The Die-back Disease of Cacoa trees and the „Brown rot" of Cacoa Fruits, caused by Diplodia cacaoicola.

by

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With Plate VIII and IX.

Introduction.

The dying off of cacoa trees-not as a result of old age, but in plantations which are still fairly young, often by groups and sometimes in such large numbers, that whole fields had to be written down-has repeatedly been observed in Surinam. It is only in the last few years, however, that more attention has been paid to this dying off, and that it has become a subject of study. In this way it was found that this dying off was not always due to the same disease, but that it had to be attributed to the attack of different parasites. For instance the „canker" which sometimes occurred in a destructive manner, was caused by a Spicaria [1]); the so-called „leaf disease", which has also caused much damage from time to time, was recognized as due to Thrips. [2])

1) A. E. de Jonge. Recueil des Travaux botaniques neérlandais. Vol. VI, 1909.

2) Inspectie van den Landbouw in West-Indië. Verslag over het jaar 1906. p. 11.

In the vast majority of cases, however, another disease was concerned, which has of late years received the name of „die-back disease", and which, after a preliminary investigation, was found to be caused by *Chaetodiplodia.* [1])

Symptoms of the Disease.

The disease is especially destructive in fields, where the trees, for some reason or other, are leafless or have only a thin foliage. Such a condition is often brought about by successive attacks of witches'brooms, by repeated shedding of the leaves in attacks by Thrips, by the eating of the leaves by ants or by the sudden exposure of the trees to sun and wind. In short, trees in such a state of complete or almost complete leaflessness, are the first to be attacked by the die-back disease. We never noticed the disease on healthy leafy branches.

There is an obvious difference between trees killed by canker (*Spicaria colorans*) or by worms (larvae of *Steirastoma depressum*) and trees which are suffering from the die-back disease; in the former cases the leaves generally dry up and in this condition they remain hanging on the dead tree, a result of the rapidly fatal course of these diseases; in the die-back disease on the other hand the leaves while still attached to the tree, first assume a yellow and sickly appearance and then they fall off, so that the tree has already lost all its leaves before it is quite dead.

Generally the disease first shows itself at the top of the twigs which have borne witches'brooms, or have suffered from Thrips or other adverse influences. Thence the

1) C. J. J. van Hall and A. W. Drost. Recueil des Travaux botaniques néerlandais Vol. IV. 1908. p. 288.
Departement van Landbouw in Suriname. Bulletin no. 16. p. 41.

disease spreads downwards into the thicker branches, passes from these into healthy lateral branches and may finally also attack the trunk. In that case the tree succumbs. Frequently, however, it happens that the disease comes to a stand-still, especially when it has reached the trunk or a thicker branch, so that it remains confined to a lateral branch with its twigs.

Very frequently branches are not attacked on the whole of their circumference, but a wider or narrower strip remains healthy. In such cases the diseased portion is, as a rule, on the lower side of the branch. Then the healthy portion often still bears green leaves, when the diseased part has already died off.

Sometimes the disease runs a very rapid course, and the discoloration which then occurs, may often spread through a distance of more than a metre in a few days.

In a few instances we observed, that healthy trunks which had been pruned on account of the witches'broom disease, were attacked by the die-back disease and killed. This, however, only occurred when the branches had been cut off in an unfavourable rainy reason, when the covering of the cut surfaces could not take place sufficiently well.

If the bark be stripped off from an attacked branch, it becomes obvious that the diseased tissue has been discoloured in a peculiar way. The diseased bark, which is still alive, shows a reddish-brown colour and forms a transition between the dull brown bark which is already dead, and the reddish yellow portion which is still healthy. Furthermore, the fibres show up more clearly in the diseased tissue than in the healthy bark; this is partly due to the fibres of the healthy tissue having the same light colour as the intervening medullary rays and phloem-groups, whereas in the diseased tissue the last-named become brown, while the fibres preserve their original

light colour; another reason why the fibres show up clearly in disease is the fact that the intervening tissue soon sinks in somewhat (fig. 1 at *a*). In the dead bark this sinking in has become much more marked, so that the fibres stand out still more; this phenomenon, however, also takes place in branches which have died from other causes, and one may therefore not assume, in examining the dead branches, that they have died of the die-back disease.

The discoloration of the bark may be best observed by cutting off a piece of it longitudinally. Not only the bark, but the wood also undergoes a change of colour, which is especially clear in transverse section; the diseased portion of the wood, while still alive, acquires a pale brown colour which after death changes to dark grey. If only a portion of the branch is attacked — a common case, which has already been described above — it becomes evident on transverse section of the branch, after the death of the diseased portion, that the change in colour extends to the centre of the wood along a definite sector. In Fig. 2 the dark sector represents the dead portion of the branch.

On the dead branches in the field there ase sometimes found the grey fruit-bodies of a *Diplodia* or of a *Chaetodiplodia;* if the branches are kept in a room, whether or not under a glass bell-jar, such fructifications are always observed after a few days.

There also occurs in the cacao-fruits a disease, which must be considered here, for, as will be seen later, its cause is the same as that of the die-back disease of the trees. On cut pods, and perhaps also on fruits which still hang on the tree — but this point is still doubtful — a brown spot sometimes arises, which very much re-

sembles petrified fruits. [1]) If such fruits are kept in the laboratory, the discoloration is seen to spread over the whole pod, which becomes covered with a coal-black mass of spores. Fruits covered with such a mass of spores are sometimes also found in the field; on examination the spores are found to belong to fructifications of *Chaetodiplodia*. This disease, called in the English West-Indian colonies „brown rot" has a close resemblance to the common „black rot" of cacoa-fruits, which is caused by another fungus, *Phytophtora* sp., and which sometimes causes considerable damage to young as well as to full-grown fruits.

Pods suffering from „black rot" (*Phytophtora*) and from „brown rot" (*Chaetodiplodia*) are similar in colour, and before the spores have been formed, it is impossible to tell with certainty from the external appearance, with which disease we are concerned.

At the beginning of the disease, when the fruit has only just been attacked, „black rot" may be distinguished from „brown rot" by cutting off a little of the rind of the pods. In the case of „black rot" alternating darker and lighter rings are often seen in the plane of section and these extend outwards from the focus of infection. We have not observed such rings in fruits attacked by „brown rot"; here the rind of the pod has a uniform brown colour when a piece of it is cut off.

„Petrified" pods and those, which have died of

1) The petrification of the pods is the symptom of a disease, caused by *Colletotrichum luxificum;* compare Van Hall and Drost, Les balais de sorcière du cacaoyer, provoqués par *Colletotrichum luxificum* n. sp. Recueil des travaux botaniques Néerlandais, Vol. IV, 1908. fig. 16.

„black rot" are, however, often infested afterwards by *Chaetodiplodia as a saprophyte*, and they thus become covered with the above-mentioned coal-black spores.

It is clear, that this may lead one to consider the „brown rot" fungus a more dangerous parasite for the fruits than it really is. This point will be further dealt with below.

Literature.

A branch and trunk disease of cacao, with the same symptoms as the one described above, also occurs in other cacao growing districts. In the West Indian Islands it is known as „die-back disease". Here it was investigated and described by Howard [1]), who observed it in Grenada, St. Lucia, St. Vincent and Dominica. He traced the cause to a fungus, which was determined by Massee at Kew, by means of material sent to him, as *Diplodia cacaoicola* Hennings. Howard found that the same fungus causes the „brown rot" of the fruits, for not only did the fructifications on the branches agree closely with those on the fruits, but he was also able to infect fruits with the fungus from branches and conversely to bring about the „die-back disease" in branches by means of spores taken from fruit suffering from „brown-rot".

He moreover found *Diplodia cacaoicola* on sugar canes from Demerara and Barbados, and proved by means of infection experiments, that this fungus can also live as a parasite on sugar canes and can thence be transmitted to cacao.

Barrett [2]), in his discussion of the „Cacoa Pests of

1) A. Howard. Annals of Botany. Vol. XV, p. 683, 1901. West-Indian Bulletin. Vol. II, p. 203, 1901.

2) O. W. Barrett. Agricultural Society of Trinidad and Tobago. Society Paper no. 280.

Trinidad" considers that 90 % of the loss of fruits by parasitic fungi is due to *Lasiodiplodia*. The disease caused by *Lasiodiplodia* is called „brown-rot" and his description agrees completely with Howard's description of the disease caused by *Diplodia*. As we shall show further, this *Lasiodiplodia* must be the same fungus as the *Diplodia* of the other West Indian islands and the *Chaetodiplodia* of Surinam.

Stockdale [1]) mentions a *Lasiodiplodia* which has as yet been incompletely investigated.

Zehntner [2]) found that in Java a *Diplodia* causes the dying back of cacao trees and also attacked the pods. Finally, according to Petch [3]), in Ceylon a *Diplodia* occurs only very rarely on the fruits, and only very few cases are known of the dying back of trees trough this fungus.

Microscopical investigation.

Microscopical examination shows that a fungus occurs in the diseased portions; the hyphae are seen in the cells of the various tissues of the bark and wood; where they traverse a cell wall they pass through the pits. The mycelium is septate and originally colourless, but when old, it becomes black. The dark colour of wood which has been killed, is also due to the numerous black hyphae traversing it. In the pods the mycelium is found in the pericarp, in the pulp and in the seeds.

The fungus was obtained in pure culture by excising a

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1) F. A. Stockdale. West Indian Bulletin. Vol. IX, p. 177, 1900.

2) L. Zehntner. Korte mededeelingen van het Proefstation voor cacao. II, p. 1, 1904.

3) T. Petch. The tropical agriculturist. Supplement, August 1907, p. 5.

small piece of the bark, from the junction of sound and diseased tissue, with a knife, which had been passed through a flame, and placing this piece of bark on a sterilized nutrient medium. This medium was generally a decoction of cacoa fruits with 2% agar-agar. The fungus developed rapidly and soon covered the medium with a grey, woolly mycelium, on which after a short time the fructifications were formed. They are depicted in Figs 5 and 6.

These pycnidia do not have the regular bottle shape of the fructifications formed on cacoapods or branches which will be described below. They are often curved; sometimes the cavity, in which the spores are formed, is also strongly curled, so that it is cut several times in transverse section (Fig. 6). The pycnidia bear hairs; the spores are at first colourless and unicellular, but afterwards dark and bicellular in consequence of the formation of a septum (Fig. 7). The structure of a pycnidium is seen more clearly in fig. 8 which was drawn from a preparation out of a cacoa fruit. The wall of the fructification in Fig. 8 consists of a pseudo-parenchymatous tissue; its outer layers are black, the inner ones colourless. In the drawing only a few spores have been indicated, but in reality they are formed in this colourless portion in large numbers on sterigmata, between which long threads, the paraphyses project into the cavity. The latter is often completely filled with spores, which at that stage are still unicellular and colourless; it is not until they have passed out, that they become bicellular and acquire a darker colour (Fig. 7). Ripe spores are 22—28 microns long and 11—14 microns broad. The neck of the pycnidium projects outside the pericarp and is covered with hairs. The round opening, through which the spores pass out, cannot be seen in Fig. 8 because the section passes obliquely through the fructification.

Whether the neck of the pycnidium is glabrous or hirsute is generally considered an important systematic character; according as the fructification is hairy or not, the fungus is placed in the genus *Chaetodiplodia* or *Diplodia*. Our culture experiments showed, however, that this character is wholly governed by external conditions.

Fruits, which had been successively treated with a 0,5 % solution of corrosive sublimate, washed with sterile water and infected through a little wound with *Chaetodiplodia* spores, were placed under a glass bell-jar. The fruits gave off enough moisture to cause dew formation on the jar and to keep the air moist during the experiment. Generally the pericarp began to turn brown round the wound after two or three days; the discoloration spread rapidly and after a few days the grey woolly pycnidia with a *hairy neck* made their appearance. The fruit had then the appearance illustrated in Fig. 3 and microscopical preparations resembled Fig. 8.

If the fruits are treated in the same manner, but if, after infection or after the appearance of the brown coloration, they are not kept in a closed space, but are laid down or hung up in the laboratory, the pycnidia do not make their appearance. A large number of small openings are indeed formed in the pericarp, and from these long, white and often strongly curled tendrils protrude. On microscopical examination these are seen to be the unripe colourless spores, which are extruded from the opening of the pycnidium and remain connected for some time as threads. They gradually become grey and then black. In this case the pycnidia themselves do not protrude so far from the pericarp. The fruit then resembles the one of Fig. 4. Often the neck of the pycnidium is quite glabrous as in Fig. 9. Sometimes it has a few hairs, but there are never enough to be recognized by

the naked eye or with a simple lens. The same condition is observed in diseased branches, which have remained dry; the white spore tendrils protrude from fissures in the bark (fig. 10) and the pycnidia are hairless. If such branches are placed in a moist atmosphere, a few hairy pycnidia are indeed formed, but the strong contamination with other fungi often prevents further observation. It results from the above, that it depends entirely on cultural conditions, whether the pycnidia are glabrous or hirsute. As the hairyness of the pycnidia is the only character, by which *Chaetodiplodia* is distinguisted from *Diplodia*, there is no reason for retaining these two as different genera and accordingly the first generic name must disappear. Our fungus differs in no constant character from the *Diplodia cacaoicola* examined by Howard and we must assume that we are dealing with the same fungus. This identity is also supported by Howard's observation regarding the fungus on pieces of diseased sugar cane, when placed in a moist chamber: „There was a considerable development of hairlike processes on the walls and round the opening of the pycnidium, giving the colonies a furry appearance which was never noted in the cane in ordinary circumstances." [1])

On branches and to a less degree on pods the pycnidia are often not found isolated in the plant tissue but then occur in groups, giving the impression that they lie in a stroma. This may already be observed in Fig. 9, but especially in Fig. 11, which was drawn from a preparation of a branch. With such a disposition of the pycnidia one would call the fungus *Lasiodiplodia*, for *Diplodia* and *Lasiodiplodia* agree completely, except as regards the grouping of the pycnidia. Whereas these are solitary in *Diplodia*,

1) A. Howard, Annals of Botany. Vol. XV, p. 686.

they are united to groups in a stroma in *Lasiodiplodia*.

On infecting fruits with spores of such pycnidia, solitary pycnidia are again formed in the fruit, a proof that we are once more dealing with only one fungus. The loose groups of cells, which lie between the pycnidia in Fig. 11, show moreover that there are transitions between solitary pycnidia and pycnidia arranged in groups. The fungus observed by Barrett, which causes in Trinidad the „brown-rot" of the cacoa pods and of which material, when sent to the Bureau of Plant Industry of the U. S. Department of Agriculture, was determined as *Lasiodiplodia*, is doubtless identical with the *Diplodia* occurring in other West Indian islands and in Surinam.

Howard [1]) also observes that the fructifications generally form colonies in the trunk and branches, and suggests, that the *Botryodiplodia*, found by Patouillard in diseased cacoa fruits in Ecuador, which possibly causes one of the forms of Mancha [2]), might be the same fungus as the *Diplodia* studied by him.

However this may be, in any case it results from the observations described, that the characters, which differentiate the genera *Diplodia*, *Chaetodiplodia* and *Lasiodiplodia* are not constant, so that the three genera should not be separated systematically but should be united in one genus *Diplodia*. Thus the genera *Chaetodiptodia* and *Lasiodiplodia* disappear, and perhaps further investigation will show that the character by which *Botryodiplodia* is distinguished (absence of paraphyses) is governed by external conditions.

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1) A. Howard. Annals of Botany Vol. XV, p. 690.
2) Lecomte et Chalot. Le Cacaoyer et sa culture p. 64, 1902.

Infection Experiments.

Infection experiments were undertaken with a twofold aim, in the first place, in order to see whether the fungus which kills the branches is the same as that found on fruits, and in the second place, to ascertain whether healthy fruits can be attacked.

In the first experiments a small piece of the bark of a trunk or branch was cut loose on three sides, so that it could be lifted up like a lid. Under this were introduced pieces of diseased tissue, taken from branches or fruits, also spores from branches or pods or from mycelium grown in pure culture. A bandage was placed round the wound, which was kept moist. None of these infections was successful, however.

In a subsequent series of experiments the tops of very young branches were cut off and the planes of cutting were infected. These infections were likewise without result.

We obtained better results by cutting or tearing pieces from branches having the thickness of a finger, infecting the extremity and making round this a damp bandage, or also by binding the extremity in a little bag of waterproof paper, into which some water was poured, so that only the surrounding air remained moist. Of the ten branches which we treated in this manner, four were attacked. After a fortnight these branches were diseased for a distance of ¼—1 metre up to the place where they joined a thicker branch. These thicker branches remained unattacked. The control branches remained healthy. Among the successful infections were three, in which the infection-material was obtained from fruits.

The *Diplodia*, which causes rotting of the pods is

really therefore the same as the one which causes the dying back of branches.

Infections of cacoa fruits were carried out in the laboratory and also in the field. The pods which were infected in the laboratory were first treated with a 0.5% solution of corrosive sublimate and were then washed with sterile water. The infections material, which was again derived from pods, branches or pure cultures, was introduced into small wounds, made in the fruit by a cut or a puncture. These infections were carried out in large numbers and were uniformly successful, no matter whether the fruits were kept in a moist atmosphere or were laid down or hung up dry. If the treatment with corrossive sublimate was omitted, the infection took place equally well, but in addition all sorts of other fungi developed, the conidia of which had been present on the fruit. The pods were however, not attacked if the infection material was placed on the *intact* fruit or if such a pod was hung in contact with a diseased one.

In no single instance did we succeed in infecting pods while they were still hanging on the trees, no matter whether younger or older fruits were taken for this purpose or whether an attempt was made in some way or other to keep the place of infection moist.

If none of the infections of fruits had been successful, it would nevertheless have been unjustifiable to draw the conclusion, that *Diplodia* does not attack pods; since, however, all fruits cut off from the tree were attacked, but none of those which were still on the tree, the supposition seems permissible, that perfectly healthy pods are scarcely susceptible to the disease, but that fruits in abnormal conditions are susceptible, like the cut fruits in the experiments. It is very probable therefore, that *Diplodia* occurs in Surinam in most cases as a saprophyte

on pods, although fruits of feeble trees, or of trees placed in unfavourable conditions, may perhaps sometimes be attacked by it primarily. Finally the experiments show, that, in the case of fruits also, the fungus probably only penetrates through wounds.

Combative measures.

It follows from the above that *Diplodia* is a wound parasite. That it can penetrate directly into living tissue, was never observed by us in the field, and may be doubted.

The loss of pods, which arises through „brown rot" is presumably small and very likely only occurs in the case of fruits which have been wounded in some way or other. In the case of the branches also we consider the fungus, as was painted out above, to be a secondary wound parasite which only attacks the branches after they have been damaged by some cause or other. The primary cause is a different one, and that primary cause the planters must reckon with in the first place, when combating the die-back disease. There is only one piece of advice which can be given for the protection of cacoa trees against the die-back disease: keep your trees in a strong, healthy state of cultivation and be on your guard against the diseases mentioned on page 3.

In Surinam the fight against the witches' brooms disease will have to be carried on first, in order to counteract the loss of trees by the die-back disease.

A second and also very important cause which favours the spread of the disease, is *Thrips*. Of late years *Thrips* has been recognized as the primary cause of the loss of large plantations of cacao trees in consequence of the die-back disease. *Thrips* chiefly attacks weak and unhealthy trees; with their mouths they make numerous small

incisions in the epidermis of twigs and of leaves, and throug these infection can take place. Really healthy trees do not suffer so much from *Thrips*. The obvious course is therefore to give the trees all they require, and to prevent everything which may affect growth adversely.

A third cause is found in strong winds, which tear the leaves from the trees or damage them; an efficient protection of the trees to windward will diminish their liability to the disease.

In those cases in which it is noticed sufficiently early that the branches have been attacked by the die-back disease, the latter can be stopped by shortening the branches down to the healthy wood. The trees then form again strong new wood and often recover from the disease.

Lesions of the trees as a result of cutting back, but also of unsuitable pruning or thinning, may affect the tree adversely and sometimes even bring about its death, if these operations are not performed at the right time. The numerous wounds, which are thus formed, give too many opportunities to the fungus of the die-back disease to infest the lesion and to enter the wood, especially in the rainy season when the coating of the wounds with tar cannot take place, or can only be done imperfectly. Cutting back and pruning should always take place in the dry season and for this reason it is distinctly objectionable in the rainy season. After the cutting off of the branches, the surface of the wound should always be tarred, so as to prevent the fungus obtaining a hold on the branch.

Since it has become evident, that *Diplodia* occurs frequently on fruits which have died from other causes, or on the skins of harvested pods, the removal of such fruits and remains of fruits must be considered one of

the necessary operations on a plantation. The great quantity of spores which develop in them constitute a source of infection for the neighbourhood. In the English West Indian islands observations would appear to indicate, that „brown rot" is especially common in the neighbourhood of so called breaking-places, where, after removal of the seeds, the husks of the cacaopods remain lying about in heaps. This is explained by the saprophytic growth of *Diplodia* on the husks, which of course causes an increase of infectious material. The burial of the husks accordingly proved to be an effective way of combating „brown rot", and may also be recommended to Surinam planters as a means of combating the die-back disease.

Department of Agriculture,
SURINAME.

EXPLANATION OF THE FIGURES ON PLATE VIII AND IX.

Fig. 1. Attacked branch, of which the bark has been cut off superficially at the place where the diseased tissue (a) passes into the healthy tissue (b).

„ 2. Transverse section of a diseased branch. The dark sector is the part killed by the fungus.

„ 3. Cacoa pod on which, in a moist chamber, the grey hairy pycnidia of Diplodia have developed.

„ 4. Cacoa fruit which was hung up in the laboratory after infection, and is now partially covered with the black spores of Diplodia.

„ 5. Pycnidia of Diplodia from pure cultures.
5*a* magnification 3 ×.
5*b* „ 10 ×.

„ 6. Section through a pycnidium grown in pure culture. Magnification 25 ×.

„ 7. Spores of Diplodia. Magnification 375 ×.

„ 8. *Hairy* pycnidium of Diplodia in the pericarp of a cacoa fruit, formed in a damp atmosphere *a* = wall of the pycnidium; *b* = pericarp of the cacoa fruit. Magnification 115 ×.

„ 9. Pycnidium with glabrous neck, formed on the pericarp in a non-enclosed space. Magnification 115 ×.

„ 10. Diseased branch, which has been in a non-enclosed space. From the fissures in the bark, beneath which are pycnidia, unripe spores protrude, still hanging together in tendrils ×. Magnification 20 ×.

Fig. 11. Section through a group of pycnidia lying in a stroma. At × there lies between the pycnidia a group of cells of the branch, which the fungus has infested. Magnification 18 ×.

Fig. 3.

Fig. 4.

Fig. 1.

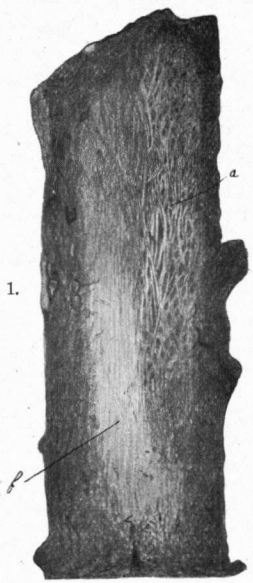


Fig. 2.

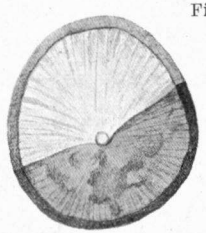


Fig. 5.

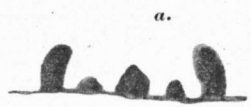


Fig. 8.

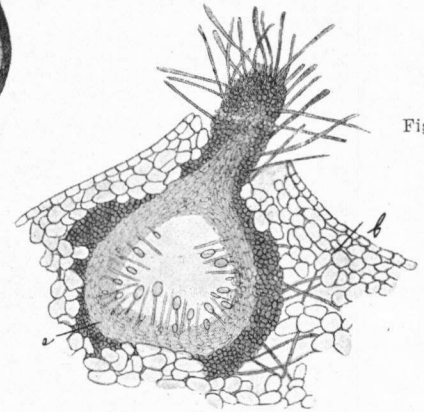


Fig. 10.

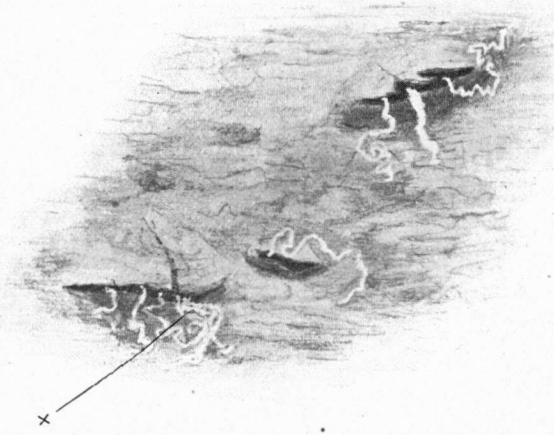
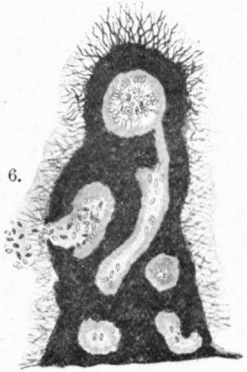


Fig. 6.



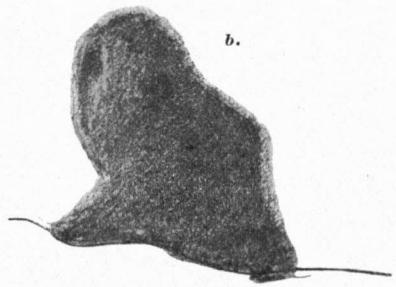


Fig. 7.

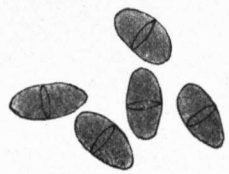


Fig. 9.

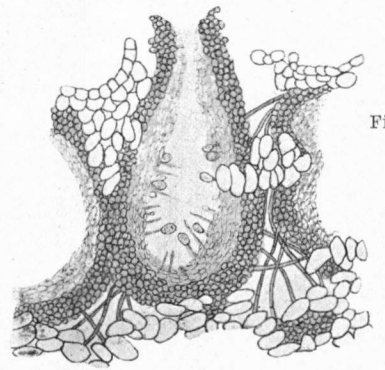


Fig. 11.