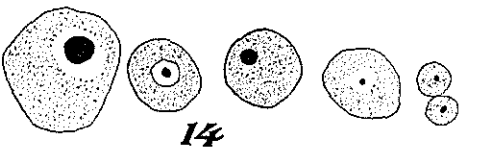
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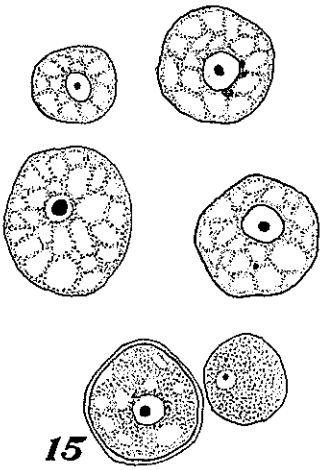
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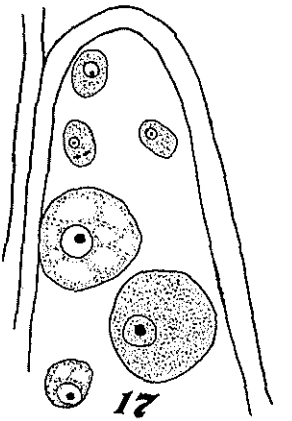
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PLATE VI.