

THE FIG "CANKER," CAUSED BY *PHOMA*
CINERESCENS SACC.

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(With Plates I and II, and 1 Text-figure.)

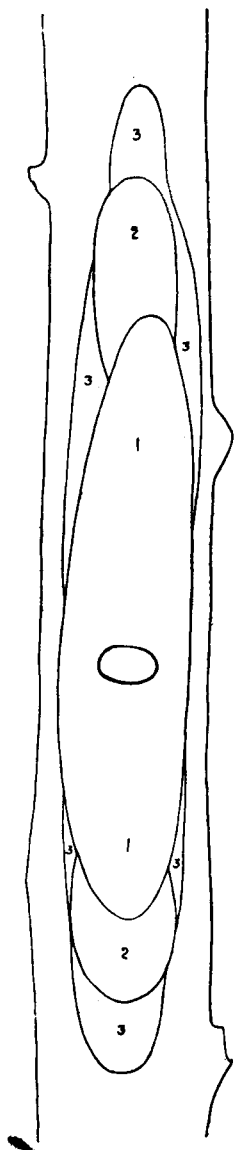
IN 1914 complaints were received by the South-Eastern Agricultural College at Wye of serious diseases affecting the plantations of fig-trees in the district of Sompting, Sussex. On a visit being paid in May 1914 to the affected plantations, fig-trees of all ages were found to be suffering from fungous attacks of two kinds. A species of *Botrytis* was found on the tips of the branches, apparently gradually killing them back. This disease is still under observation and will be the subject of a further communication.

The second disease which occurred was a "canker" of the branches. In some cases, where old trees were badly attacked, numerous "cankers" were found, both on the younger branches, and on the old, main branches, often quite close to the ground. With the progress of the disease, the "canker" area enlarges, until the tissues extending through the branch are killed, with the result that the parts above die. With the successive removal of these "cankered" branches the productiveness of the tree is soon seriously impaired. The majority of the trees in these plantations are of the variety known as "Brown Turkey," but a few are of the "White Marseilles" variety. It was obvious from the effects produced that this "canker" disease was of serious economic importance; it was the opinion of the farmer of the largest fig-plantations that unless the cause of the disease could be discovered and a remedy devised the whole future of Fig-growing in that district was threatened.

The constant occurrence of a fungus with pycnidial fructifications on the cankered area was noted in the field, and the following notes were made from the examination of the material collected.

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One of the "cankers" which may be taken as typical of those found on the larger branches is shown in the photograph at Fig. 1, Plate I, where the characteristic cracking of the bark in the older portions is seen.



It will be observed that near the centre of the "canker" is the old base of a smaller branch and it is probable that that was the place where the fungus made an entrance. This particular canker was evidently the result of three distinct periods of activity of the causal organism. The oldest portion, recognized by the fact that the bark is very much cracked and separating from the wood, was elliptical and measured 16.5×5 cm. At each end of this ellipse the canker extended 5 cm. upward and 2.5 cm. downward, these extensions corresponding to the second period of activity. The check to the normal growth in thickness of the branch induced by the presence of the organism caused these affected areas to be depressed below the general surface of the branch. The youngest portions of the canker extended still further upward and downward and though they were but indistinctly marked off from the adjoining healthy portions of the branch they bore numerous pycnidia. The accompanying diagram of the canker shows the areas, affected during the three periods of activity, numbered respectively 1, 2 and 3; this diagram should be compared with Fig. 1, Plate I, which is a photograph of the same canker.

The portion of the branch bearing the "canker" was placed in a damp chamber and in twenty-four hours "tendrils" of conidia were issuing from the youngest portions of the canker but not from the rest. No conidia were obtainable from the older portions and there the fungus was either dead or had ceased to produce conidia, although these areas were still dotted over with old pycnidial pustules. The tendrils were at first of a pale orange colour but when fully protruded were almost or quite white. When a tendril or a portion of one is placed in a drop

of water the mucilaginous matrix binding the conidia together is dissolved and the conidia themselves stream apart and become diffused through the water; a slight "Brownian movement" is to be detected when the conidia are mounted in water.

Transverse sections through the bark show the fungal fructifications to be pycnidia. These bodies are more or less circular in outline and are from $250\ \mu$ to $600\ \mu$ in diameter; they are somewhat flattened and are therefore lenticular, appearing elliptical in transverse section. The pycnidia are produced a little below the surface, but on approaching maturity each develops a short neck which ruptures the outer layers of the bark and the tendril emerges through an apical pore. The wall of the pycnidium is lined with the conidiophores which are from $15\ \mu$ to $25\ \mu$ in length and which abstrict the conidia from their apices.

The conidia are continuous, ellipsoidal to fusiform, often with one extremity more rounded and broader than the other. Their dimensions are $6.5\text{--}13 \times 2.4\text{--}3.6\ \mu$. Usually they are about $9 \times 3\ \mu$ and as a rule an increase of length is associated with a decrease in the width; thus the following dimensions are typical: 6.5×3.6 , 9.2×3 , 11.5×2.5 , 13×2.4 . With medium magnification they appear to be biguttulate, but by employing an oil-immersion objective this appearance is seen to be due to *two groups* of minute guttules. The two groups may merge into one another but usually they are quite distinct and situated at opposite ends of the conidium (see Fig. 10, Plate II).

The conidia are capable of germinating in water. When placed in a hanging-drop of distilled water and examined after remaining three days at the temperature of the laboratory (about 18°C.) a few of the conidia had germinated. They showed no appreciable increase in size before the protrusion of the germ tubes. The latter were at this stage one to four times the length of a conidium and frequently were more or less geniculate; they emerged laterally, sometimes at or near the middle of the conidium, at others more towards one or the other extremity, but none was seen truly polar.

In June of the same year (1914) Mr F. J. Chittenden sent us a specimen of the Fig "canker" from the glass-houses at Wisley; this bore pycnidia and conidia similar to those described above.

The pycnidial form of fructification and the spore-characters referred the fungus to the genus *Phoma*, and reference to Saccardo's *Sylloge Fungorum* enabled us to identify it with *P. cinerescens* Sacc. in *Mich.* 1, p. 521 (1879). The specific diagnosis given is as follows: "Peritheciis gregariis, globoso-depressis, atrolivaceis, subcutaneis; sporulis

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fusoideis, biguttulatis, $6-8 \times 2-2.5$, hyalinis. *Hab.* in ramis corticatis v. demum decorticatis *Fici Caricae*, in Italia et Gallia. *Spermogonium* *Diaporthes cinerescens* Sacc."

To make certain of its specific identification, an example was sent to Prof. P. A. Saccardo, who replied, "J'ai examiné votre specimen. C'est sans doute mon *Phomopsis cinerescens*. Ma diagnose est incomplète, car les *sporophora* (comme il se fait très-souvent) étaient déjà disparus; aussi les *guttulae* dans votre specimen sont peu distinctes, mais cela est aussi variable." The author who transferred the present fungus from *Phoma* to *Phomopsis* was J. B. Traverso (in his *Flora Italica cryptogama*, vol. II, fasc. I, p. 278 (1906)), who after giving a description of *Diaporthe cinerescens* Sacc. merely remarks: "Status pycnidicus verisimiliter *Phomopsis cinerescens* (Sacc.) Trav. sporulis fusoideis, hyalinis, biguttulatis, 8×2 ." In general appearance and structure the present fungus agrees so well with other parasitic species which are still named *Phoma* that we retain it in that genus.

In searching for records of the occurrence of a Fig-tree "canker" in England, we met with the description given by Mr Massee first in the *Gardeners' Magazine*, for July 23 (1898), and later in his *Text-Book of Plant Diseases*, p. 431 (1903). In the former place Mr Massee wrote: "A disease presumably of old standing has of late years proved very injurious to fig-trees, and one remarkable feature in connection with this disease is the fact that it is most prevalent and destructive in those cases where the trees have received the greatest amount of attention, pruning more especially favouring its extension. The most usual symptoms of its presence are a cankered or ulcerated appearance of the bark, which frequently becomes eaten away in large patches, or variously cracked. In the majority of cases it is very evident that the canker first starts at a point where a branch has been cut away or accidentally broken off, and in all instances it appears that a broken surface of the bark is absolutely necessary to enable the fungus causing the disease to gain a foothold." While the general description of the disease given here agrees exactly with what we have found, the description of the fungus which Mr Massee gives as the causal organism is quite different from that of *Phoma cinerescens*. Mr Massee places his fungus in the *Melanconiaceae* ("perithecia absent; conidia produced on a more or less developed cushion or stroma formed beneath the surface of the matrix, and becoming erumpent") as a new species of the genus *Libertella*, viz. *L. ulcerata* Mass. In the *Gardeners' Magazine*, l.c., it is stated: "Finally the fruit of the fungus, which is formed in

minute pustules, below the epidermis, bursts through to the surface, the exceedingly minute spores, only about 1–3,000 of an inch in length, oozing out through minute cracks in the epidermis, under the form of very slender, white, gelatinous threads or tendrils.” The figure given of these spores is reproduced at Fig. 11, Plate II. It will be seen from Fig. 11, traced from Mr Massee’s figure of the Fig canker *Libertella* in the *Gardeners’ Magazine*, that the length of the conidia is about 1–3,000 of an inch (i.e. 8–9 μ), or approximately that of the conidia of *Phoma cinerescens* (compare Figs. 10 and 11, allowing for the difference in magnification). In *A Text-Book of Plant Diseases*¹ Mr Massee in describing his species under the name *Libertella ulcerata* Massee (sp. nov.) says, “Conidia fusiform, ends acute, continuous, curved, hyaline, 55–60 \times 4 μ ,” and this description is repeated in his *Diseases of Cultivated Plants and Trees*². This discrepancy in the accounts of the dimension of the conidia of *Libertella ulcerata* we are unable to account for. Our fungus, i.e. *Phoma cinerescens*, belongs to the *Sphaeropsidiaceae* (“perithecia containing conidia borne at the tips of slender conidiophores”) and its conidia are ellipsoidal to fusiform, straight or slightly curved, ends usually rounded, averaging 9 \times 3 μ , and never exceeding 13 μ in length.

The Herbarium at Kew was found not to contain the type specimen of *L. ulcerata* Mass. In March 1915, Mr Massee kindly forwarded to us an example of his fungus, writing “Enclosed is a fragment of the type specimen,” and adding as a postscript, “I am almost certain that the *Libertella* is followed by a *Phoma* stage, but have not been able to get one from the other in cultures.” The portion of the type specimen sent consisted of some hundreds of fructifications, mostly with dried-up tendrils of conidia still attached, and proved to be entirely *Phoma cinerescens*, with all the characters as described above. A thorough search of this type material showed no conidia resembling those described by Mr Massee, or any fructification of the type found in *Libertella*.

With reference to the parasitic nature of his fungus, Mr Massee states: “Experiments conducted on healthy young fig-trees show that the spores of the fungus—a species of *Libertella*—will not cause the disease when placed on the unbroken surface of even very young branches, whereas when the spores are placed on the end of a cut branch or on injured bark, inoculation always followed, and the mycelium was found in abundance at the expiration of 10 days. In one experiment

¹ Massee, G., *A Text-Book of Plant Diseases*, p. 431 (1903).

² *Idem*, *Diseases of Cultivated Plants and Trees*, p. 448 (1910).

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a badly diseased branch, showing numerous threads of spores, was cut through; immediately afterwards the same knife was used for making an incision in the bark of a branch of a healthy young plant of *Ficus religiosa*. At the expiration of 10 days the wound showed undoubted symptoms of disease and at the end of five weeks the white threads of spores were found. This experiment proves that the disease may be imparted to healthy plants by using a knife that had previously been used for pruning diseased plants, and an examination of various diseased plants suggests the idea that this method of spreading the disease is not an unusual one."

Since the above work was not conducted with a pure culture of the fungus, and especially since we had found associated with the Fig "canker" disease a quite distinct fungus from that described by Mr Masee, it was important to obtain scientific evidence of the parasitism of *Phoma cinerescens*.

It was found that pure cultures could be obtained by two methods:

(1) A portion of a tendril obtained from the canker shown in the illustration (Plate I, fig. 1) was placed in a drop of sterilized distilled water; after dilution a small drop containing conidia was transferred to a tube of melted agar (prepared with an extract of prunes as nutrient) and a "poured plate" made. One such plate produced six sporelings; growth was rather slow and after eleven days the mycelial masses only measured from 4 to 7 mm. in diameter. From each of two of these a tube culture on an agar slant was prepared. In the tube cultures growth continued with more vigour and in seven days each showed a rather dense, flat, hyaline disc of mycelium about 2 cm. in diameter with a raised white ring of aerial hyphae midway between the point of origin and the periphery. Later these slant cultures consisted of a rich growth of dark brown mycelium with a few whitish tufts.

(2) An alternative method of obtaining cultures of the fungus was also adopted. The canker referred to above was cut across and transverse sections made through the discoloured wood. In this wood the vessels were found to be blocked with hyphae, tyloses and "wound-gum." Two small fragments of such sections made with a sterile razor were each transferred by means of a flamed platinum wire to an agar slant. The resulting growth resembled that obtained under similar conditions with mycelium produced from the poured plate of conidia, the white ring of aerial mycelium at a short distance from the point of inoculation again being a characteristic feature. The inference is that the mycelium in the affected tissues of the xylem is that of the

fungus seen on the exterior and indicates that it is possible to obtain a pure culture directly by transferring fragments of infected wood to a culture medium.

The medium used in these primary cultures was a decoction of prunes containing $1\frac{1}{2}$ per cent. agar and although it was found to be quite suitable for vegetative growth, no reproductive bodies were produced under those conditions. Sub-cultures made on sterilized cylinders of potato in Roux's tubes and on slabs of wood (obtained by making transverse slices¹ of a branch from a fig-tree) placed in Petri dishes and autoclaved at 115° C. for 30 minutes, readily produced pycnidia and "tendrils" of conidia. On potato the pycnidia were numerous and in some cases well-developed "tendrils" emerged, while in others the conidia accumulated at the mouth of the pycnidium in a globular mass, the latter condition obtaining when the moisture within the tube was too great for the typical tendrils to retain their characteristic form. The "tendrils" and globules of conidia were at first of an orange colour changing later to a dark red. The conidia were similar to those obtained originally directly from the canker in their size, shape and guttulation.

On the slices of fig wood the fungus grew vigorously as a white mycelium and eventually produced a few scattered "tendrils": the latter were larger (stouter and usually longer) than those normally produced under natural conditions and were dark red in colour, the conidia however were similar to those obtained from the canker itself. It is interesting to note in this connexion that the "tendrils" were more numerous on the under surface of the wood, *i.e.* where it came in contact with the inner surface of the Petri dish, than on the upper free surface.

INOCULATION EXPERIMENTS ON FIG-TREES.

Young fig-trees in pots were obtained for inoculation with pure cultures of the *Phoma* and were kept in a greenhouse throughout the experiments.

Experiment I. In this preliminary experiment a plate culture of the fungus was started on June 8, 1914 as a sub-culture from one of the tube-cultures mentioned above as obtained from a "poured plate" of conidia. At the end of eight days (June 16) there was sufficient mycelium for use in the following inoculations:

¹ These slices were 4-5 cm. diam. and about 1 cm. thick.

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(1) A V-shaped cut was made through the bark of a branch of one of the young fig-trees; the triangular tongue of bark was turned back and a little agar bearing mycelium was removed from the plate culture and inserted between the bark and the wood. The free portion of bark was then pressed back into its place and the wound was covered up with cotton wool and tinfoil to prevent possible infection from air-borne spores. This precaution was perhaps unnecessary for as shown in Exp. II exposed wounds remained uninfected.

(2) From another branch a small portion of the bark (about 1 cm. \times 0.5 cm.) was entirely cut away and agar with mycelium placed on the exposed tissues, after which the wound was bound up as in the previous case.

On September 16 the coverings were removed and the following results observed:

(1) Round the inoculated wound was a depressed very dark elliptical portion of bark 2.5 \times 1.2 cm.; the tissue bordering the canker was pale in colour and thus showed strong contrast with the sunken canker. The latter bore a few pycnidia which appeared on the surface as small protuberances with incipient "tendrils" consisting of conidia which on microscopic examination were indistinguishable from those obtained from natural cankers.

(2) The result of the second inoculation was similar to the one just described except that the canker was smaller, its dimensions being 1.8 \times 0.8 cm.; tendrils with typical conidia were again produced.

In both cases the infected branch was seen in September 1914 to be slightly swollen when viewed in a direction perpendicular to the surface of the canker. No further increase in the size of these cankers was subsequently observed and in the following season both branches bore fruit and leaves. In February of the present year (1916) leaves and fruit again appeared but by the middle of March the secondary branch immediately above the canker of the second inoculation, and on the same side of the main branch as the canker itself, showed signs of wilting for the leaves began to droop and turn yellow, indicating that the transpiration current was interrupted in the neighbourhood of the canker.

Experiment II. Semicylinders of potato were placed in Roux's tubes and sterilized in an autoclave by steaming at a temperature of 115° C. for 30 minutes; they were inoculated from an agar slant culture on June 22, 1914. The fungus grew vigorously on the potato; pycnidia were readily produced and by July 15 "tendrils" of conidia began to

appear. Later the latter were further developed and on September 29 were used for a second series of inoculations on fig-trees. Two of the "tendrils" were removed on a sterile hooked platinum wire and placed in a small tube of sterile distilled water which was then agitated in order to diffuse the conidia throughout the liquid. Wounds are made on branches as in Exp. I, but instead of inserting agar and mycelium, a drop of the water containing the conidia was transferred to the wound by means of a pipette (previously sterilized) and allowed to flow in between the bark and the wood. In the case of control wounds a drop of sterile water only was inserted in a similar manner. Some of the wounds were bound round with damp cotton wool (previously sterilized in autoclave) and tinfoil secured by raffia in order to ensure favourable conditions for the germination of the conidia: the other wounds were left uncovered. In other cases again water containing conidia and fragments of tendrils was dropped on damp cotton wool which was then bound round certain internodes with tinfoil and raffia as before, but without injuring the bark.

Eight of the largest branches on two young fig-trees, var. White Marseilles, were selected and treated as follows, the branches being from 0.8 cm. to 2 cm. in thickness.

- No. 1. Inoculated; wound covered with cotton wool and tinfoil.
- „ 2. As in 1.
- „ 3. Inoculated; wound left uncovered.
- „ 4. Control; not inoculated but branch cut and wound covered with cotton wool and tinfoil.
- „ 5. Control as in 4.
- „ 6. Control; wound left uncovered.
- „ 7. Spores placed on damp cotton wool which was then wrapped round an internode.
- „ 8. As in 7.

Eight branches were similarly selected on two young trees of the Brown Turkey variety and respectively treated as those of the White Marseilles. Thus in this experiment six branches were wounded and inoculated, with an equal number of control branches also wounded, and in four cases conidia were applied to uninjured branches.

To test the viability of the conidia used in the experiment a drop of the conidia-containing water (as used in the inoculations) was used in preparing a "poured plate" which was then placed in the greenhouse in close proximity to the trees. No growth was to be observed in the

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plate for several days but on October 9 (*i.e.* when ten days old) numerous small mycelial masses were conspicuous.

Positive results were obtained in each case where the conidia had been inserted in the wound. The rest all yielded negative results.

The trees were examined from time to time and the progress of the disease in the inoculated branches noted as follows.

White Marseilles.

No. 1. (Branch about 1 cm. diam.)

Jan. 15, 1915. No change to be observed.

Feb. 16. No visible canker: no discoloration of the bark but "tendrils" were seen in the neighbourhood of the wound: they were arranged in the form of an irregular ellipse 2.2 cm. \times 1.4 cm., elongated in a direction parallel with the axis of the branch.

Jan. 25, 1916. Distinct sunken canker 4 cm. long and extending laterally three-quarters round the branch.

No. 2. (Branch about 2 cm. diam.)

Jan. 15, 1915. No change to be observed.

Feb. 16. " " "

March 25. A single tendril of typical conidia was seen at 2 mm. above the wound; otherwise there was no change externally.

April 20. The bark round the wound was slightly depressed below the general surface; the affected area measured 1.5 \times 1.5 cm.

Jan. 25, 1916. Distinct sunken canker 1.5 cm. long and extending one-third round the branch.

No. 3. (Branch 1 cm. diam.)

Jan. 15, 1915. No distinct canker (*i.e.* no sinking or cracking of the bark) but round the wound and extending 1 cm. above and below and 0.5 cm. laterally were numerous small protuberances; one of these pustules removed and mounted in water proved to be a pycnidium containing conidia as described above.

Feb. 16. Tendrils were issuing from the pustules which now covered an area 3.5 \times 1.8 cm.

Jan. 25, 1916. Distinct sunken canker 5 cm. long and extending three-quarters round the branch.

Brown Turkey.

No. 1. (Branch 1 cm. diam.)

Jan. 15, 1915. No change to be observed.

Feb. 16. " " "

Mar. 3. A rather indistinct zone of discoloured bark (darker than the rest) was observed at 3-5 cm. from the wound.

Mar. 25. A single tendril of typical conidia was found at 2 mm. from the point of inoculation.

Jan. 25, 1916. Distinct sunken canker 2 cm. long and one-third round the branch.

No. 2. (Branch 0.8 cm. diam.)

Jan. 15, 1915. No change to be observed.

Feb. 16. The bark was discoloured for 2-3 mm. round the wound; at 4 mm. from the point of inoculation was a small group of pustules.

Mar. 3. A tendril was observed projecting from one of the pustules; it was removed and microscopic examination showed conidia as before.

Jan. 25, 1916. The affected area, now 2 cm. long and extending three-quarters round the branch, was only slightly depressed below the general surface but was distinguished from the rest by its darker colour.

Feb. 24. Branch dead above the canker, as shown by absence of any growth, the rest bearing leaves and young fruit.

No. 3. (Branch 1 cm. diam.)

Jan. 15, 1915. No external change.

Feb. 16. No canker and no discoloration noticeable but round the wound was an irregular circle (about 1 cm. diam.) of tendrils.

Jan. 25, 1916. The affected area appeared as a slightly sunken canker 2 cm. long and extending half-way round the branch.

Thus in the two series of experiments eight inoculations through wounds were made in all, and in every case not only was there a "canker" formed round the point of inoculation but the fruiting fungus appeared in the affected tissues and produced tendrils of conidia resembling those found on "cankers" arising from natural infections. Since "cankers" were so readily induced when the fungus, either in the form of conidia or of mycelium, was introduced into the internal tissues through wounds, the negative results in those cases in which conidia were applied to the uninjured bark indicate that in all probability the fungus is solely a wound-parasite.

Preventive Measures.

From our knowledge of the biology of the parasite, it is very unlikely that spraying with any fungicides will hold the disease in check. The direct measures should consist of a search for all "cankers," and the cutting of them out, down to the sound tissues, at a time when the disease is dormant; it will be advisable to paint over the wounds made with Stockholm tar. All dead branches must be cut out and *promptly burned*, as otherwise the fungus producing conidia on the dead wood might constitute a dangerous source of infection. The indirect measures

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which should be employed—and these will be no less important than the direct measures—must consist of the prevention as far as possible of wounds to the bark. The making of wounds by the boots of men climbing in the branches when gathering the figs must be rigorously prevented, as well as those caused by careless hoeing or digging round the trees or due to the attacks of animals. The minimum amount of pruning to the older branches should be employed until the disease has been stamped out or reduced to a small amount.

DESCRIPTION OF PLATES I, II

PLATE I

1. Canker on a large branch of a fig-tree. $\frac{2}{3}$ natural size.
2. Lower half of the same canker, natural size, showing the small wart-like protuberances produced by the pycnidia.
3. Section through the bark of a canker; the pycnidial form of the fructifications is evident. $\times 60$.

PLATE II

4. Portion of a canker with "tendrils." $\times 4$.
5. Transverse section, natural size, through the middle of the canker shown in Fig. 1; the affected tissues were dark brown in colour.
6. *Phoma cinerescens* in pure culture on sterilized potato, natural size.
7. Two "tendrils" of *Phoma cinerescens* produced in pure culture on a slice of sterilized fig wood. $\times 4$.
8. Branch of fig-tree after inoculation with conidia taken from a pure culture, natural size; an irregular ring of "tendrils" at some distance from the wound is shown.
9. Another branch after inoculation with conidia; the protuberances produced by the pycnidia are seen around the wound.
10. A camera-lucida drawing of conidia of *Phoma cinerescens*; each conidium is provided with two groups of guttules. $\times 1000$.
11. Drawing traced from Mr Massee's illustrations, in the *Gardeners' Magazine*, of the conidia of the Fig canker *Libertella*. $\times 500$.