The Panama Disease.

BY

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With Plate XXVIII.

I

THIS disease broke out among the banana plantations in Panama about five years ago. Considerable damage to the crops was only done in a certain district, where the disease appeared when the fields were five to six years old. From certain spots it spread all around, affecting at last a considerable area. The fields, when ten years old, were absolutely worthless. Thirty miles from this district, however, the plague makes its appearance, but sporadically.

In Costa Rica it badly rages only in spots; there are still magnificent plantations covering an area of $\pm 60,000$ acres, where the disease does not spread and no real damage is done.

In neither of these countries were such serious consequences experienced as in Surinam, where it appeared on fields but one year old, and the loss amounted to 25-75 % of the second and third crop. On some plantations even entire fields were annihilated.

Different varieties of banana and plantain have been cultivated in this country for a century or more, but not so extensively as now; and as they were grown for shade of other crops—the plantain for food at the same time—they were not allowed to remain any longer on the same field than two years. This is the main reason why the disease never attracted the attention of the planters, and it will explain why the attack became such a serious and general one as soon as the same areas were covered by extensive fields of one of the most susceptible varieties, the Gros Michel.

As I was informed, the following varieties also are more or less susceptible: the Indian or Wine-coloured Banana, the Silverskin, the Apple, and the Horse Banana, the dwarf, and some varieties, unnamed, of plantain; resistant were the Ladies'-finger, the Congo variety, and the ordinary plantain.

The disease manifests itself by a peculiar withering of the leaves along the margin; along a mid-dorsal line on the midrib discoloration may be observed. Sometimes only the bud (the youngest convolute leaf) withers, not being able to unfold, whereas the older leaves are healthy; sometimes

the older leaves show signs of decay, whilst the buds develop for a certain length of time, depending of course on the direction followed by the germ in propagating through the rhizome: this may be called the first stage. In the second stage development stops, the leaves droop, the plant looks water-starved, wrinkles appear on sheath and midrib of the leaves, which gradually dry up, and finally the pseudo-stem bends down along a line of least resistance.

As soon as the disease becomes evident, one is sure to find the tuberous rhizome, when cut asunder, showing on the exposed surfaces more or less signs of putrefaction. The healthy whitish colour is replaced by a yellowish hue with reddish brown spots or streaks scattered through the infested parts, and yellowish or brownish mucilage exuding from the slime canals. The roots do not participate in the process of decay before the tissue at their base is affected, proving that the germ does not enter by them, and since the starting-point of disintegration could be traced to an old wound-surface there is reason to assume that the fungus at the start behaves as a wound parasite or saprophyte, living at first on the exudation and by degrees preparing its way up into the damaged vessels.

Sections made from the rhizome showed at once that a fungus was the probable *causa morbi*. The mycelium is mainly massed in the wood vessels and the immediately surrounding tissue (see Pl. XXVIII, Fig. 1). The water-starved appearance of the affected plant is readily explained by the enormous amount of hyphae and spores in the vessels, which must seriously interfere with the passage of water. But a more effective impediment to the transpiration current and assimilation in general are the at first mucilaginous bodies plugging the wood vessels and sieve tubes and filling up cells and intercellular spaces of the parenchyma. These cartilaginous bodies I shall henceforth term sclerotia,¹ which I shall prove them to be.

The damage done by the fungus is not only of a mechanical but mainly of a physiological nature, as may be concluded from the wood vessels being discoloured and the contents of the sieve tubes, &c., resorbed by the hyphae running through them. The changes in the parenchyma seem at first to be slight, and manifest themselves by an unusual turbidity of the protoplasm, apparently caused by the action of an enzyme secreted by the fungus, to which enzyme also the brown discoloration and slimy degeneration of the cell-walls may be due. By degrees the contents of the cells are absorbed and replaced by the cartilaginous sclerotia. Some details of the formation of the resting mycelia may be mentioned here; on hyphae passing from cell to cell comparatively large, bladder-like structures arise, which, sometimes wrinkled and lobed, then again split in tufts and divided, seem to exercise a haustorial function. The enzyme alluded to before is probably secreted by these structures, as may be inferred from the

¹ Another name for these structures is perhaps desirable.

fact that the turbidity of the protoplasm is only noticed in cells where they are found, and that the sclerotia formed in these cells often show a marked resemblance in outlines to the said structures (see Pl. XXVIII, Fig. 2).

Transverse sections through the leaf-sheath showed that the hyphae entering the wood vessels send out branches in a direction vertical to the surface. They terminate in the intercellular spaces between the sub-epidermal layers, where oblong sclerotia are formed or irregularly shaped ones, which entirely fill up the sinuosities of the spaces (see Pl. XXVIII, Fig. 3). The spore-clusters arising from these sclerotia again give the impression of *Sorosporium* among the Ustilagineae. Some, very small and breaking up into minute spores, made me think of *Physoderma* among the Chytridiaceae.

Transverse sections through the midrib displayed a peculiar formation of the sclerotia in the stellate cells of the parenchyma composing the partition walls between the large air-spaces. The hyphae entering the cell send branches into the lobes, where they turn to sclerotia which give rise to minute spores at last (see Pl. XXVIII, Fig. 4). From these tiny sclerotia results the brown dotted appearance of the septa in an affected leaf.

The conditions met with in transverse sections of the lamina are essentially the same as in the sections of the sheath, but the vertical course of the fertile hyphae is more obvious. They push through or between the cells of the palisade parenchyma, some not beyond the back-wall of the inner layer of the subepidermal cells, gradually increasing in thickness and turning into a slimy tube or rod, which hardening into sclerotia may after a time of rest give rise to spores, as will be seen in Pl. XXVIII, Fig. 5. The spores may retain the position of the sclerotium or they may, in consequence of the tension in the surrounding tissue, be squirted into the overlying cells, from whence they are liberated after the decay of the leaf. Again, the hyphae may at once push through the subepidermal layers, filling the cells with sclerotia, or they may run out at the surface of the leaf as tiny brown gall-like swellings (mycocecidia). Then again traversing and rupturing the epidermal layer or entering the stomata, the hyphae reach the surface of the leaf, and branching in all directions give rise to numerous sickle-shaped conidia.

The slime canals are apparently attacked by the fungus; that means that hyphae may be running along or through them, and the possibility of the fungus deriving some benefit from this intimate contact may be inferred from the fact that the colour changes into some shade between yellow and brown—explaining why the mucilage exuding from the slime canals of an infected rhizome shows a corresponding hue—whilst spores may be seen to arise on or in the mass.

The process of chlamydospore-formation out of the sclerotia is of a marked Ustilaginoid character: the spores loosely adhere to each other,

forming clusters as in *Sorosporium*. Very frequently globular transparent bodies are found in the sclerotia, reminding one of the water-clear bubbles which Meyen discovered between the spores of *Ustilago longissima*. Their nature is difficult to explain; in some cases, however, I found them containing crystals of calcium oxalate. Besides the ordinary chlamydospores, large spore-shaped bodies of a smoky or shining black colour are seen to arise within the sclerotia. I shall later on try to give an explanation of their origin and nature (see Pl. XXVIII, Fig. 3).

During the final stage of the development of the disease all parts of the host plant are attacked by the fungus, which, enclosing the tissue-remnants in sclerotia, adapts itself to a saprophytic mode of life. Numerous spores—chlamydospores and conidia—are formed and crowded in pycnidia-like cavities. The pycnidia containing chlamydospores mainly form within the more compact inner portions of the mass, whereas those containing conidia arise on the subaerial parts, now and then with chlamydospores scattered between them. In the leaves also I found similar pycnidia between the subepidermal cells, containing chlamydospores, with no visible aperture for the escape of the spores; the walls in both cases were formed symphyogenetically.

All through the plant multicellular chlamydospores are met with. It will be shown later on that they are chlamydospores by their mode of origin and germination.

PURE CULTURES.

The little-discoloured parts of the rhizome, abutting on those where putrefaction had gone to a great length, appeared to be most suitable for the preparation of the inoculation fluid, since the bacterial colonies did not appear in such an overwhelming number as to overrun the mycelia; moreover, the inoculation was carried out on large Drigalsky plates (Agar). Using a favourable dilution, what appeared to be two distinct kinds of mycelia and two kinds of bacterial colonies were obtained. The difference between the two kinds of mycelia was pronounced, in the colour and the mode of growth; they were transferred to separate Petri dishes.

The one kind of mycelium was hyaline and silvery shining; the hyphae were thin and the septa wide apart. On the fifth day a thickening of special hyphae had taken place, and a greater amount of transverse walls had divided them into a large number of isodiametric cells. Many branches were given off in monopodial fashion, and more or less vertical to the main axes. On the seventh day tiny protuberances arose, which the next day had cut off single multicellular *Fusarium* conidia and smaller bicellular conidia, which were slightly sickle-shaped or straight and always grouped in yeast-like fashion or in heads or bundles of 8–20. The following day the *Fusarium* conidia were seen to arise in heads of 10–50 on an incon-

spicuous, unbranched conidiophore as in *Gibberella cyanogena*. At the same time thin curling branches appeared, at the apices of which arose round cup-shaped chlamydospores with granular, richly vacuolated protoplasm. On the tenth day some thick hyphae appeared, giving rise to the same chlamydospores, intercalary as in *Chlamydomucor*. In one case they were wide apart, in another in immediate contact with each other, so that fusion took place, producing multicellular chlamydospores as alluded to before. On another culture they were seen to arise on the apex of short thick branches, as in *Hypomyces ochraceus* (see Fig. 7).

Anticipating this kind of chlamydospore formation, the protoplasm of the hyphae was drawn to favoured sites, where the spores would arise, and the interspaces were left entirely empty and finally collapsed, liberating the chlamydospores. Their colour when mature is dark-brown or violet; the exosporium is generally smooth, but now and then adorned with numerous warts. In some instances the hyphae did not produce resting spores, but broke up into their component cells, giving rise to oidia-like reproductive organs.

The sclerotia arose on extremely thin hyphae as transparent yellowish saucer-shaped bodies, measuring $\pm 100\mu$ across. The cultures were then nearly five weeks old. Sometimes they stood solitary, then again they were crowded together, and finally fused into a massive gelatinous body, in which no separating membranes, but often the above-mentioned globular bodies, were visible. Maturing, they assumed a darker colour and hardened into the identical gristly structures met with in the histological preparations. In the same way they broke up into identical chlamydospores. In the absence of fusion some of the single scyphoid sclerotia matured into large, dark-coloured, spore-like bodies, as mentioned before on p. 346; I shall term them giant chlamydospores. They may be expected to have the potentialities of a single scypho-sclerotium or of a group of chlamydospores that could arise out of such a sclerotium. The other mode of sclerotium formation hinted at in the description of the sections is readily explained by Figs. 1 and 6.

The process of chlamydospore-formation is initiated by pellucid rings within the sclerotia. The mass then splits in polygonal plates, each containing one or more spores; at last they are completed, still surrounded by a thin layer of mucilage, which is finally resorbed. Indeed, the whole process is identical with that of the Ustilagineae.

The second kind of mycelium was marked off from the first one by the dusky colour of the central underlying part and the conspicuous hemispherical shape. The dark colour was due to the basal growth, consisting of thick hyphae of a smoky hue. They terminated, however, in hyaline slender apical portions, and the secondary mycelium arising on them was also perfectly hyaline. On the seventh day chlamydospores arose, cut off

at the summit of thick, short side branches, or intercalary as in *Entyloma*. On the eighth day the conidia made their appearance on the hyaline parts of the mycelium; but few sclerotia were formed. From the tenth day onwards no real change was perceptible until, in the fourth week, the mycelium broke up into a dense mass of chlamydospores. At all events, it was shown that the difference of colour of what appeared to be two distinct kinds of mycelia was only due to a slight modification of the nature of the spores from which they arose.

Cultures on gelatine medium. It was not easy to produce a solid gelatine medium on account of the high temperature reigning in the tropics. Still, the mycelia raised in the semi-fluid medium excellently established the facts gathered from the agar cultures. One special feature of the gelatine cultures, however, was the appearance of the hyaline, bladder-like structures already described as haustoria. They arose and remained submerged in the semi-fluid medium, performing their haustorial function. Comparing the results of the histological research with the facts gathered from the pure cultures, one cannot fail to see the identity of the structures found in the plant tissues and those developed on the Petri dishes. The figures will fully explain the matter.

CULTURES IN LIFE-BOX.

From one of the most flourishing cultures a sufficient quantity of mycelium was transferred with the aid of a sterile, spatulate platinum wire to a tube containing ± 10 grains of sterilized water and divided by agitating moderately. A drop was brought on a thoroughly clean cover-slip, a drop of sterilized water placed on the bottom of the life-box, and the cover-slip then fixed on by means of some vaseline. Three other life-boxes were prepared for the sake of comparison. As a matter of convenience in describing the different modes of germination, I shall take the liberty of making some distinction between chlamydospores, gemmae, and oidia. By chlamydospores I designate the spores arising from the sclerotia; by gemmae, those formed in the course or at the apices of fertile hyphae; and by oidia, the spores which are set free by the breaking up of the fertile hyphae into the component, isodiametric cells.

The gemmae were the first to germinate; some gave rise to short, club-shaped tubes, at the apices of which one or two small conidia were cut off; another group grew tubes of 3–7 cells, small bicellular conidia arising apically and laterally. These conidia were often loosely joined together in pairs or in bundles of three or more, the adherence probably being caused by a thin layer of mucilage enveloping them. In another case the conidia were thrown off by the gemmae at once, without the interpolation of a promycelium, reminding one of the germination of *Entyloma*, which very often takes place on the host plant, as could be seen in this case

also. In yet another case a yeast-like budding took place, giving rise to a promycelium, which may laterally and apically throw off conidia or grow into a dark-coloured mycelium as met with before (see Pl. XXVIII, Fig. 7).

The chlamydospores may arrange themselves in beadlike fashion; some of the spores then emit germ tubes, which grow at once into a mycelium; others produce promycelia with conidia or conidia alone. Finally they give rise to branched promycelia, on which chlamydospores and conidia, arising on the extreme tips of the branches, put a stop to their further development (see Fig. 8).

The oidia did not differ in their mode of germination from the first-mentioned kind of gemmae, with which they are, in fact, identical, as could indeed be inferred from their mode of origin (see Fig. 8).

The bicellular conidia produced branched mycelia, which grew out at one or both ends; but in some cases fusion was seen to occur between two of these conidia; one of them assumed the nature of a chlamydospore, giving rise to a branched promycelium with conidia constricted off at the apices of the hyphae.

The *Fusarium* conidia, germinating under ordinary conditions, did not present any essential difference. The mycelia mostly arose on the apical cells, each giving off sometimes two germ tubes. But in many cases the protoplasm was drawn to one or two of the cells, which rounded off and then germinated exactly as the gemmae. The same transformation of *Fusarium* conidia into gemmae was noticed on an old culture, where, after two months, these conidia were replaced by gemmae; this surely throws some light on the occurrence of gemmae in the pycnidia and the prominence of the chlamydospore fructification in general.

The multicellular chlamydospores or gemmae presented the same peculiarities in germinating as mentioned in the case of the single cells.

Bits of sclerotium were seen showing a differentiation into spores; after a few days they were formed, still enveloped in mucilage. As soon as the mucilage was resorbed they started germinating in the same way as mentioned under chlamydospores. In another bit, germ tubes arose in the still undifferentiated mass as stout, highly refractive rods, which gradually emerged from the sclerotium, producing promycelia as before (see Pl. XXVIII, Fig. 8). This goes to show that the sclerotium is able to propagate the fungus in the same way as the chlamydospores. Another function of the sclerotium is made plain in the following account of the formation of what I think I am justified in considering a fruit body; for although it was not obtained under sterile conditions, and its premature withering prevented me from controlling my conclusion by raising new mycelia from the ascospores, it is plain that the identical *Fusarium* conidia and gemmae could not arise except on a part of the fungus under investigation.

A sucker of the banana in the second stage of the disease, from which

the upper part was cut off so as to expose a comparatively large surface, was left on my table. Within a few days I noticed that the process of putrefaction was rapidly advancing, turning the upper surface to a black corrugated mass. Shortly after that it was covered with silvery shining mycelium, upon which the already known gemmae were found when some small bits were brought under microscope. Later on I discovered two branched, antler-like, hairy, orange-coloured bodies arising on little protrusions bare of hyphae. One lobe of each was cut off and examined under the microscope; it consisted of closely packed hyphae, of which some were fertile, bearing conidia and gemmae—the identical *Fusarium* conidia and gemmae treated of before—whereas the remainder were sterile, the free ends sticking out and producing the hairy appearance of the upper part of the lobes.

The bodies were allowed to develop; they grew in a fortnight to double the size, when they were seen to wither and crumple, probably for want of moisture. Taken off with a portion of the underlying tissue and brought under low power, the basal part proved to be a bit of sclerotium. Sections were then made of the entire bodies, in the one case longitudinal, in the other transverse sections. Remarkable developmental changes had taken place; across the apices of the lobes a pseudo-parenchyma of a pink colour had arisen; the conidia had disappeared, and only a few gemmae were still to be found in the sinus between two lobes.

The stalk, $\pm \frac{1}{2}$ cm. high, consisted of a bright, yellowish brown pseudo-parenchyma, and was perfectly sterile. The broader upper half was enclosed in a sheath of closely packed paraphyses with free terminations, supporting, as it were, the pink-coloured stroma; they themselves were of a bright orange-red colour. The transverse as well as the longitudinal sections showed that the fruit body was not solid all over, but that in the median part a large air-space was partly filled up by strands of pseudo-parenchyma connecting the opposite walls of the cavity and producing a kind of spongy parenchyma.

I gather from the foregoing facts that I am justified in considering the structures in question as prematurely withered, ascigerous fruit bodies, which would have produced the asci in or on the pseudo-parenchymatous terminal discs (stromata), if the conditions had been favourable to their development. It has, of course, yet to be proved, and fully occupies my attention still; but since the raising of fruit bodies of Ascomycetes is not always possible in the laboratory, and the systematic position of the fungus could be determined from the pronounced pleomorphism, the development of a brightly coloured stroma, and in general from the great resemblance to many members of the well-defined homogeneous order of the Hypocreaceae, I think it right to classify it with this order; and on account of the formation of chlamydospores out of sclerotia, the mode of germination of these

spores, and the morphological peculiarities of the stroma, I propose to give it a place beside *Ustilaginoidea*. As far as I could find out we are dealing with a fungus not yet described, for which, on account of its great resemblance to *Ustilaginoidea* and its harmfulness to the banana, I propose the name *Ustilaginoidella musaeperda*.

BACTERIA.

On account of the assertion of the expert of the United Fruit Co., that Bacteria were the cause of the Panama disease, I thought it necessary to ascertain—although the *Ustilaginoidella musaeperda* was shown to be undoubtedly connected with the disease—whether Bacteria also had anything to do with the plague, as a primary or secondary cause, besides the fungus. The bacterial colonies were isolated in the ordinary way; for the sake of convenience I shall indicate them by *a* and *b*.

The *a* colonies appeared on one of the plates only, so that it proved to me at once that this bacterium could not be the *Krankheitserreger*. Pure cultures, however, were raised on banana agar with the special purpose of identifying the micro-organism. The colonies grew into roundish elevations of a citrine colour measuring $1-1\frac{1}{2}$ mm. in diameter. On the fifth day they flattened down, showing a slight indication of sliminess on the top, and the original granular consistency was only discernible along the somewhat crenate margin.

Stab cultures. Granular, yellow at the margin, merging into citrine in the centre, margin a little elevated.

Bouillon cultures. Slightly turbid, with slimy sediment.

Milk culture. Clotting after twelve days; acid reaction.

Potato culture. Citrine colour; sinuous margin; flat.

Glucose culture. No gas formed.

Staining. Gram-positive.

Microscopic appearance. Round cocci of $\pm 1\mu$ diam., in some cases joined in packets of two or four. It is clear that we are here dealing with one of the forms of *Micrococcus sulfureus*, a common micro-organism in air and soil.

The *b* colonies attained their maximum development after five days, presenting a great diversity of shape and size. Some were roundish, others irregular and lobed; in the one case the margins were crenate, in the other case moruloid. The longest axis of a colony was about 2 mm. The colour was greyish white, with faint, bluish-green fluorescence on the agar.

Stab culture. Thread-like, with a slight funnel-shaped dilatation near the surface; granulation scarcely perceptible. After twenty-four hours a circular, shining, thin, slimy layer around the inoculation spot, upon which arose (forty-eight hours) the greyish-white colony, evenly spread; agar fluorescing faintly blue-green.

Bouillon culture. Yellowish-green fluorescence; sediment in condensation liquid abundant, slimy; can only be divided by agitating well. Skin on the surface, yellow-green colour.

Milk culture. Clotting after nine days; acid reaction.

Potato culture. Flat, dull grey; margins sinuous. No gas and no indol formed.

Microscopic appearance. In hanging drop, short but slender rods, sometimes long threads and stout forms, all of which displayed great motility.

Staining. Gram-negative. One terminal cilium found by using Bunge's mordant and staining with fuchsin.

It is plain that this micro-organism is nothing but a form of *Bacterium fluorescens*. Reasoning from facts gathered during my research, it looks to me at least improbable that Bacteria have any share in the ruinous war waged on the banana plantations in Surinam.

To satisfy all conditions, however, I inoculated four suckers with the fungus-spores, four with Bacteria, and four with fungus-spores and Bacteria combined. Which will prove to be the mischief-maker time will tell.

EXPLANATION OF FIGURES IN PLATE XXVIII.

Illustrating Mr. Ed. Essed's paper on the Panama Disease. Part I.

Fig. 1. (*w.*) wood vessels full of hyphae spores, and sclerotium forming; (*s.*) sieve tubes plugged by sclerotium, spores forming; (*sc.*) scyphoid sclerotium.

Fig. 2. Haustoria in parenchyma. $\times 105$.

Fig. 3. (*a*) spore-clusters; (*b*) sclerotium in intercellular space broken up into chlamydospores; (*c*) giant chlamydospores.

Fig. 4. Stellate cell in which sclerotia and spores are formed.

Fig. 5. Section through leaf, showing sclerotia and spores.

Fig. 6. Explained in text.

Fig. 7. Explained in text.

Fig. 8. Explained in text. $\times 255$.

Fig. 9. (1) Fruit body on sclerotium. $\times 10$. (2) Young stroma with spores and paraphyses. (3) Nearly mature stroma.

3.

5.

7.

9.

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