On *Diplodia cacaoicola*, P. Henn. ;
a parasitic Fungus on Sugar-Cane and Cacao
in the West Indies.

BY

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With Plate XXXVII.

WHILE engaged from time to time since December, 1899, in a search, which has so far been unsuccessful, for the perithecia¹ of *Trichosphaeria Sacchari* (Masse), among diseased sugar-canes in Barbadoes and elsewhere, a Fungus was very commonly met with which was distinguished by having brown elliptical bi-septate spores developed in pycnidia just underneath the rind of the cane, and which ruptured the surface when ripe. To the naked eye there is little difference between this Fungus and the *Melanconium* stage of the 'rind Fungus' described by Masse, and it was observed that when this new form predominated on diseased canes it was locally regarded as 'rind Fungus.' Indeed in the West Indies at the present time it is customary to regard all cane diseases as either 'rind' or 'root' disease, and many are of opinion that both these diseases are caused by one Fungus. It is very probable, however, that there are several rind, leaf, and leaf-sheath

¹ On *Trichosphaeria Sacchari*. Ann. Bot., vol. vii, p. 128, 1893.

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diseases, and that 'root' disease is nothing more than a general term covering several apparently distinct maladies.

During the early part of last year cultivation-experiments were performed with the Fungus, and no difficulty was experienced in obtaining hanging-drop cultures containing a single spore. The germination of the spores was observed in several culture-media, and the Fungus was grown in flasks, in tubes, and on sterilized wood. At this time frequent absence from the laboratory and pressure of other duties prevented a complete study of the Fungus being made, and it was not till last December, when an outbreak of what was described as 'rind' Fungus occurred in Demerara and diseased canes were forwarded to the Department of Agriculture by G. S. Jenman, the Government botanist of British Guiana, that the study of this form was resumed.

It was then found that the predominant Fungus on these canes was identical with the pycnidial form noted earlier in Barbadoes, and since many of the canes appeared to contain this Fungus alone, experiments which are described below were undertaken to follow the development of this form under artificial conditions, and to prepare pure cultures.

About this time the cacao planters in Grenada had their attention directed to the diseases of cacao by the spread of 'thrips,' which did some slight damage to the pods and the young leaves of the trees. It was observed that several trees on which 'thrips' had been seen died off rather rapidly, and considerable alarm was caused by the belief that these insects might destroy the cacao industry in that island. An investigation on the spot, however, showed that it was highly probable that the trees believed to be dying through the attack of the thrips were really being killed by Fungi, and that the insects were not more numerous on the diseased trees than on others which were quite healthy. Portions of the branches of such trees were collected and sent to Barbadoes for examination, when it was found that in all cases they contained the same Fungus, and that this was apparently identical with that found on the diseased sugar-canes from

Demerara. Experiments, which are described below, were undertaken to follow the development of this cacao Fungus, starting from a single spore. The behaviour of the two forms was therefore studied side by side, and the results are given in the following.

In February and March of the present year I was requested by Dr. Morris, the Imperial Commissioner of Agriculture for the West Indies to visit Grenada and to report on the nature and extent of the damage done to cacao trees by fungoid diseases. While engaged on this mission I found that in addition to its occurrence on cacao trees, the Fungus was common on growing cacao pods, where it was apparently the cause of a rather serious disease. Infection-experiments with pure cultivations of the Fungi found on canes and on the cacao tree, on their respective host-plants, and also cross-infection experiments were carried out during this time, and are described below. Later in the present year a Fungus on cacao was noted in St. Lucia, St. Vincent, and Dominica, which proved to be identical with that found in Grenada.

THE SUGAR-CANE FUNGUS.

The external appearance of a sugar-cane in which this Fungus occurs is not unlike that of a cane attacked by the *Melanconium* stage of *Trichosphaeria Sacchari*. The rind is ruptured by the growth of dark bodies underneath, which are arranged in more or less vertical lines (Pl. XXXVII, Fig. 1). Transverse sections through these black areas show that the disturbance is caused by the development of colonies of true pycnidia formed just beneath the rind, and which are in connexion with a dark-brown septate-branched mycelium abundant in the tissues of the cane (Fig. 2). The wall of the pycnidium is seen to be made up of two distinct layers, an outer *peridium* made up of dark-coloured, thick-walled hyphae forming a definite tissue, and an inner hymenium of thin-walled colourless cells, charged with granular contents, which are no doubt destined for spore-formation. In the cavity of the pycnidium short conidiophores, 20-40 μ in length, bearing

elliptically shaped spores in all stages of development, and numerous paraphyses, are to be seen. The detailed structure of a pycnidium seen under the high power is shown in Fig. 3. It agrees closely with that described by Bauke¹ in the case of a *Diplodia* on *Cornus sanguinea* and figured in Zopf².

In cases when pieces of diseased cane, on which this Fungus was found, were placed in a moist chamber, it was observed that there was a considerable development of hair-like 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.

The spores are liberated from the mature pycnidium by means of a circular ostiole at the apex (Fig. 4), and are at first greyish and unicellular. In a short time, however, the exospore rapidly darkens and becomes dark brown, while at the same time a transverse wall is formed across the equator of the spore. The mature spores are very constant in size and measure $20 \times 10 \mu$.

The development of the Fungus was next studied, starting from a single spore. No difficulty was experienced in obtaining hanging-drops containing a single spore by the method described by Marshall Ward³ in his investigation on the ginger-beer plant. The spores germinated quite readily in such media as 15 to 20 per cent. gelatine raisin-extract or cane-juice, but the best results were obtained in the following food-material which is referred to later as the cane-extract medium:—

Cane sugar	.	.	.	5 grams.
Gelatine	.	.	.	15 „
Tartaric acid	.	.	.	1 „
Peptone	.	.	.	5 „
Cane-extract	.	.	.	100 c. c.

The cane-extract was obtained by soaking small pieces

¹ Beitr. z. Kenntniss der Pycnidien, Nova Acta, Bd. xxxviii, No. 5.

² Zopf, Die Pilze, p. 60.

³ The Ginger-beer Plant and the organisms composing it. Phil. Trans. B., vol. 183, pp. 130-2, 1892.

of ripe sugar-cane in distilled water for half an hour, boiling, and then filtering. It was found that the addition of the small quantity of tartaric acid was sufficient to check the development of Bacteria in the hanging-drops, and to permit of the mycelium developing in such a way as to obtain pure cultures from the drops.

The ripe spores germinate in from three to six hours after sowing by sending out a colourless hypha from one of the compartments of the spore, which contains finely granular protoplasm (Fig. 4). The hypha grows in length, slowly at first, afterwards much more rapidly, and then branches: about twenty-four hours after sowing septa begin to appear.

In cases where spores are sown which are still colourless and have not yet developed the transverse wall, germination is more rapid than in the case of ripe spores, and takes place in less than two hours after sowing. The earlier stages in the germination of such a spore are shown in Fig. 5.

Two days after sowing, fusion of hyphae was common in the drops. A young hypha approaches very close to one somewhat older, and the advancing tip of the young hypha curves round to meet a short branch pushed out by the older filament, and fusion rapidly takes place (Fig. 6). Where hyphae have crossed in the older regions of the colony a short process is sent out near the crossing from each of the hyphae, and these fuse.

About this time the hyphae commence to grow down into the moist air below the drop, and their development goes on until the mycelium reaches the water on the floor of the culture-chamber, where considerable subsequent growth takes place. The appearance of these positively hydrotropic columns of mycelium reminds one somewhat of the hanging roots of the banyan: the appearance is shown in Fig. 7. From the point of view of pure cultures this aërial development is an obvious advantage, since further cultures which proved to be free from Bacteria were obtained by infecting flasks of sterile food-material, and tubes containing sterile wood, with portions of this mycelium developed from a single spore.

When three days old the mycelium showed signs of darkening in hue, and this tendency increased till the colour was dark olive-green when seven days old. During this colour-development oil-drops appeared in the hyphae, the contents of which gradually collected into certain portions of the mycelium, leaving the rest empty (Fig. 8).

No further developments were observed in hanging-drops, except that the thick-walled hyphae became covered with a dark-brown resin-like covering, like that mentioned by Bauke in the case of a *Diplodia*¹. The old mycelium in the cane near mature pycnidia shows this brown covering also.

In plate-cultivation made with spores from a diseased cane by the dilution-method, using the cane-extract medium, pure cultures were obtained containing only one colony, which in three days developed a copious greyish mycelium, and this, in spite of Bacteria, covered the plate and grew into the air, forming a dense pile about a quarter of an inch in height. When four days old, dark circular hairy bodies were noted, which proved to be pycnidia containing the characteristic spores of the Fungus when examined on the ninth day.

When grown in flasks or tubes of the cane-extract food-material the development is very similar to that on plates, except that when the cultures are free from Bacteria the mycelial development is more luxuriant and the surface of the food-material becomes covered with a dense felted mass of mycelium about half an inch thick, which soon turns black, and in which numerous colonies of pycnidia are embedded.

Such luxuriant development, however, is not so satisfactory as that obtained on sterile oak- or cacao-wood infected with some of the aërial mycelium from hanging-drops containing a single spore. Such cultures, on account of their slow development, were found to be of service in maintaining a stock of fairly fresh pure-cultures during the progress of the investigation, and for use in the infection-experiments. Pycnidial formation does not begin in this case before from six to ten days, and spores cannot be usually detected in

¹ De Bary, Comparative Morphology and Biology of the Fungi, p. 10.

them in less than two or three weeks. In one case, however, a pycnidium was examined from a ten-days-old culture on oak-wood, and stages were observed in spore-formation. The spores are liberated after vacuoles have appeared in the conidiophores. A portion of the hymenium of this artificially grown pycnidium showing spores and paraphyses, is also represented in Fig. 9.

No further developments were noted in the various cultures, which were kept under observation for some months.

It now became necessary to carry out infection-experiments with pure cultivations of the Fungus, and to determine to what extent it can behave as a parasite. Fully developed and extremely vigorous seedling-canes of the variety known as 'Barbadoes 347' or 'B. 347' were selected for the experiment. Incisions were made with a sterile knife in the centre of one of the internodes, about halfway up the stem of the six canes selected for the experiment, after cleaning the internode carefully with alcohol. A small chamber was cut under the rind, into which portions of mycelium from a seven-days-old pure culture of the Fungus in the cane-extract medium were introduced, together with some of the food-material. The wounds were then bound up with budding-tape. Four canes were infected with mycelium, while the other two were treated similarly except that no mycelium was introduced and thus served as control canes. The result was most marked. In four days all four inoculations of the infected canes had taken, and the mycelium could be traced in the parenchyma of the internode for about two inches in all directions round the point of infection. In two cases pycnidia had formed under the rind near the point of infection. The control experiments gave negative results, and no reddening of the tissues was observed in the four infected canes.

This result entirely confirmed previous infection-experiments made on sugar-canes in Barbadoes in the early part of 1900, when the Fungus was found to behave parasitically.

THE CACAO FUNGUS.

The Fungus found on the pods and branches of the cacao tree agrees, as far as the characters of the pycnidia and spores go, exactly with the form described above on the sugar-cane. In the case of the pods the pycnidia are often formed singly just under the epidermis, and the latter is ruptured by the growth of these structures. In diseased branches, or on the trunk of the tree, the pycnidia occur usually in colonies just under the bark. The appearance of a branch attacked by the Fungus, which has been kept in a moist chamber a short time, is shown in Fig. 10, and a transverse section through one of the colonies is given in Fig. 11.

Some general indication of the appearance of cacao trees and pods apparently attacked by this Fungus may be of interest. It is quite common in Grenada to see cacao trees dying back to a slight extent at the extremities of the branches, a phenomenon probably due to poverty of soil, wind, drought, or defective root-action, or perhaps to a combination of these causes. In all such cases there is a sharp line of demarcation between the dead and living tissues, and although several Ascomycetes are to be found in the dead wood, they appear to be purely saprophytic in character. In many cases, however, dying back goes on to a very great extent, extending to the larger branches and the trunk, and, in spite of the production of suckers at the base, the trees are often killed outright. In such cases there is no boundary line between dead and living tissues, but an intermediate zone, often as much as two feet in length, always occurs between the obviously dead and living tissue. Mycelium can be easily detected in the young wood in this transition-region, and the pycnidia referred to above are to be found under the bark. The mycelium can be detected in the wood at some distance in advance of the point at which it seems to end in the bast, a point which seems to indicate the saprophytic origin of the Fungus. The mycelium makes its way in the wood from element to element by means of the pits in the walls of the

vessels and cells, and where the hyphae have commenced to darken in colour this point can be determined without staining (Fig. 12).

In cases where pods are apparently attacked by the Fungus this is very common near the 'breaking-grounds,' where the 'beans' are extracted by the pickers, and where it is the custom to leave the empty husks on the ground in heaps. The husks speedily become covered with the spores of the Fungus, as this form lives on them as a saprophyte. The rind of the pod turns brown and mycelium soon spreads to the mucilage surrounding the seeds, completely destroying the pod and its contents, usually in from six to ten days. The diseased areas commence as a brown spot, as a general rule either at the free end of the pod, or in the groove round the insertion of the stalk, or at the point where the pod comes in contact with the branch. These places are those which are liable to be moist long after the rest of the pod is dry, and indicate the probability that infection may here be effected by spores without any previous wounding.

The artificial cultivation of the Fungus was carried out in an exactly similar manner to that employed in the case of the sugar-cane Fungus described above, and as far as possible similar cultures of the two forms were made and examined at the same time.

Stages in the germination of the spore are shown in Fig. 13. The germ-tube grows out into a long hypha, at first slowly, but afterwards much more rapidly, and extensive branching eventually takes place. Septation of the hyphae was not noted before twenty hours after sowing, and after two days fusions of the hyphae were common: about the same time the mycelium commenced to grow down towards the water on the floor of the moist chamber, reaching it four days after sowing (Fig. 7). When three days old the mycelium gradually changed colour, passing through various shades from light yellow to olive green, and at the same time the hyphal contents began to aggregate in certain portions of the mycelium, leaving the rest empty. Oil drops also made their appear-

ance. The appearance of the mycelium, when eight days old, closely resembles that shown in Fig. 8. No further development was noted in hanging-drops, except that the thick-walled oil-containing hyphae became dark brown three weeks after sowing, and appeared identical with the old mycelium described above in the case of the cane Fungus. As in the sugar-cane Fungus, advantage was taken of the aërial development of mycelium in single-spore hanging-drops to prepare cultures free from Bacteria.

In plate-cultures in the cane-extract medium a copious development of greyish mycelium was obtained on the third day, which formed a dense velvety pile, a quarter of an inch in height, on the surface of the gelatine, and in which dark bodies could be detected with the naked eye. When examined on the sixth day these dark bodies were found to be pycnidia in which paraphyses and spore-formation could be detected, after the manner indicated in Fig. 9.

On sterilized cacao- and oak-wood small dark bodies were noted in nine days, and these when twenty days old proved to be the pycnidia of the Fungus.

It will be seen therefore that the development, under artificial conditions, of the Fungus of the cacao tree corresponds exactly in all its details with that found on the sugar-cane, so that morphologically regarded the two forms are identical.

It was now necessary to perform infection-experiments on cacao trees and pods, with pure cultivations of the Fungus. These were as follows:—

1. On cacao pods:

(a) Two nearly ripe pods were selected for the experiment, and were washed with alcoholic corrosive sublimate, and at the points where incisions were to be made small cavities were made in the rind by lifting the surface and cutting out a small portion of the tissues underneath. Into one of these chambers actively growing mycelium, three days old and from a pure culture, was introduced and the pod was bound up with budding-tape. The other pod was treated in a similar way, except that no mycelium was introduced and thus served as

a control. Five days afterwards, about a quarter of the surface of the infected pod had turned brown, and in eight days after infection the whole of the surface was deep brown, and there was a considerable development of pycnidia for some distance round the point of infection. The protrusion of the spores as a greyish 'tendril,' visible to the naked eye, from the ostiole of the pycnidium, and their gradual darkening were beautifully shown on this pod. Near the point of infection the spores were visible as a black dust on the surface of the pod, and in most cases the 'tendrils' had broken down into their constituent spores, each showing the transverse wall and the dark-brown colouration under the microscope. Further away the colour of the spores became lighter and 'tendrils' were more numerous. These were composed of spores loosely cemented together, in which the transverse wall had not yet appeared. The control pod in this experiment showed no infection.

(b) The above preliminary experiment was repeated, and in this case two nearly ripe pods were infected with mycelium, and a third was used as a control. Distinct infection took place in three days, while the control gave negative results.

(c) Next, four half-grown pods were selected for experiment, in order to determine whether the spread of the Fungus is as rapid here as in nearly ripe pods. In each case small cavities were made in the rind, and in the first pod ripe spores from the infected pod in experiment (a) above were placed in the cavity. In the second pod a portion of the rind containing growing mycelium from the infected pod (a) above was introduced, and in the third pod actively growing mycelium from a pure culture was used for infection. The fourth pod served as a control. Seven days afterwards distinct infection was noted in the first pod, the rind having turned brown about a quarter of an inch all round the cavity, and in the discoloured tissue mycelium was extremely abundant. In the second pod infection had proceeded further, about one square inch of the surface being attacked. In the third pod about six square inches of the surface was decayed, and

it was found that the mycelium had penetrated to the mucilage surrounding the seeds and had completely invaded the interior of the pod. Numerous pycnidia were observed under the epidermis near the point of infection. The control pod gave negative results. This experiment is of some interest as it throws light on the steps by which a saprophytic form may gradually become parasitic, and confirms previous observations on the influence of a nutritious food-material in increasing the activity of a Fungus.

(*d*) Next, a preliminary experiment was performed on two nearly ripe cacao-pods, in order to determine whether infection could be produced by spores without previously wounding the rind. A drop of sterile water containing spores was placed on the surface of the pod, and was covered by a small glass cell which was sealed on to the rind by means of budding wax. The cell was covered with a dark bandage to shield the spores from direct sunlight. Seven days afterwards the spores had developed a mycelium on the pod, but penetration of the intact rind by mycelium was not noted in either case. Unfortunately time did not permit of carrying out further experiments to settle this point definitely; but a consideration of natural infections seems to indicate that pods at any rate are capable of being infected by germinating spores directly. If this were not the case it is difficult to explain why infection almost always begins at those points on the pod which are moist for the longest time, viz. the free end of the pod, the groove at the insertion of the stalk, and the place where pods come in contact with the branches.

2. On the cacao trees:

In the infection-experiments performed on the cacao tree itself, a small portion of the outer dry bark was carefully removed and the exposed bast washed with sterile water. A small chamber was made by raising the bark and cutting out a small portion of the bast down to the cambium. After introducing the infecting material, the bark-lid of this chamber was depressed and the whole covered with a water-

tight bandage of budding-tape. The control plants were treated in the same way, except that no mycelium or spores were purposely introduced into the cavity.

(a) Two branches of a healthy cacao tree were selected for the preliminary experiment. In one cavity actively growing mycelium in the cane-extract medium was introduced, while the other branch was used as a control. Eight days after infection the Fungus had killed the bast up to about eight inches above and below the chamber, at which point the branch was nearly ringed. The mycelium could be traced more than a foot above and below the chamber, both in the bast and in the wood, and to a considerable depth in the latter. Numerous pycnidia were developed under the attacked bark, some of which were liberating their spores. The control showed no infection.

(b) Eight healthy cacao plants about eight months old, growing in bamboo pots, were next selected for an experiment. Nos. 1 and 2 were infected with spores taken from a pod attacked by the Fungus, 3 and 4 with portions of the diseased rind of a pod containing actively growing mycelium, 4 and 5 with vigorous mycelium from a pure culture in cane-extract and 7 and 8 were control plants. After binding up the wounds the plants were placed in the shade and watered daily. Eight days afterwards it was found that infection had taken place in all the plants from 1 to 6, while the control plants showed no infection. There was scarcely any difference in the amount of infection when spores or diseased cacao-pod rind were used, except that plant No. 1 was killed outright by the Fungus. When culture-mycelium was used infection was more extensive, one of the plants being killed while the other was evidently dying. In each case (5 and 6) pycnidia were formed under the rind.

(c) Four vigorous young trees about eighteen months old were now selected. The first was used as a control, the second was infected with spores, the third with a portion of a diseased pod taken from near the still healthy tissue, and the fourth with pure culture-mycelium. Eight days

afterwards distinct infection was noted in No. 2, while in Nos. 3 and 4 the trees were nearly ringed near the infection-chamber, and the mycelium could be traced in the bast and wood as far as six inches above and below this point. Pycnidia were noted under the bark near the chamber. The control tree showed no infection.

(d) Attempts to induce infection by spores growing in water on the bark, in a manner similar to that used in experiment 1 (b) above, failed. The spores germinated, but I could not detect any penetration of the living tissues by the hyphae.

These infection-experiments show that the Fungus can behave as a parasite towards cacao pods, and is a dangerous wound-parasite of the cacao tree itself. The nature of one of the tree-diseases in Grenada, and of an important pod-disease, is therefore placed beyond doubt.

ONE FUNGUS OR TWO?

Up to this point it had been demonstrated that the Fungi on sugar-cane and on the cacao tree, as dealt with above, are both capable of pronounced parasitism on their respective hosts, and in addition are morphologically identical. It became therefore an interesting question to determine whether or not parasitism on these widely different hosts had become so closely adapted to these hosts that cross-infection was impossible. To answer this question the following series of infection-experiments was carried out, in which an attempt was made to infect cacao trees and pods with pure cultures of the sugar-cane Fungus, and also to try the effect of the cacao Fungus on the sugar-cane.

Infection of Cacao Tree and Cacao Pods with the Sugar-Cane Fungus.

(a) Two branches of a healthy cacao tree were infected with pure culture-mycelium of the sugar-cane Fungus, while a third was used as a control. Four days afterwards the

invading mycelium could be distinguished both in the wood and bast as far as six inches from the point of infection.

Cultivations made by infecting sterile tubes of food-material with small portions of tissue taken from the bast at this distance from the wound, developed the characteristic pycnidia and spores of the Fungus. The control experiment showed no infection.

(b) Three green pods were selected for this experiment. In a cavity of two of these, mycelium of the Fungus, seven days old, was introduced, while the third was used as a control. In three days the inoculated pods showed that the mycelium had spread through about a quarter of the pod, and cultures of a portion of the diseased tissue, taken as far as possible from the point of infection, developed the characteristic organs of fructification of the Fungus. No infection was detected in the control pod.

Infection of the Sugar-Cane with the Cacao Fungus.

Three healthy B 347 canes were selected. In two of them pure culture-mycelium was introduced into a small cavity made under the rind, and the third served as a control. In eighteen days the mycelium had spread into the tissues of the internodes above and below that in which the infecting chambers were cut, and the characteristic pycnidia of the Fungus were formed in large numbers under the rind in the internode in which infection was made. No infection was detected in the control cane.

These cross-infection experiments leave no doubt that the two Fungi are identical, and that the Fungus has not yet adapted itself to its host closely enough to give rise to two physiological species.

PROPHYLAXIS.

In dealing with the remedial measures which might be adopted on estates to check the ravages of this Fungus, it is well to remember that it readily lives as a saprophyte on old cacao-pod husks, and on the dead wood which is usually

left under the trees. Great care should therefore be taken to bury all the old pods, and to collect and burn all diseased pods noticed on the trees, together with all the dead wood found in the plantations. The use of tar after pruning would also to a great extent check infection of growing trees. In sugar-cane cultivation, if the diseased canes were regularly collected and burnt at the reaping season there would be less chance of infecting the next crop. The cacao planters in the West Indies are now beginning to adopt these measures, with the result that the pod-disease described in this paper is much rarer on such estates than on others where no precautions are taken.

SYSTEMATIC POSITION OF THE FUNGUS.

In the absence of any higher fructification than the pycnidia described above, the Fungus must be referred to the Fungi Imperfecti, and, on account of the character of the pycnidia and the brown two-celled spores, falls into the division *Sphaerioidaceae-Phaeodidymae* of the *Sphaeropsidales*, which includes the genus *Diplodia* and its allies. Specimens of the Fungus were referred to Kew, where it was determined by Masee as *Diplodia cacaoicola*, P. Henn., a form occurring on dead cacao branches in the Cameroons¹. In the classification given by Lindau in 'Die natürlichen Pflanzenfamilien'² mention is made of an allied Fungus, *Botryodiplodia Theobromae*, Pat., as occurring on cacao fruits in Ecuador. Lecomte and Chalot³, in their book on Cacao, in discussing the South American diseases known as *Mancha*⁴, state that Patouillard found this Fungus on diseased cacao-pods from Ecuador, and that probably one of the forms of *Mancha*⁵, in which the rind turns brown and the pods dry up, may be due to this Fungus.

¹ Engler's Jahrb., Bd. xxii, p. 80, 1895.

² Engler and Prantl, Die natürlichen Pflanzenfamilien: Fungi, I. Theil, 1. Abteilung, p. 372.

³ Lecomte and Chalot, Le Cacoyer et sa culture.

⁴ Sodiro, Observaciones sobre la enfermedad del Cacao 'Mamada,' &c. Quito, 1892.

⁵ De Lagerheim, Pflanzenpathologische Mitteilungen aus Ecuador. 1892.

On referring to Lindau's classification of the *Sphaeroidaceae-Phaeodidymae* given in Engler and Prantl, it appears that the group is divided up according to the occurrence of the pycnidia singly or in colonies, and according to the presence or absence of a stroma.

In *Diplodia* the pycnidia are free from each other, occurring singly, and there is no stroma. In *Botryodiplodia* they are arranged in colonies and a stroma is present. In considering the behaviour of the Fungus under discussion under artificial conditions and on the host-plants, it will be seen that there is great variation in the arrangement of the pycnidia, as they sometimes occur alone, at other times in colonies. There is, besides, a good deal of variation in the amount and arrangement of the hyphae surrounding the pycnidia. In some cases the latter appear to be embedded in a stroma, in others to stand in a web of hyphae. It would therefore appear likely that the Fungus on cacao pods in Ecuador is identical with that which is so common in the West Indies.

The Fungus does not appear to have been noted in Java on the sugar-cane, as no mention is made of it in Wakker and Went's¹ book on the diseases of the cane or in Krüger's 'Das Zuckerrohr.'

It appears, however, to have been noted previously in the West Indies in 1878, when specimens were sent to Kew for examination. Some confusion in the literature seems to have arisen from these specimens, as will be seen by the following note by Massee in the Kew Bulletin²:—

'Specimens of diseased sugar-cane were sent to Kew in 1878 from Porto Rico for investigation. These were submitted to the Rev. M. J. Berkeley, who gave the MS. name of *Darluca melaspora* to the Fungus present on the canes. The Fungus was afterwards very briefly described under Berkeley's name by Cooke in 'Nuovo Giornale Bot.,' vol. x, p. 26 (1878), where the locality was incorrectly given as Australia, instead of Porto Rico. Saccardo has added to

¹ Wakker and Went, *De Ziekten van het Suikerriet op Java*. Leyden, 1898.

² Kew Bulletin, p. 86, 1895.

the confusion by changing the name to *Coniothyrium melasporum*, and by quoting Cooke's diagnosis incorrectly in Syll. Fung., vol. iii, No. 1799.

'Finally, Prillieux and Delacroix, in their paper on Sugar-cane Diseases (Bull. Soc. Mycol. de France, tom. xi, p. 75, 1895), have fallen into the error of considering the *Melanconium*-stage of *Trichosphaeria Sacchari* (Massee) to be synonymous with *Coniothyrium Melasporum*, (Berk.) Sacc. Examination of Berkeley's type specimen shows that the Fungus is a *Diplodia*.'

There can hardly be any doubt that this *Diplodia* is identical with the species discussed in this paper.

BARBADOES.

August 2, 1901.

EXPLANATION OF FIGURES IN PLATE XXXVII.

Illustrating Mr. Howard's paper on *Diplodia cacaoicola*.

Fig. 1. Portion of the stem of a sugar-cane, showing the pycnidial colonies of the Fungus breaking through the rind. Nat. size.

Fig. 2. Transverse section of the sugar-cane stem, showing pycnidia. Zeiss. *a a*.

Fig. 3. Transverse section of a pycnidium breaking through the rind of the sugar-cane. Zeiss. D D. Drawn with the help of a *camera lucida*.

Fig. 4. Stages in the germination of a spore obtained from a pycnidium on a diseased sugar-cane. The sowing was made at 9.30 a.m. Dec. 22, *a*, at 3 p.m.; *b*, at 3.55 p.m.; and *c*, at 5 p.m. the same day. Temp. 27–29° C. At 8 a.m., Dec. 23, a hypha had developed from the other half of the spore, but the single-spore colony was too complex to draw. $\times 375$.

Fig. 5. Stages in the germination of a young spore in which the transverse septum has not yet appeared. The spore was obtained from a pycnidium from a diseased sugar-cane, and the sowing was made at 9.30 a.m., Dec. 22; *a*, at 11.30 a.m.; *b*, at 12 noon; *c*, at 1.30 p.m.; and *d*, at 4 p.m. the same day. Temp. 27–29° C. *a, b, c* $\times 375$. *d* $\times 60$.

Fig. 6. Stages in the fusion of hyphae in a hanging-drop, two days after sowing: *a*, at 2.5 p.m.; *b*, at 4.45 p.m.; and *c*, at 5.0 p.m., Dec. 24. Fusion was complete at 5.30 p.m. the same day. $\times 375$.

Fig. 7. Culture-tube, showing the passage of mycelium from the hanging-drop to the water on the floor of the moist chamber in a four-days-old culture of a spore obtained from a diseased cane. A similar development was noted in cultures of spores obtained from a diseased cacao branch. Nat. size.

Fig. 8. Mycelium from a seven-days-old hanging-drop culture in a resting condition. The darker hyphae are olive green in colour, and contain numerous oil-drops; the others are empty. $\times 375$.

Fig. 9. *a*. Stages in the formation of spores in a pycnidium from a ten-days-old culture on oak-wood.

b. A portion of the hymenium of a pycnidium from the same culture, showing conidia and paraphyses. (The parent spore of this culture was obtained from a sugar-cane.) $\times 375$.

Fig. 10. A portion of a cacao branch attacked by the Fungus (which has been kept in a moist chamber for a short time), showing the pycnidial colonies breaking through the bark. Nat. size.

Fig. 11. Transverse section through a pycnidial colony bursting through the bark of a cacao branch. The second pycnidium on the left hand shows the ostiole. Zeiss. *aa*.

Fig. 12. Portion of a tangential section of a cacao branch, showing the mycelium passing through pits in the walls of the vessels. A portion of a medullary ray is shown on the right-hand side. The mycelium is brown, and shows numerous oil-drops. Zeiss. *E*.

Fig. 13. Stages in the germination of a spore obtained from a pycnidium on a diseased cacao branch. The sowing was made at 2 p.m., Dec. 18: *a*, at 4.5 p.m.; *b*, at 4.45 p.m.; *c*, at 6.45 p.m.; and *d*, at 11 p.m. the same day. Temp. 27-29° C. At 7.0 a.m., Dec. 19, there was a copious development of a branched, rarely septate mycelium in the drop, which was far too complicated for me to draw. *a, b, c* $\times 375$; *d* $\times 60$.

HOWARD. — ON DIPLODIA.

Howard, Albert. 1901. "On *Diplodia cacaoicola*, P. Henn.; a parasitic fungus on sugar-cane and cacao in the West Indies." *Annals of botany* 15, 683–701.
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