INVESTIGATION OF VASCULAR-STREAK DIEBACK OF COCOA IN PAPUA NEW GUINEA

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Abstract

Vascular-streak dieback of cocoa in Papua New Guinea has different symptoms from the diseases commonly referred to as cocoa dieback in other cocoa-growing countries. The symptoms of this destructive disease of cocoa have not previously been reported outside Papua New Guinea. A species of tulasnelloid fungus, *Oncobasidium theobromae* (Talbot & Keane), was consistently associated with the disease. It grew specifically within xylem vessels of diseased stems and leaves, preceding symptom development. It was the only fungus which was consistently isolated from, and sporulated on, living diseased cocoa stems: it grew from xylem vessels exposed on leaf scars formed by the abscission of diseased leaves and formed white, effused, adherent fruit bodies exclusively on these leaf scars and their adjacent bark. When spores were shed from these fruit bodies on to expanding leaves of young seedlings, the disease symptoms developed after 3 months and the fungus was re-isolated. *O. theobromae* therefore appears to be the cause of vascular-streak dieback. The fungus did not sporulate in culture but conditions necessary for fruit body formation and sporulation were studied in the field and fluctuations in fruit body numbers were correlated with fluctuations in disease incidence.

I. INTRODUCTION

Turner (1967, 1968), in reviewing cocoa dieback as reported from most cocoa-growing countries, considered that the name had been applied to symptoms with a number of different causes. He described the first symptoms as browning and wilting of the growing tips followed by wilting and fall of leaves leading to death of twigs and branches progressing from the tips. Turner (1967, 1968) listed several possible causes of cocoa dieback; these included various suboptimal environmental factors and insect attack, sometimes associated with invasion by saprophytic or weakly parasitic fungi.

Shaw (1962) described two types of cocoa dieback in Papua New Guinea, "ordinary dieback", caused by environmental factors alone, and "*Botryodiplodia* dieback" which was then thought to be caused by invasion of weakened or wounded branches by the fungus *Botryodiplodia theobromae* Pat. For the latter disease Shaw (1962) listed several symptoms in addition to tip death; these were brown streaking of wood, sponginess of bark, enlargement of lenticles and growth of lateral buds. Bridgeland, Richardson, and Edward (1966*a*, 1966*b*, 1967) gave a more complete description of the symptoms of "*Botryodiplodia* dieback" and emphasized the differences between this disease and tip dieback caused by environmental factors.

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The present work concerns the disease then known as "*Botryodiplodia* dieback". As *B. theobromae* was not demonstrated to be the cause of this disease the common name "*Botryodiplodia* dieback" was a misnomer. The disease reported here is clearly distinguishable from tip death caused by environmental factors as reviewed by Turner (1967, 1968): it has much more complex and definitive symptoms which usually develop on the second or third flush behind the growing tip many months before the tip is affected. Leaf death is preceded by a much more distinguishable pattern of chlorosis than the wilting mentioned by Turner (1967, 1968) and leaf shed usually occurs first on the second or third flush behind the tip, not at the tip. Tip death is very much a secondary symptom following development of the disease further down the branch. In order to distinguish this particular disease from general tip-dieback conditions we propose to refer to it as "vascular-streak dieback". In Papua New Guinea this is a most destructive disease of cocoa, killing many trees and young replants, as well as reducing the productivity of surviving infected trees, in the affected areas. It is similar in severity to the more acute forms of cocoa dieback reported from other countries but this particular symptom complex has not been described from any other cocoa-growing country.

II. Symptoms

Symptoms are the same whether the disease occurs on the main stem of a seedling or on a branch of an older tree. The first symptom is the chlorosis of one leaf, usually on the second or third flush behind the tip (Fig. 1); a few enlarged lenticels may be evident on the stem immediately below the petiole of this leaf. The characteristic pattern of chlorosis, with islets of tissue remaining green, is shown in Figures 1, 2, and 4. Within 2 or 3 days the chlorotic leaf is shed and subsequently leaves above and below it turn chlorotic in the same way and are shed (Figs. 2 and 3); lenticels may become noticeably enlarged and roughen the bark in the affected region (Fig. 10). Axillary buds along the stem often begin to grow (Fig. 6). The disease spreads into lateral branches, particularly those formed by the growth of axillary buds on a diseased stem, and on such branches leaves turn chlorotic and drop off in succession from the base. Leaves in the latest flush of a diseased seedling or branch often show interveinal necrosis ("oakleaf pattern") symptomatic of calcium deficiency (Fig. 5). Eventually leaf fall occurs right to the growing tip which then dies, followed by the rest of the seedling or branch; from such a branch the disease may spread to other branches or the trunk and kill a mature tree. Disease progression from the first affected leaf to death of the growing tip usually takes about 5 months in a branch 1 m long on a mature tree but may take only a few weeks in a young seedling.

Leaf scars resulting from the fall of chlorotic leaves were sometimes covered by a white, effused adherent fruit body (Fig. 6) of the fungus *Oncobasidium theobromae* described by Talbot and Keane (1971). Despite extensive searching on healthy cocoa and other plants these fruit bodies have only been found on leaf scars and their adjacent bark in the diseased region of cocoa stems.

III. Examination of Diseased Tissues

(*a*) *Materials and Methods*

To examine affected cocoa tissues for the presence of microorganisms, longitudinal and transverse sections were cut freehand and mounted in lactophenol cotton blue.

(*b*) *Results*

As the roots of diseased seedlings appeared healthy, attention was concentrated on the region of the plant showing disease symptoms. Cambium in this region browned abnormally fast when exposed to air and the underlying xylem was discoloured by

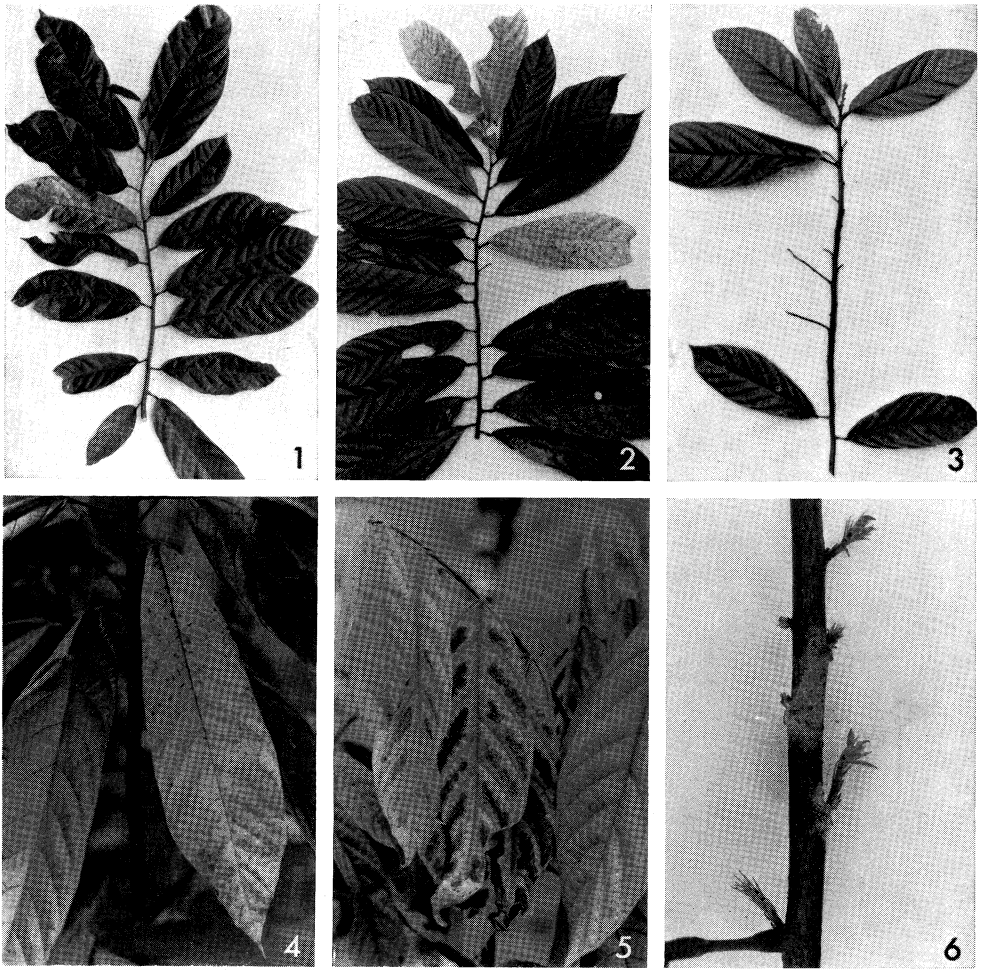


Fig. 1.—Diseased cocoa branch showing typical position and chlorosis of the first leaf to show disease symptoms.

Fig. 2.—Diseased cocoa branch with leaf fall and typical chlorosis symptoms confined to one side of the branch.

Fig. 3.—Diseased cocoa branch with leaf fall extending nearly to the growing tip with several small axillary twigs already dead.

Fig. 4.—Typical chlorosis of diseased cocoa leaf, with islets of tissue remaining green.

Fig. 5.—Unhardened, terminal leaves of a diseased cocoa seedling showing interveinal necrosis.

Fig. 6.—Diseased cocoa stem showing growth of axillary buds and white, effused, adherent fruit bodies of *O. theobromae* on the leaf scars.

brown streaks (Fig. 7). Fungal hyphae were regularly present in the discoloured wood and chlorotic leaves; in both, hyphae were confined to xylem vessels, whether reticulately or spirally thickened (Fig. 8). Hyphae were often located up to 10 cm beyond the upper and lower extremities of obvious streaking. A proportion of infected vessels were discoloured and blocked by gum-like deposits. This discoloration, with the discoloration of some surrounding cells, produced the macroscopic streaking.

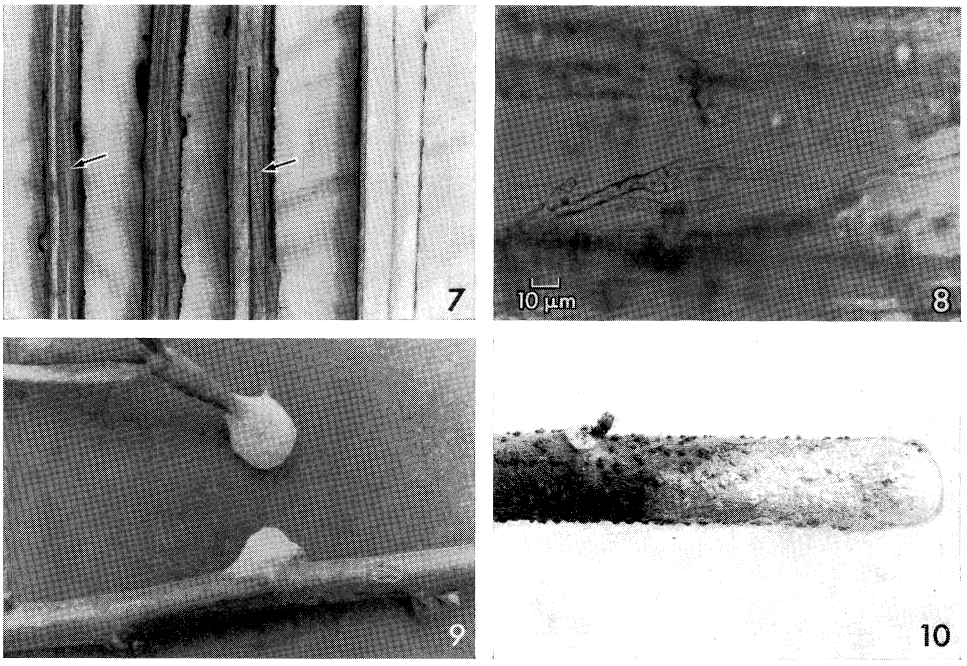


Fig. 7.—Diseased and healthy (far right) cocoa stems split longitudinally. Note discoloration of xylem and cambium (arrowed) of diseased stems.

Fig. 8.—Photomicrograph of longitudinal section of diseased xylem showing hyphae growing in a vessel. Note characteristic branching habit of hyphae.

Fig. 9.—Emergence of mycelium from vascular traces exposed by the abscission of a diseased leaf.

Fig. 10.—Fruit body of *O. theobromae* formed on cut end of a diseased cocoa stem. Note markedly swollen lenticels.

In the chlorotic leaves, even those with a barely perceptible paling in colour prior to development of the more charactertistic chlorosis, hyphae were present in xylem vessels throughout the main and lateral veins and petioles. Distribution of hyphae did not correspond with the pattern of green spotting on the leaves.

When there was only one chlorotic leaf on a stem, hyphae in a few cases were confined to the leaf, but usually they occurred through the petiole, in the stem from a few centimetres up to 30 cm above and below the axil of the leaf, and also in the petioles and leaf bases of adjacent leaves. In early stages of disease, streaking and fungal infection in the stem were usually restricted to the three vascular traces of the

diseased leaf. Sometimes the leaves on only one side of a stem were diseased (Fig. 2) and in such instances streaking and fungal growth were also limited to that side. These observations suggest that fungal infection may occur through the leaf although disease incidence was not associated with any gross wounding or weakening of the first affected leaf.

Hyphae appeared to be of one type (Fig. 8) with a diameter of 3·7–7·5 μm, with cells up to 200 μm long, and with dolipore septa. Hyphal branches originated near the end of a cell and the first septum in a branch was usually formed within 5–10 μm of the point of origin of the branch. Hyphae were hyaline except in badly discoloured vessels where they were brown.

Hyphae were not found in tip leaves suffering interveinal necrosis, indicating that this may be a secondary symptom following the blockage of xylem vessels further down the stem, which would restrict the upflow of mineral salts to the tip leaves.

IV. Isolation of Fungus

(*a*) *Materials and Methods*

Isolation was attempted from pieces of bark, xylem, and intact stem from the diseased region. Initially small pieces of tissue were surface-sterilized and placed on agar media but as the surface sterilization prevented emergence of hyphae, particularly from xylem, a procedure which exposed uncontaminated tissue was used. Strips of bark, sticks of bare xylem, and sticks of intact stem all about 20 cm long were surface-sterilized for 10 min in 70% alcohol, washed in sterile water, and cut with flamed secateurs into pieces 1–2 cm long which were placed on agar media. Healthy bark, xylem, and stems were treated similarly as controls.

To overcome problems of desiccation and contamination all culturing was done on agar slants in test tubes with cotton-wool plugs. Cultures were kept at 24°C. Several standard mycological media were used but potato sucrose agar with 0·1% yeast extract and 0·1% beef extract gave rapid growth of emerging hyphae and so was used in routine isolations.

Subculturing away from cocoa wood was attempted on to potato sucrose agar and sweet potato (*Ipomoea batatas*) sucrose agar (1·0% sucrose) with and without extracts of yeast and beef (each 0·1%), Czapek Dox agar, and tap water agar. Mycelium was first grown from leaf scars on to water agar and small squares of this were transferred to the various media.

Freshly cut pieces of intact diseased stem were placed on moist filter paper in Petri dishes and fungal growth from the newly exposed tissue was examined.

Stems with attached chlorotic leaves were surface-sterilized for 10 min in 70% alcohol and washed in sterile water. Each chlorotic leaf was then broken from its stem at the abscission zone and both leaf and stem were incubated at high humidity in a sterile glass jar; fungal growth from freshly exposed leaf scars was examined.

Pieces of stem each bearing a fruit body of *O. theobromae* were attached to the lids of Petri dishes containing water agar. Moist filter paper was placed around the stems. Hyphae developing from the fruit bodies grew on to the agar and were examined and subcultured to potato sucrose agar with yeast and beef extract, 0·1% malt extract agar, or neutral Dox yeast agar.

Fungal nuclei were revealed by staining mycelium for 3 min in 0·01% acridine orange made up in 0·07M phosphate buffer at pH 6·0; the mycelium was then washed in buffer, differentiated for about 30 sec in 0·1M calcium chloride solution, and mounted in buffer for examination under a Zeiss ultraviolet microscope using excitation filter 1 and barrier filter 53. Nuclei fluoresced bright green, cell walls pale green, and cytoplasm orange-red. Fluorescence of cytoplasm was reduced by treatment with calcium chloride solution. The HCl–Giemsa technique did not consistently stain nuclei but it clearly stained parenthesomes around dolipore septa. Dolipores were also clearly shown by phase-contrast microscopy.

(b) Results

Mycelium identical to that seen in xylem vessels was the only type consistently isolated from diseased xylem; microscopic observation of hand-cut sections of the xylem clearly showed that the mycelium had grown from xylem vessels. This mycelium, which was composed of binucleate cells, was not isolated from diseased bark nor from healthy tissues. Several fungi, including a species of *Fusarium*, a species of *Phomopsis*, and *Botryodiplodia theobromae* grew from both diseased and healthy bark, often overgrowing mycelium emerging from xylem during culturing from intact diseased stems. These were assumed to be common inhabitants of the outer layers of bark, although the species of *Fusarium* did have some association with diseased bark in that it consistently formed sporodochia in the enlarged lenticels. These sporodochia were also commonly observed in the field on stems killed by dieback; in the field the sporodochia were eventually replaced by perithecia of *Calonectria rigidiuscula* (Berk. & Br.) Sacc. However, sometimes sporodochia and perithecia were seen in enlarged lenticels on stems which had died from causes other than dieback. Several bacteria occurred in cultures from both diseased and healthy tissues and were assumed to be common inhabitants of both healthy and diseased stems.

Mycelium isolated from diseased xylem was yellowish white if grown in light or white if kept in the dark. The wispy, superficial growth filled the air space above the agar slant and penetrated into the agar; it extended about 3 cm from the xylem in less than 2 weeks. Hyphal characteristics were identical to those of hyphae observed in xylem vessels. In older cultures small clusters of monilioid cells occurred frequently on the agar surface but fruiting did not occur in culture even after 12 months' incubation. Subcultured mycelium grew fastest on sweet potato sucrose agar, extending about 3 cm in 5 weeks. This growth was much slower than the growth from the diseased wood. The addition of extracts of yeast and beef did not enhance fungal growth.

Mycelium identical to the above in hyphal characteristics, septation, nuclear number, and growth on nutrient media grew in nearly every instance from xylem at the cut ends of pieces of diseased stem incubated on moist filter paper in Petri dishes, from vascular traces exposed on both leaf scar and petiole when a diseased leaf was broken from its stem and the two were kept at high humidity (Fig. 9), and from fruit bodies of *O. theobromae* incubated at high humidity above water agar. Microscopic examination showed that the mycelium emerging from cut stems and from leaf scars was in fact growing only from exposed xylem vessels.

In the field this mycelium was also observed to grow from newly exposed dieback leaf scars and from recently cut dieback stems during moist weather. Under drier conditions the mycelium settled down on the leaf scar or cut and the surrounding bark, forming a fruit body of *O. theobromae* (Figs. 6 and 10) consisting of monilioid cells similar to those seen in cultures.

From these observations it is concluded that the mycelium growing in the xylem vessels of diseased cocoa is the vegetative stage of *O. theobromae*. This conclusion is supported by the binucleate nature of the cells constituting both the hyphae emerging from xylem and the fruit body of *O. theobromae*, and by the presence of dolipores

in the hyphae growing in xylem, which is consistent with their connection with *O. theobromae*, a species of Basidiomycotina.

V. Biology of the Fungus

(*a*) *Materials and Methods*

Fall of chlorotic leaves, emergence of mycelium from the resulting leaf scars, and the development of fruit bodies of *O. theobromae* on these scars were studied under natural conditions. The fruit bodies on 10 diseased cocoa trees were counted over a 4-month period beginning in August 1970.

Conditions suitable for the growth of mycelium from leaf scars on diseased stems were studied in the laboratory. Stems with attached diseased leaves were surface-sterilized in 70% alcohol and washed with sterile water. The leaves were broken from their stems at the abscission zone and the stems were kept at different humidities in glass jars containing various saturated solutions. The relative humidity above each solution was determined with an aspirator psychrometer and radial growth of mycelium from the freshly exposed leaf scars was measured.

Spore shedding by *O. theobromae* was first studied by attaching pieces of stem, each bearing a fruit body, to the lids of Petri dishes containing tap water agar. The fruit bodies were maintained at different degrees of wetness by surrounding them with varying amounts of moist filter paper and spores were shed on to the agar. The diurnal cycle of spore shedding was determined by suspending a fruit body above a Cellotape strip wrapped, with its adhesive facing outward, around a recording drum set to rotate once in 24 hr. Hour marks were traced on the Cellotape and the fruit body and drum were enclosed in a moisture chamber. At the end of a run the Cellotape was cut into sections and mounted in lactophenol cotton blue and adhering spores were counted. Spore shedding under natural conditions was studied by enclosing the recording drum in a plastic cover which had a small slit above the strip of Cellotape; the slit was placed below a fruit body in the field so that spores gravitated to the Cellotape.

The germination of spores shed on to free water and water agar was observed. In order to study their further development spores were transferred from water agar to the following media—soil rice agar (Echandi 1965), potato dextrose agar, potato sucrose agar, potato sucrose agar with 0·1% yeast extract and 0·1% beef extract, potato sucrose agar with 0·1% Casamino acids, potato sucrose agar with 0·1% Bactopeptone, potato sucrose agar with 0·1% Proteose-peptone, potato xylose agar, potato arabinose agar, 0·1% malt extract agar, and tap water agar containing surface-sterilized cocoa wood. Spores were picked up with fine, hooked needles made by pulling out glass rod over a flame.

(*b*) *Results*

Results given in Figure 11(*a*) indicate that rapid emergence of mycelium from freshly exposed leaf scars occurred only at relative humidities above about 95%. This is consistent with the observation that mycelium grew from leaf scars in the field only during wet weather. Results given in Figure 11(*b*) suggest that at least a 30-hr period with relative humidity near saturation is necessary for mycelium to become established on a leaf scar; observations in the field, where at least one wet day was required for obvious growth of mycelium from a leaf scar, supported this.

Mycelium grew most readily from leaf scars formed by the fall of chlorotic leaves during moist weather; hyphae rarely emerged from leaf scars which had been hardened by exposure to hot, dry weather. The emerged vegetative hyphae extended further over the bark with successive moist periods. Drier conditions stimulated the emerged hyphae to form a compact layer of shorter, wider cells on the surface of the leaf scar and surrounding bark; after the change to a reproductive growth phase the fungus did not extend further from the leaf scar. These cells eventually produced

basidia of *O. theobromae*. Under suitable conditions about a week elapsed between emergence of hyphae and formation of a fertile fruit body. Fruit bodies have persisted in fertile condition up to 30 days. If dry conditions prevailed for an extended period the fruit bodies dried and could not be induced to shed spores by removal to a moist chamber in the laboratory. Fruit bodies also dried and collapsed when branches bearing them began to wither and die.

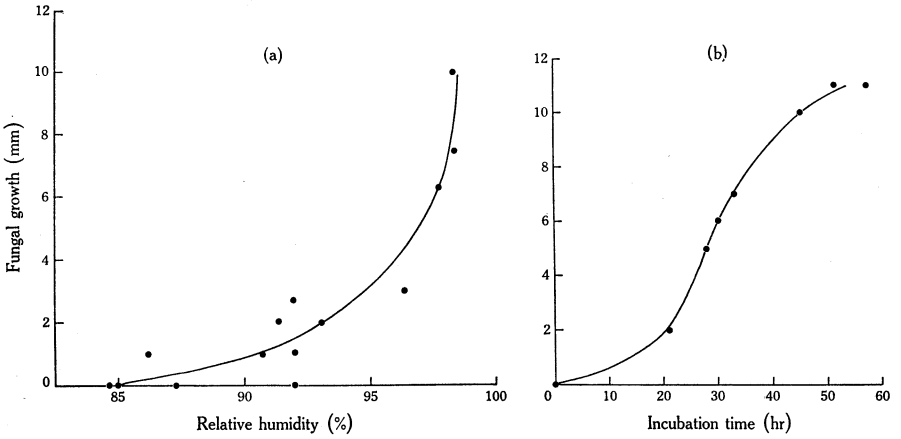
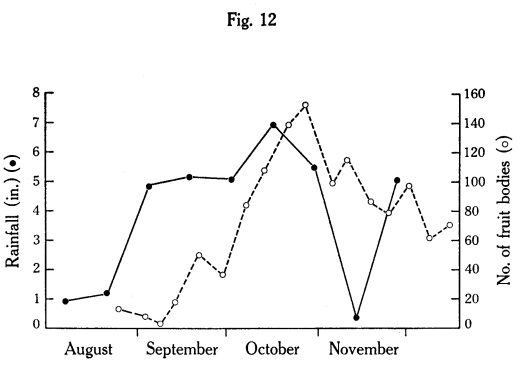


Fig. 11.—Fungal growth from newly exposed leaf scars kept at various relative humidities for 72 hr (*a*) and at 98% relative humidity (*b*).

Fluctuations in the number of fruit bodies on 10 dieback-diseased trees are shown in Figure 12. Rainfall is plotted as 2-weekly totals in order to average out the



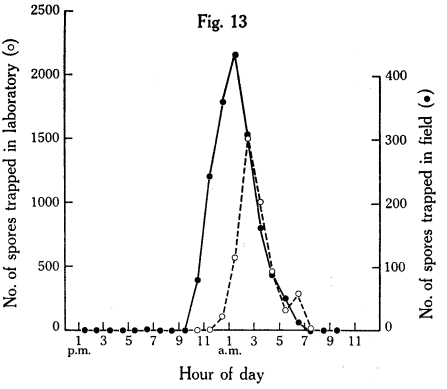


Fig. 12.—Fluctuations in rainfall (2-weekly total) and in number of fruit bodies of *O. theobromae* on 10 diseased cocoa trees over a 4-month period.

Fig. 13.—Diurnal rhythm of spore shed of *O. theobromae* in laboratory and field.

violent daily fluctuations which occur in this region. As would be expected from the above observations, fruit bodies were much more prevalent after a period of moist weather such as that during September and October.

Fruit bodies shed spores in Petri dishes only when thoroughly wetted and held at a high humidity maintained by wet filter paper placed around them. Basidia were formed and spores were shed mostly at night. Spore trapping on Cellotape gave the results set out in Figure 13; spore shedding commenced at about 10 p.m., reached a peak between midnight and about 4 a.m., and diminished through to dawn. Fruit bodies shed spores most prolifically on nights which followed afternoon or early evening rain; spores were rarely shed during nights with dew following dry days, indicating that dew did not wet fruit bodies sufficiently for them to sporulate.

Spores were not shed in Petri dishes after the diseased stems had begun to blacken and wither, which was usually about four nights after they had been collected from the field. In the field, too, spores were not trapped on Cellotape placed under fruit bodies on dead or dying branches.

Almost 100% of *O. theobromae* spores germinated immediately on falling into free water on agar. However, germ tubes never grew longer than about the 400 μm attained in the first 24 hr after germination, and never branched. Further growth was not sustained on any of the test media to which spores were transferred. It seemed that a nutrient factor required for further fungal growth was lacking. Cocoa wood browned slowly in the agar medium and it is likely that the browning products would have inhibited fungal growth regardless of the nutrients present.

VI. Pathogenicity Tests

(*a*) *Materials and Methods*

Spores were shed on to agar, Cellotape, and sterile water from fruit bodies of *O. theobromae* collected in the field. In all cases only spores of *O. theobromae* were found; apparently in the material used no other fungi were shedding spores.

Spores shed into sterile water were concentrated by withdrawing some of the water through a Millipore filter (pore size 0·45 μm). This suspension was injected into stems, petioles, leaves, and growing points of 1- and 6-month-old cocoa seedlings in an attempt to induce symptoms of dieback. Mycelium isolated from diseased xylem was inserted into cuts made in stems of cocoa seedlings; the cuts were bound with cotton wool and sealing tape in order to maintain moist conditions around the fungus.

Spores were also shed on to the young expanding leaves of the growth flush of 6-month-old cocoa growing in polythene bags. Before spore shedding the leaves of the growth flush were either left untreated or were treated by lightly rubbing both upper and lower surfaces with (1) a dry Kleenex tissue or (2) a Kleenex tissue to which had been added a few drops of chloroform. Fruit bodies on small pieces of diseased cocoa stem were held a few centimetres above the growth flush of each seedling and the fruit bodies and growth flush were wetted just before nightfall; the upper part of the seedling and the fruit bodies were covered with a plastic bag to ensure that spores shed during the night fell on to the growth flush. A piece of wire 0·4 cm in diameter and with Cellotape wrapped around it, adhesive facing outward, was held in a horizontal position immediately above the growth flush. Similarly treated seedlings were wetted and held overnight under plastic bags as controls. The following morning before direct sunlight had fallen on the seedlings the plastic bags were removed and the leaves of the growth flush were tagged to indicate their length at the time of spore shedding; the Cellotape strips were mounted in lactophenol cotton blue and examined for the presence of spores. The seedlings were kept in the shade for several weeks before being planted under *Leucaena leucocephala* remote from other cocoa plantings.

Detached expanding leaves from the growth flush of cocoa seedlings were lightly rubbed with Kleenex tissue dampened with a few drops of chloroform. These were each held on wet filter paper in a Petri dish; a piece of diseased stem was attached to the lid of the dish so that a leaf scar formed by the removal of a chlorotic leaf was just above the leaf and mycelium emerging

from the leaf scar grew over the treated surface. After 48 hr the leaves were cleared for 2½ hr in glacial acetic acid and ethanol (50 : 50 v/v) and were immersed for 2 days in 0·4% trypan blue in 45% acetic acid before being mounted for microscopic examination in water.

(*b*) *Results*

Disease symptoms were not induced by injecting spores nor by inserting mycelium of this fungus into young cocoa seedlings. Dissection of cut or injection wounds in cocoa seedlings several weeks after they were made revealed extensive blackening in the vicinity of the wound. The fungus had not penetrated beyond the discoloured region.

Typical symptoms of vascular-streak dieback began to appear in seedlings on which spores of *O. theobromae* had been shed 2½–3 months after spore shedding. The first symptoms were the chlorosis, with small spots of tissue remaining green, of the growth flush leaves which had been tagged and the enlargement of lenticels at the base of these leaves. Within a week these chlorotic leaves were shed and in some cases the emergence of mycelium of *O. theobromae* from the leaf scars was observed. Mycelium of *O. theobromae* was isolated from a sample of diseased leaves, emerging only from the cut ends of vascular tissue in the leaves; microscopic examination showed that mycelium was confined to xylem vessels in the diseased, living leaves and stems. None of the control seedlings showed symptoms of vascular-streak dieback and no mycelium grew from a sample of leaves taken from control plants. Spores were trapped on Cellotape above 47% of the seedlings kept overnight under fruit bodies but, because of the small area of the trapping surface, an absence of spores would not necessarily indicate that none had been shed on to the seedling. Of the seedlings kept overnight under fruit bodies, 36% developed symptoms of vascular-streak dieback; spores had been trapped above 70% of these seedlings. There was no indication that the rubbing or chloroform treatments increased the susceptibility of seedlings to the disease.

Mycelium growing from diseased cocoa stem over the surface of unhardened leaves rubbed with a trace of chloroform penetrated the cuticle either from a single, club-shaped, terminal cell or from an appressorium formed by the loose aggregation of mycelium. Below the cuticle the mycelium ramified rapidly with prolific branching compared with the sparse branching observed above the cuticle and in culture. However, penetration of xylem vessels was not observed in this preliminary study, but it would appear that penetration can occur readily through the undamaged, unhardened leaves and this is almost certainly the mechanism of entry following spore germination.

VII. General Discussion

The preliminary pathogenicity test indicates that *O. theobromae* is the cause of vascular-streak dieback of cocoa. The shedding of spores of the fungus on to the growing tips of cocoa seedlings resulted in the development of disease symptoms in 36% of the seedlings and *O. theobromae* was isolated from the diseased tissues. In addition there is much previously accumulated evidence that *O. theobromae* is the primary cause of the disease and not just a secondary invader. Obvious or macroscopic weakening or mechanical damage of plants seemed to be unnecessary for fungal

infection and the presence of the fungus actually preceded the development of disease symptoms—it was usually found beyond affected regions of a stem and was occasionally observed over a distance of up to 60 cm in plants showing no sign of debility other than a faint chlorosis of one leaf. Within diseased plants fungal growth is confined to xylem vessels, which in itself strongly suggests a pathogenic association. Furthermore *O. theobromae* is the only fungus seen to sporulate consistently on young, living diseased plants; fertile fructifications of this fungus have been found only on diseased, but living, cocoa stems and then only on the leaf scars of those stems. This fruiting habit is more likely to be that of a pathogen than that of a secondary invader. The pathogenicity test established an incubation period of $2\frac{1}{2}$–3 months and indicated that infection probably occurs through unhardened flush leaves. A similar incubation period can be calculated from the rate of fungal growth, measured as the rate of emergence of hyphae from a leaf scar [3 mm per day, Fig. 11(*b*)], and the extent of fungal growth (30–60 cm) in plants showing the first symptom, assuming that the fungus penetrated in the centre of the infected region. The same incubation period was also indicated by the occurrence of a peak in disease incidence about 3 months after the wet season, when there would have been a peak in the sporulation of *O. theobromae*.

The incubation period of 2–3 months following infection through unhardened flush leaves explains the appearance of initial symptoms in a leaf on the second or third flush behind the tip: the fungus penetrates the soft tip leaves but before it ramifies through the leaf sufficiently to induce chlorosis the infected branch produces a further one or two growth flushes.

The mechanism of fungal penetration following spore germination has not been determined but it seems likely from the results of the initial pathogenicity test that penetration occurs through the undamaged, unhardened leaves.

VIII. Acknowledgments

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