THREE LEAF SPECKLE DISEASES OF THE BANANA IN QUEENSLAND

By W. PONT, B.Sc.*

SUMMARY

Leaf speckling diseases of the banana caused by *Chloridium musae* Stahel, *Ramichloridium musae* Stahel and *Mycosphaerella musae* (Speg.) Syd. are described.

The first two diseases appear to be confined to the tropical north of Queensland where they are present as relatively superficial leaf blemishes in rain-forest plantations or on native bananas. Mycosphaerella speckle, however, causes serious damage in plantations in Queensland and New South Wales.

A conidial stage of *M. musae* has not yet been found. The disease is disseminated by ascospores ejected from perithecia in the dead leaf tissue. Germination takes place on the lower surface of the leaves. After a lengthy period of epiphyllic existence, at least 5-6 weeks, appressoria are formed and the fungus penetrates the leaf through the stomata on the under-sides.

In experiments with protectant fungicides, Mycosphaerella leaf speckle was controlled by a single application of either copper oxychloride or zineb to the lower surfaces of leaves not showing visible symptoms. Other sprays which controlled Mycosphaerella speckle when applied to the under-sides of the leaves were white oil emulsion, white oil emulsion/malachite green, phenyl mercuric chloride and a straight paraffin oil. Treatments applied to the upper surfaces of the leaves were ineffective.

Tests with eradicant sprays showed that sodium pentachlorphenate almost completely inhibited ascospore discharge when sprayed onto the lower surfaces of dead leaves containing mature perithecia.

I. INTRODUCTION

Leaf speckling diseases have been described on several banana varieties in the West Indies and Australia. In Queensland a leaf speckle was first described on the Cavendish variety by Simmonds (1933), who found a species of *Mycosphaerella* to be consistently associated with the disease. In Trinidad the fungi *Nigrospora oryzae, Gloeosporium musarum, Stachylidium* sp., *Fusarium* sp. and *Penicillium* sp. were isolated from a characteristic leaf mottling or speckling of leaves of Cavendish, Gros Michel and Lacatan bananas by Wardlaw (1935), who suggested that this might be the same disease as described by Simmonds.

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Two distinct leaf speckle diseases of the Congo banana caused by the closely related fungi *Chloridium musae* Stahel and *Ramichloridium musae* Stahel were later described from Surinam (Stahel 1937). Stahel suggested that Simmonds' Queensland speckle might be similar to that caused by *Ramichloridium musae* in Surinam. *Chloridium musae* was subsequently recorded in Fiji (Parham 1938) and Brazil (Bitancourt 1940). The list of speckling diseases was further extended when Martyn (1945) described a "flecking" caused by *Cladosporium musae* Mason. Martyn also confirmed the presence of Stahel's Chloridium speckle in Jamaica and described a peculiar epiphyllic fungus commonly found on banana leaves in Jamaica and which was named *Zygophiala jamaicensis* by E. W. Mason of the then Imperial Mycological Institute.

In Queensland the leaf speckle originally recorded by Simmonds (1933) and referred to in this article as Mycosphaerella speckle is a common cause of defoliation. Simmonds contended that in South Queensland it sometimes was "equally if not more responsible for the leaf damage occurring" than leaf spot (*Mycosphaerella musicola* Leach). In North Queensland, where leaf spot outbreaks are more virulent and more sustained, the importance of Mycosphaerella speckle has, until recently, been overshadowed by that of leaf spot. Improved control measures for leaf spot now in use in this region of the State have been responsible for a marked reduction in the amount of damage due to this cause. However, in many cases and for reasons which will be discussed later, the incidence of leaf speckle has not been similarly depressed by these measures and the damage attributable to this disease has become more obvious. The increased importance of Mycosphaerella speckle necessitated further investigations into its cause and control and these questions are the main subjects dealt with in this paper.

In the course of this work it became apparent that two other leaf speckle diseases often occur in North Queensland banana plantations, viz. those originally described by Stahel in Surinam and caused by *Chloridium musae* and *Ramichloridium musae* respectively. These two disorders are referred to in this article as tropical leaf speckles. This is the first Australian record of *C. musae* and appears to be the only published record of *R. musae* since it was described by Stahel.

It is of interest to note here that the epiphyllic fungus *Zygophiala jamaicensis* Mason has also been recorded on leaves of Cavendish and Mons Mari bananas in North Queensland.

II. TROPICAL LEAF SPECKLES

(a) **Chloridium musae** and **Ramichloridium musae**

Both these diseases are commonly found in plantations in the high-rainfall area which extends from Tully to Deeral in North Queensland. Such plantations are located on clearings in rain-forest and the protection afforded by the trees favours speckle development presumably because of the limited air movement and

high humidity. The two diseases have been found on the Mons Mari, a Cavendish mutant commonly grown in this area, and also on a native species, *Musa banksii* F. Muell. Both fungi are often seen in mixed culture on the one leaf.

(b) Symptoms and Effect on the Host

(i) Chloridium musae

This fungus produces on green leaves chlorotic blotches roughly circular in outline and up to 1½ in. in diameter. On the upper surface of these blotches dark-brown or black pin-point sized specks are clearly visible and are thickly distributed (Figure 1). On the lower surface of the lesions the specks are not nearly as prominent and when an affected leaf is viewed from underneath the disease appears as tan-coloured blotches. If a piece of speckled leaf tissue is bent around the finger with the lower surface of the leaf uppermost and held against the light it is easy to see, even with the naked eye, the densely packed, bristle-like sporophores of the causal fungus.

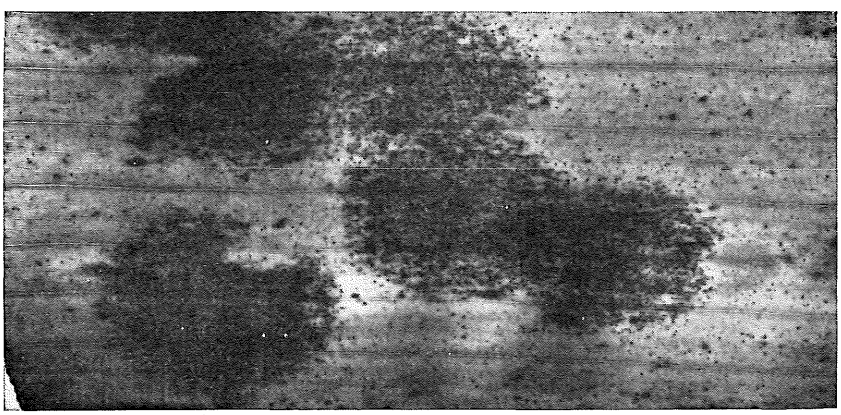

Fig. 1.—Upper surface of leaf of Mons Mari variety showing lesions of *Chloridium musae*.

(ii) Ramichloridium musae

On green leaves the lesions are quite obvious as irregularly circular, dark-grey to black patches on the lower surfaces (Figure 2). These patches can be resolved with a hand lens into densely aggregated, minute black specks. The individual speckled areas are smaller than those produced by *C. musae* but often they merge into quite extensive smoky blotches. The blotches are still discernible but less distinct on the upper surfaces of the leaves and here a speckled pattern cannot be distinguished. The sporophores are clearly visible on the lower surface of the lesions as a dense, almost velvety coating.

These fungi are weak parasites and are seen only in unsprayed plantations. Their effect on the host is hard to assess because damage from either leaf spot or Mycosphaerella speckle is always present on the older leaves and confuses the

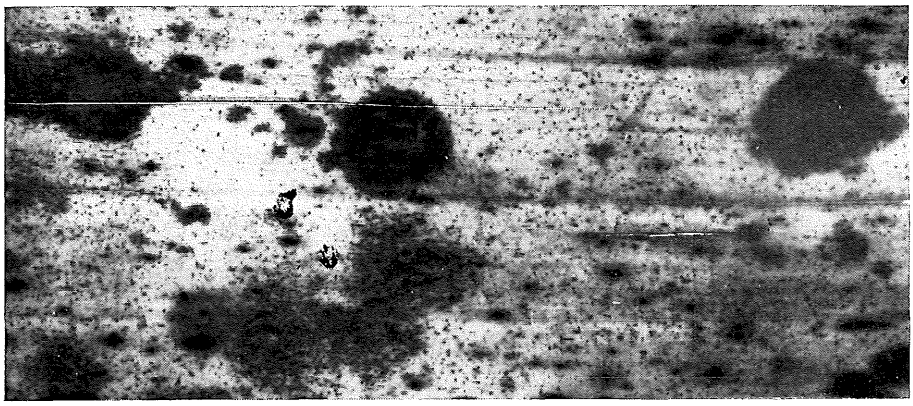

Fig. 2.—Lower surface of leaf of Mons Mari variety showing lesions of *Ramichloridium musae* (above) and *Chloridium musae* (below).

issue. Lesions of these tropical speckles are often seen on young foliage, even as high on the plant as the third fully expanded leaf, where leaf spot or Mycosphaerella speckle symptoms are not yet evident. However, no extensive necrosis or breakdown of leaf tissue which could lead to premature defoliation has ever been associated with infection by either organism.

(c) Cultural Characteristics

Both fungi can be easily isolated by removing fruiting structures from a lesion with a sterile needle. Both can be cultured on potato dextrose agar. Colonies of *R. musae* on this medium grow slowly; they are dark-grey with an elevated centre on which, as the cultures age, a lighter grey aerial mycelium is produced. *C. musae* grows faster and forms flat or only slightly elevated colonies which are wrinkled and of a similar dark-grey colour (Figure 3). Both fungi produce sporophores and spores on potato dextrose agar; the sporophores of *R. musae* are, however, usually only sparingly branched in culture.

(d) Growth-Temperature Relationships

The temperature relationships of these fungi were determined by exposing potato dextrose agar cultures in a multi-temperature incubator giving 14 temperature regimens ranging from 10°C to 37°C. Uniform squares of inoculum were placed in a central position on this medium in 4 oz clear bottles and incubated at room temperature for three days. The cultures were then examined and their outlines were marked. Two bottles were placed in each of the 13 chambers of the multi-temperature incubator. The growth was measured after 7 and 14 days' exposure (Figure 3). The results are shown in Figure 4, where the average increase in colony diameter expressed in millimetres is plotted against temperature.

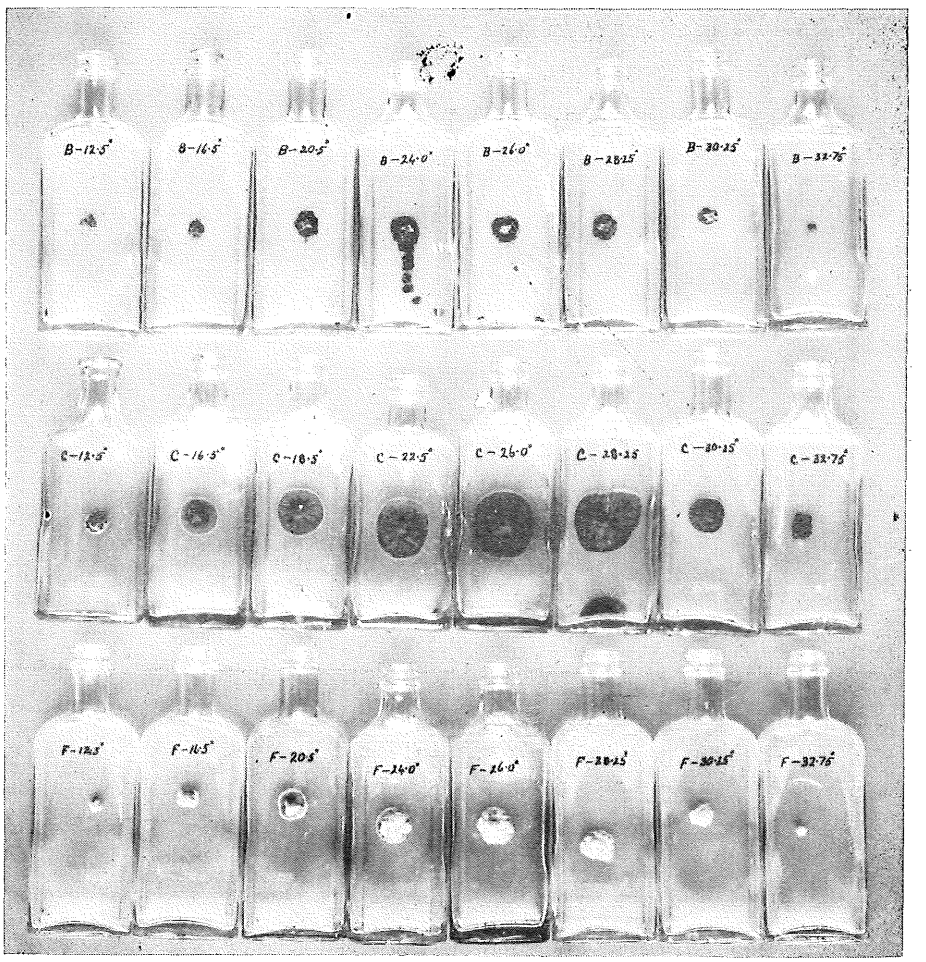

Fig. 3.—Effect of temperature on growth in culture of leaf speckle fungi. Above, *Ramichloridium musae.* Middle, *Chloridium musae.* Below, *Mycosphaerella musae.*

The optimum temperature for the development of *R. musae,* 27°–28°C (82°F), was slightly higher than that for *C. musae,* 26°C (79°F), which in this trial had the same optimum as *Mycosphaerella musae.* Growth of both species was negligible below 10°C and above 35°C.

The curves for the three species *R. musae, C. musae* and *M. musae* were very similar in shape, and difference in temperature requirements for growth therefore appears an unlikely reason for the restricted distribution of the two tropical species.

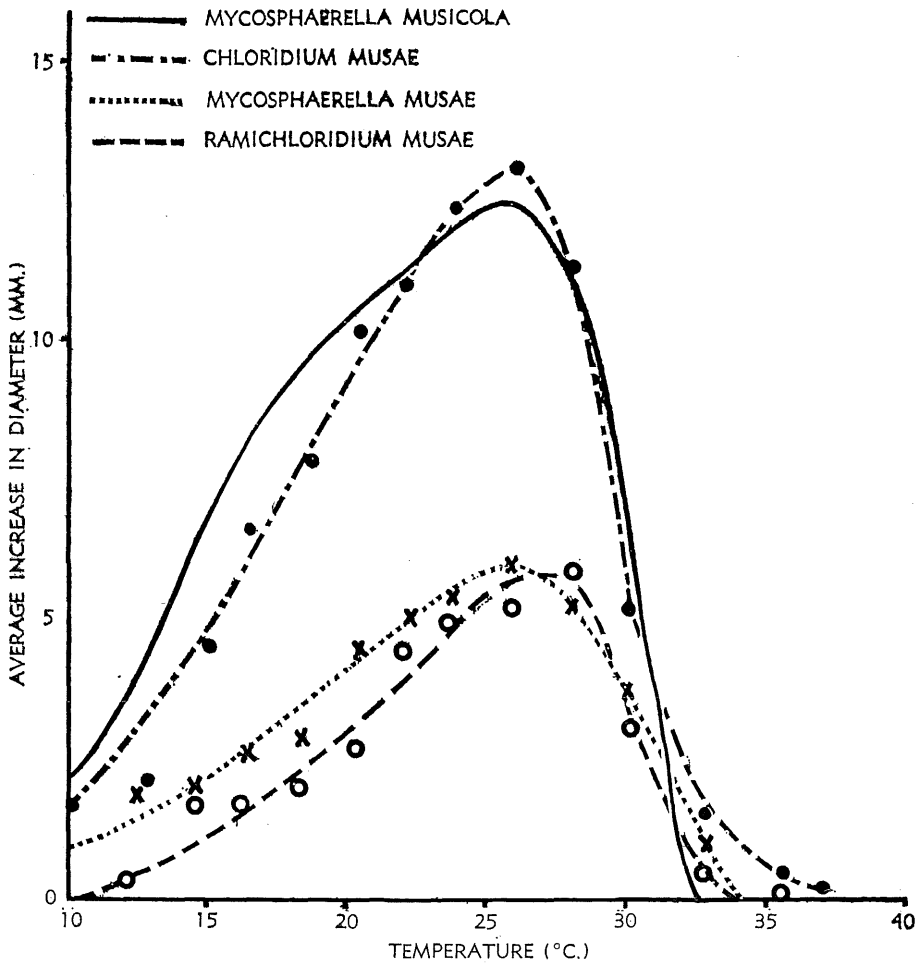

Fig. 4.—Growth-temperature curves. Each 7 days' growth on potato dextrose agar except *Mycosphaerella musicola,* which is 24 days' growth. (*M. musicola* values after Simmonds 1933).

(e) Morphology

The dimensions of the fruiting structures of the two fungi present in North Queensland are compared with those given by Stahel for his Surinam collections in Table 1.

The spores of *C. musae* are oval and those of *R. musae* are elliptical. The sporophores of the latter are longer than those of the former. The conidia of *C musae* have the papilla described by Stahel at the point of attachment to the sporophore.

TABLE 1

Dimensions of Fruiting Structures of C. musae and R. musae, North Queensland and Surinam Collections

Organism	Origin	Spores (μ)	Sporophores (μ)
Chloridium musae	Surinam	6–7·5 x 2·5–3	100–300
	North Queensland ..	6–7 x 3–4	118–291
		Average (50) 6·4 x 3·1	
Ramichloridium musae	Surinam	6–7·5 x 1·5–2·5	200–500
	North Queensland ..	6·4–9·6 x 2–3	342–411
		Average (50) 7·2 x 2	

III. MYCOSPHAERELLA SPECKLE

(a) Distribution

The only definite descriptions of this disease are from Australia, where it is present in northern and southern Queensland and in New South Wales. Material typical of the leaf speckle present in New South Wales and collected at Tullera, near Lismore, was recently examined. It was indistinguishable in appearance from the disease in North Queensland.

It is uncertain whether the leaf speckling of the West Indies referred to by Wardlaw (1935) is identical with the Queensland disease, as he made no record of a Mycosphaerella amongst the various associated organisms. The causal organism of the Queensland speckle had, however, previously been described from the Dominican Republic in the same general region (Ciferri and Fragoso 1927).

Simmonds collected material closely resembling the Queensland speckle at Peradeniya, Ceylon, in 1932 (Simmonds 1933). Recent examination has shown a Mycosphaerella closely resembling *M. musae* to be associated with this material (Table 3), and it would appear that the two diseases are identical. In spite of the paucity of references to its occurrence, Mycosphaerella speckle is therefore widely distributed throughout the banana-growing regions of the world.

(b) Host Range

All the varieties commonly grown on a commercial scale in Queensland, viz. the Cavendish and its two mutants Mons Mari and Williams Hybrid, also the Lady Finger and Sugar, are susceptible. The variety called Ducasses in Queensland, which is immune to leaf spot (*Mycosphaerella musicola*), is susceptible. The disease is also commonly recorded on a native banana (*Musa banksii* F. Muell).

(c) Symptoms

The first signs of infection on a leaf are clearly visible early in the morning when dew is present or during rainy periods as watersoaked patches which exude droplets of moisture. In the absence of moisture the infected areas can often first be distinguished on the lower leaf surface as light-brown or tan-coloured irregular blotches which may show on the upper surface as smoky patches. As the disease progresses the blotches on the lower surface darken in colour, become visibly stippled due to necrosis of the stomata and cells of the spongy parenchyma, and eventually become irregularly shaped speckled areas visible on both leaf surfaces (Figures 5 and 10). The colour of these speckled patches ranges from dark purple to black. By this time the leaf tissue in and

Fig. 5.—*Mycosphaerella musae.* Natural infection on lower surface of leaf of *Musa banksii.*

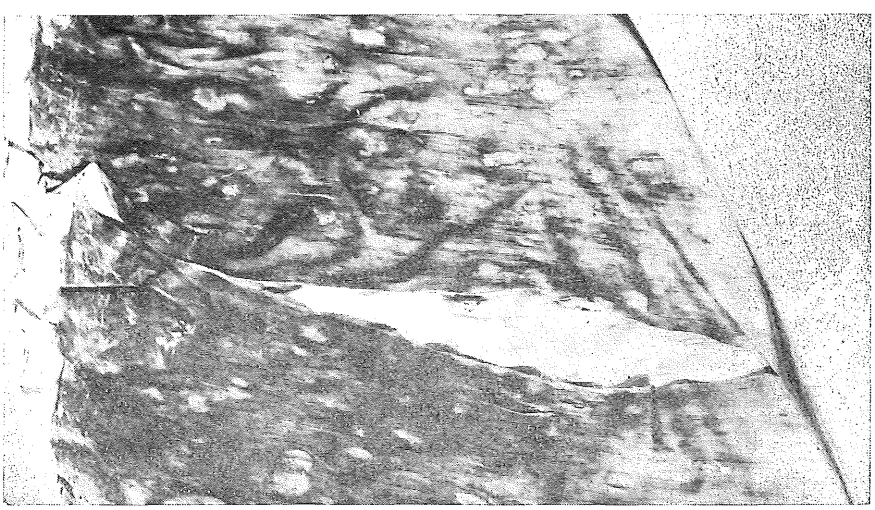

Fig. 6.—*Mycosphaerella musae.* Upper surface of dead leaf of Ducasses variety.

around the speckled area is chlorotic. As the disease progresses the individual specks of necrotic tissue coalesce to give large necrotic blotches which bleach with age as the affected area dries out and become grey on the lower surface and straw-coloured on the upper surface. Usually at this stage the perithecia first become visible on the lower leaf surface.

Sometimes on the variety Ducasses local patches of speckle may give rise to small necrotic spots with a dark-brown or black margin, light-tan on top and grey below (Figure 6). Perithecia are numerous on the lower surfaces of such spots but sparse on the upper surfaces.

On dead leaves the speckled areas stand out, being visible on the undersides of the leaves as grey blotches studded with minute perithecia (Figure 7). These structures are much less numerous on the upper surfaces of the blotches, which are usually straw-coloured.

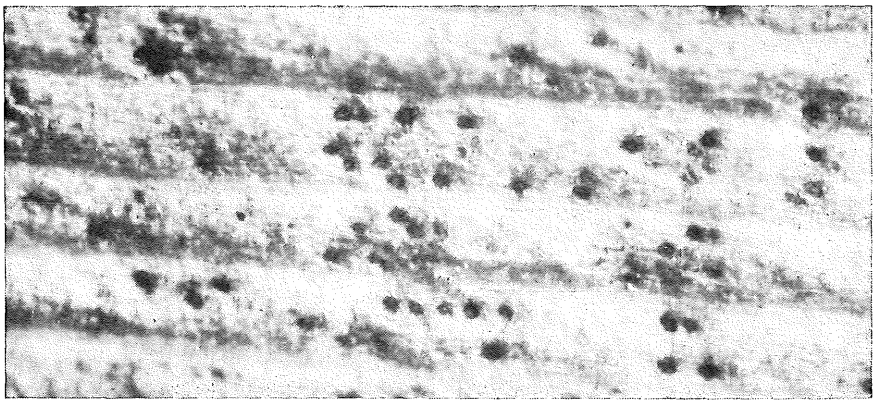

Fig. 7.—Lower surface of leaf of Ducasses banana showing perithecia of *M. musae* (X52).

Except under unusual circumstances, such as on unthrifty plants during humid, rainy weather, visible speckle lesions seldom appear above the fifth or sixth fully opened leaf and extensive necrosis and death of leaf tissue are not often seen above the ninth leaf. However, after a plant has flowered the leaves are killed off successively from the oldest upwards and quite extensive defoliation can result before the bunch is mature.

(d) Etiology

In Queensland the presence of a fungus in speckle-affected tissue was noted by Simmonds (1933). He was able to isolate consistently an organism which produced on potato dextrose agar a mound-like and slowly spreading colony with a grey compact mycelium which may later develop pinkish areas. In addition, he recorded the association of small perithecia of the Mycosphaerella type with "speckle infected areas on leaves which have been dead and dry for some time."

In New South Wales (Anon. 1945) the view was originally held that speckle was a secondary development of the injury caused by red spider (*Tetranychus* sp.). More recently observations in that State have shown that serious infection occurred in a relatively dry season in the apparent absence of red spider (Anon. 1958).

(e) Isolation of the Causal Organism

The variety Ducasses was used as a source of ascospores in order to avoid confusion with *Mycosphaerella musicola.* Spores were obtained by placing pieces of dead leaf tissue containing perithecial material into the lids of 4 in. petri dishes over a shallow layer of moist cotton wool so that when the lids were fitted the pieces of leaf were exposed above either slides coated with plain agar (2 per cent. water agar) or a film of plain agar in the bottom of the dishes. A single-spore isolator (Leach 1955) was used to remove the ascospores.

The material used for tissue isolations was always taken from definite speckled patches on living leaves. Such material was surface sterilized by dipping in a 0·5 per cent. aqueous solution of mercuric chloride/hydrochloric acid (20 g–100 c.c.) for 5 min, then washed with 70 per cent. alcohol and dried. Small pieces of the leaf tissue were crushed and transferred to plain agar in petri dishes. The resulting colonies were sub-cultured to potato dextrose agar. All isolates were cultured on this medium for purposes of comparison.

The "mound-like" fungus colony described by Simmonds was consistently isolated from speckle lesions on the Ducasses, Cavendish and Mons Mari varieties. In addition, a similar fungus was obtained when ascospores ejected from perithecia on dead speckled leaves of Ducasses and Lady Finger plants were used as inoculum. No spores are produced in culture.

(f) Pathogenicity Tests

Inoculations to prove pathogenicity were made in several ways, using vegetative and ascospore material.

(1) *Vegetative Material.*—Small pieces of agar and mycelium were transferred from cultures on potato dextrose agar to cuts made with a sharp scalpel on the lower surface of a leaf along the junction of lamina and midrib. The cuts paralleled the midrib and were essentially slits into which a wad of inoculum was placed.

(2) *Ascospore Material.*—Leaf discs were cut from infected dead leaves of the Ducasses variety showing abundant development of perithecia. These leaf discs were fitted into the lids of 4 in. petri dishes over damp cotton wool as before. The lids were inverted and clamped tightly onto the surface of a leaf with the aid of small G cramps (2 in.). A 4 in. square of $\frac{3}{16}$ in. plywood

pressed against the opposite leaf surface provided a base on to which the lid could be clamped (Figure 8). If the lower leaf surface was inoculated the lamina was inverted so that this surface was uppermost. To provide a check on the identity of spores which landed on the leaf beneath the leaf disc, $\frac{7}{8}$ in. glass coverslips were stuck to the leaf with a small drop of mucilage. When the leaf was uncovered, the coverslip was removed, flooded with cotton blue in lactophenol, inverted and placed on a slide for examination.

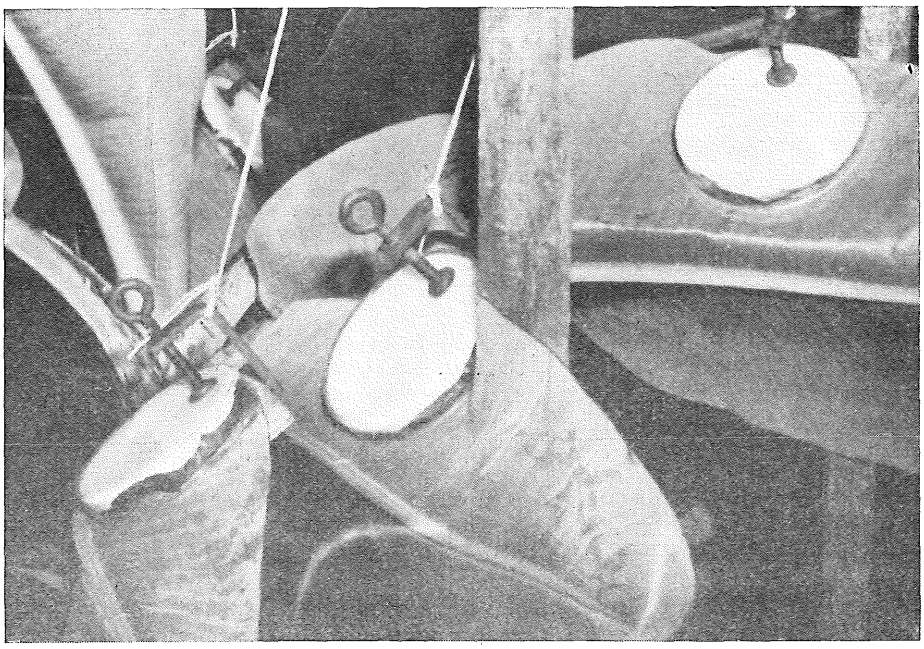

Fig. 8.—Inoculation technique used with *Mycosphaerella musae.*

In an alternative method, ascospores were collected by exposing suitable leaf discs over a very small quantity of water in the bottom of a petri dish. The spore suspensions from a large number of such dishes were combined and atomized onto the inoculation sites with a nebulizer. The suspension was allowed to dry on the leaf, then the inoculation site was moistened by atomizing with sterile water and a petri dish lid was tightly clamped as described above over the inoculated area.

Early experiments were carried out on plants in the open and some confusion was caused by natural infection. To reduce to a minimum the chances of infection from airborne spores, inoculations were carried out subsequently on two Cavendish plants which were growing in a lath-house situated in a sheltered position. The results of some of these pathogenicity tests are summarized in Table 2 and illustrated in Figures 9 and 10.

TABLE 2

Results of Pathogenicity Tests with *Mycosphaerella musae*

Date of Inoculation	Type of Inoculum	No. of Inoculations and Site	No. of Positive Infections	Incubation Period (days)
17–xi–1956 ..	Vegetative. Culture 1101 ex leaf lesion on Ducasses	8 wounds along midrib of youngest expanded leaf (lower surface)	8	102
17–xi–1956 ..	Vegetative. Culture 1112 from ascospores ex Ducasses	8 wounds along midrib of youngest expanded leaf (lower surface) (Fig. 9)	8	102
17–xi–1956 ..	Ascospores collected from Ducasses	Atomized onto four 4 in. dia. circles on youngest expanded leaf (lower surface) (Fig. 10)	1	102
13–xii–1956 ..	Ascospores collected from Ducasses	Atomized onto three 4 in. dia. circles on youngest expanded leaf (lower surface)	2	80
12–iv–1957 ..	Vegetative. Culture 1101 ex leaf lesion on Ducasses	12 wounds along midrib of 8th expanded leaf (lower surface)	5	86
12–iv–1957 ..	Vegetative. Culture 1104 from ascospores ex Ducasses	12 wounds along midrib of 8th expanded leaf (lower surface)	1	86
12–iv–1957 ..	Vegetative. Culture 1234 Re-isolation from positive inoculations made with ascospores 17–xi–1956	12 wounds along midrib of 8th expanded leaf (lower surface)	6	86
21–i–1958 ..	Ascospores from Ducasses	Leaf discs exposed on two sites on each of :—		
		1st expanded leaf, lower	1	83
		2nd expanded leaf, lower	2	
		6th expanded leaf, lower	1	
29–i–1960 ..	Ascospores from Ducasses	Leaf discs exposed on :—		
		1st expanded leaf, lower*	1	98
		1st expanded leaf, upper		
		2nd expanded leaf, lower		
		2nd expanded leaf, upper		
		3rd expanded leaf, lower*	1	
		3rd expanded leaf, upper		
		4th expanded leaf, lower		
		4th expanded leaf, upper		

TABLE 2—*continued*

Results of Pathogenicity Tests with *Mycosphaerella musae*

Date of Inoculation	Type of Inoculation	No. of Inoculations and Site	No. of Positive Infections	Incubation Period (days)
22–iii–1960 ..	Ascospores from Ducasses	Leaf discs exposed on :—		
		1st expanded leaf, lower†	Blotch stage only	45
		2nd expanded leaf, lower†	1	45
		3rd expanded leaf, lower		
		4th expanded leaf, upper†		
		4th expanded leaf, lower†	1	45
		5th expanded leaf, upper		
		5th expanded leaf, lower		

*The inoculation sites marked thus were injured by pricking the lower leaf surface with a battery of pins three weeks after inoculation.

†The inoculation sites marked thus were exposed to a saturated atmosphere each night for the duration of the experiment by covering with a petri-dish lid containing damp blotting paper.

Many of the inoculations made in the course of this work were inconclusive and are not recorded here. Vegetative material planted in wounds very often gave rise to a watersoaked lesion on and around the wound site. Ascospore inoculations similarly in many cases often failed to reproduce typical speckle lesions. Despite this fact, when inoculated leaf surfaces were examined large numbers of germinated spores and a profuse development of epiphyllic mycelia and appressoria could be seen. Often such artificial infections reached the stage where a light-tan blotch became visible on the inoculation site and no further development ensued.

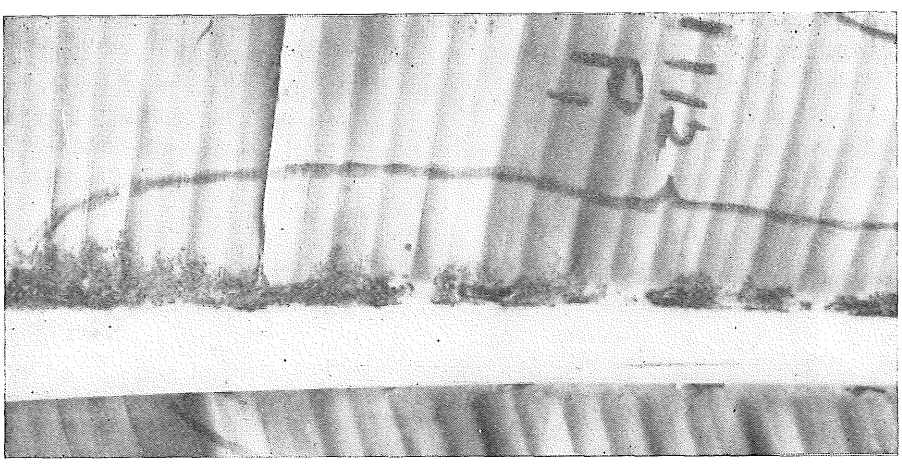

Fig. 9.—*Mycosphaerella musae*. Result of inoculating mycelium into wounds along the midrib of the youngest expanded leaf. Inoculated 17.xi.1956. Photographed 14.iii.1957.

Fig. 10.—*Mycosphaerella musae*. Result of inoculating ascospores onto the lower surface of the youngest expanded leaf. Inoculated 17.xi.1956. Photographed 14.iii.1957.

The typical light-grey fungus producing a mound-shaped colony was usually re-isolated from the lesions produced. However, in the case of re-isolations from inoculations into wounds it was difficult to obtain a culture free from bacterial contamination.

Proof of pathogenicity is considered to be provided by the tests of November 17, 1956, and April 12, 1957, in which it was demonstrated that ascospores collected from speckle lesions on dead leaves of a Ducasses plant produced typical symptoms of the disease on a Cavendish plant. The fungus which was isolated from the lesions on the latter plant in turn reproduced the usual symptoms when it was inoculated into leaf tissue of a Cavendish banana through wounds. In the course of these pathogenicity tests the disease was never reproduced by the inoculation of the upper surface of a leaf with ascospores.

(g) Cultural Characteristics and Morphology

All fungus mounts were made in cotton blue/lactophenol. In order to examine the development of the fungus on the leaf surface two types of preparation were employed. In the first method the leaf portion was placed on a sheet of plate glass with the surface to be examined—usually the lower leaf surface—facing downwards. As much of the mesophyll and vascular strands as could be removed without rupturing the lower epidermis was then taken off by abrading with the blunt side of a scalpel blade. Sample pieces of the tissue remaining, cut to fit under a $\frac{7}{8}$ in. coverslip, were then cleared by warming

in lactophenol and stained in cotton blue. In the second method whole leaf pieces were decolourized by boiling in a solution consisting of equal parts of absolute alcohol and glacial acetic acid or of 5 per cent. lactic acid in absolute alcohol. The leaf pieces were then cleared by heating in lactophenol in a water-bath and stained in cotton blue before mounting in lactophenol; or alternatively, they were transferred from the decolourizing solution to cotton blue in lactophenol and heated for a few minutes before they were mounted in lactophenol.

Transverse sections made to investigate the development of the fungus within the leaf and to show details of the fruiting structures of the causal fungus were cut by hand with a blade razor and stained in cotton blue before mounting in lactophenol. In these preparations mycelia and other fungus structures stained a darker blue than did the leaf tissue.

A colony of the speckle fungus on a potato dextrose agar slope first assumes an irregularly circular outline and a smoothly convex elevation, giving it a characteristic domed shape. The colony consists of a thin layer of compact, light-grey, aerial mycelium on a hard, black, sclerotium-like hump, and its colour is grey when viewed from above and black when seen from below (Figure 3). The aerial mycelium consists of densely aggregated, sparingly branched, septate hyphae not exceeding 3μ in diameter.

As the culture ages its outline becomes more irregular and tends to elongate along the length of the tube. The edges are crenate in places. Droplets of dark-brown or black fluid may exude from any part of its elevated surface. After 6–8 weeks a pink colour develops in the grey surface mycelium. The colony on potato dextrose agar is very similar to that of the related banana leaf spot organism, *Mycosphaerella musicola.*

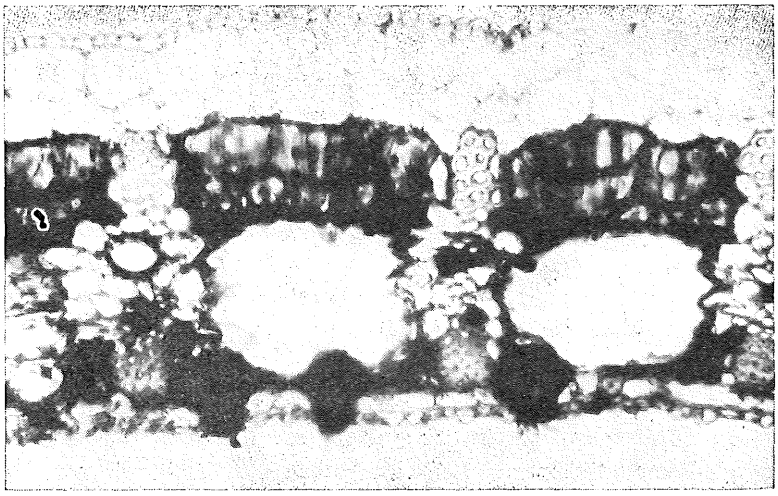

Fig. 11.—Freehand section of banana leaf with perithecia of *M. musae* on lower surface (X120).

No fruiting structures have been noted in culture. On the plant there is no apparent conidial stage and the fungus reproduces by means of ascospores. The absence of a conidial stage is not unusual, for it has been pointed out that some species of *Mycosphaerella* do not possess conidial stages (Wolf and Wolf 1947).

Fig. 12.—Freehand section of banana leaf including a perithecium of *M. musae* (X285).

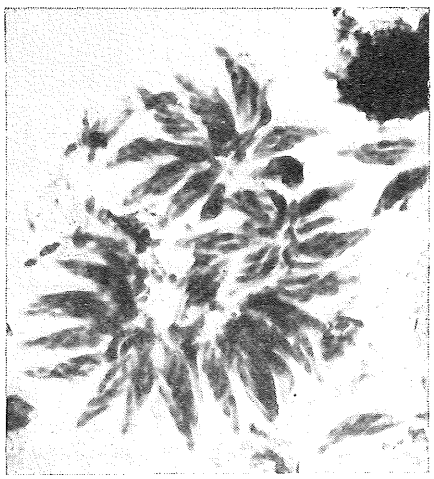

Fig. 13.—Squashed perithecium of *M. musae* showing asci (X285).

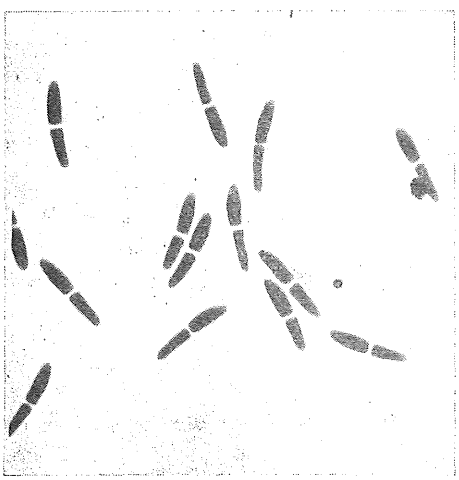

Fig. 14.—Ascospores of *M. musae* (X570).

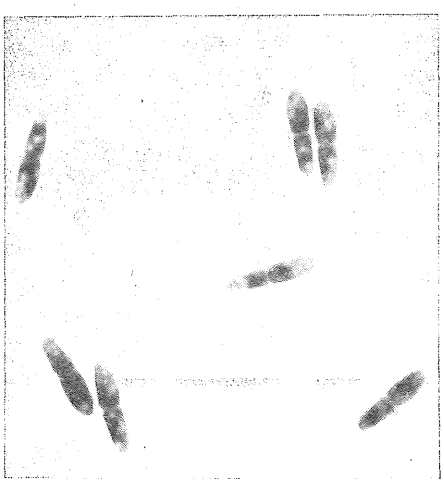

Fig. 15.—Ascospores of *M. musicola* for comparison (X570).

The perithecia are minute (34–99μ dia.), thickly scattered, immersed, sub-globose, ostiolate, black, thin-walled and aparaphysate (Figures 11, 12). The asci are obclavate, measure 24–44μ x 8–12μ and contain 8 ascospores (Figure 13). The ascospores are hyaline, obtuse fusoid or cylindrical, 2-celled with one cell commonly broader than the other; the septum is very prominent and there is no constriction. They measure 8·8–16μ x 2·0–3μ (Table 3 and Figures 14, 15).

TABLE 3

Dimensions of Fruiting Structures of Various Species of *Mycosphaerella* on Banana

Fungus and Authority	Perithecia (μ)	Asci (μ)	Ascospores (μ)
1. *Sphaerella musae* Spegazzini Spegazzini, C. 1909. Ann. Mus. Nac. Buenos Aires XIX, p. 354	80–90	38–40 x 6–8	12–13 x 2
Ciferri, R. and Fragoso, R. G. 1927. Bol. R. Soc. Esp. Hist. Nat. 27 : 165–77			13 x 3
2. *Sphaerella musae* Saccardo Saccardo, P.A. 1917. Notae Myc. XXIII Accad. Veneto-trent, p. 67. (from Sylloge Fungorum 24 : 879)	150–180	45–50 x 7·5–9	10–12 x 2·3–2· 5
3. *Mycosphaerella Musae* (Speg.) Syd. Sydow, H. and P. 1914. Philipp. J. Sci. 8 : 482 ..		35–48 x 12–16	12–15 x 3–4
4. *Mycosphaerella musicola* Leach Leach, R. 1941. Trop. Agriculture, Trin. 18 : 91–5	46·8–72 Av. 61·8	28·8–36 x 8–10·8	14·4–18 x 3–4 Av. 16·7
W. Pont, North Queensland material (Fig. 15)			14·4–19·2 x 3·2–4·8 Av. (50) 16·3 x 3·8
5. *Mycosphaerella minima* Stahel Stahel, G. 1937. Trop. Agriculture, Trin. 14 : 257–64	25–37	24–28 x 10–12	20–22 x 5–6
6. *Mycosphaerella* sp. from Queensland speckle.			
J. H. Simmonds (3 collections)	34–81 x 45–99 Av. (84) 56 x 69·8	24–44 x 8–12 Av. (41) 30·1 x 9·1	10–15 x 2–3 Av. (63) 12·2 x 2·6
W. Pont (2 collections) (Fig. 14)	60–90		8·8–16 x 2–3 Av. (75) 13·2 x 2·7
7. *Mycosphaerella* sp. from Ceylon speckle. Collected by J. H. Simmonds, 1932.			
W. Pont	41–80	28·8 x 9·6	9·6–12·8 x 2·5–3

The spores germinate on the leaf surface. The germ-tubes grow out from both ends of the ascospore, often simultaneously. They are fine structures not exceeding 1·5μ in width. They grow quickly and branch often, giving rise to extensive, equally fine, much-branched epiphyllic mycelia. After a prolonged period of epiphyllic existence, generally of 5–6 weeks' duration, but sometimes shorter or considerably longer, appressoria are formed and the fungus penetrates into the leaf by way of the stomata (Figures 16, 17). The appressoria are stout, brown, lobed or digitate structures which are formed around or on

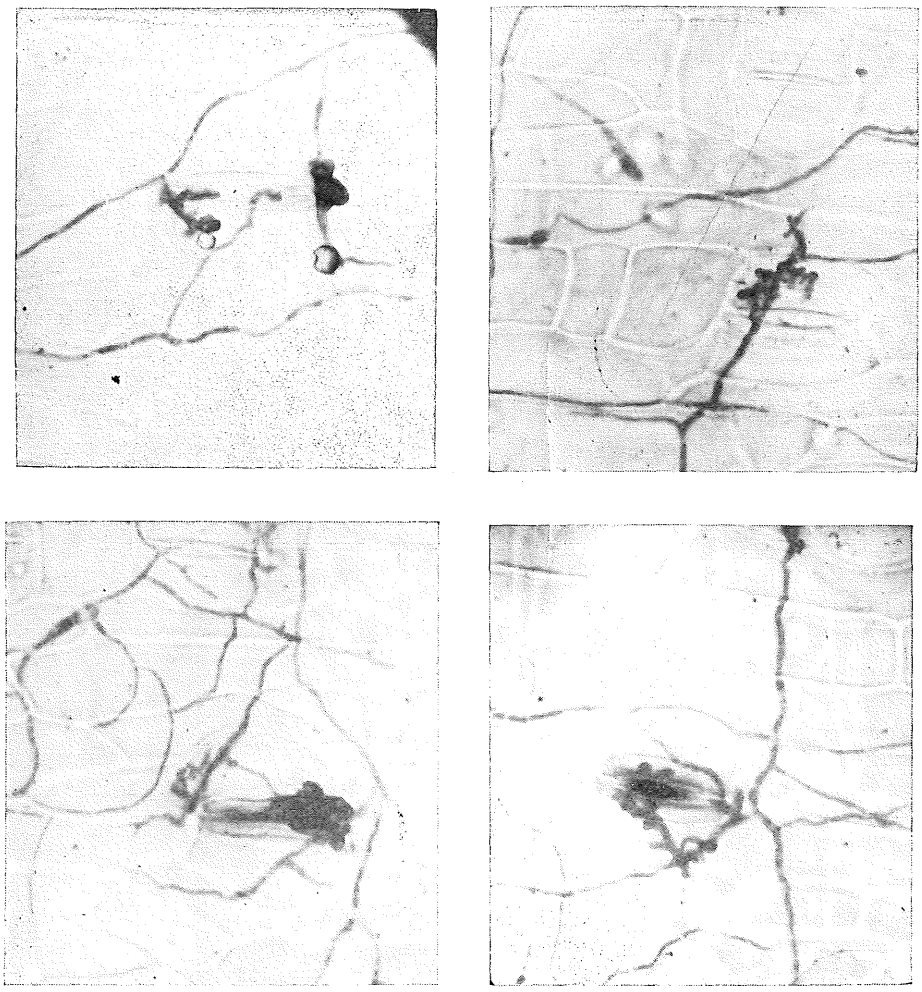

Fig. 16.—Artificial infection with *M. musae*. Germinated ascospores, epiphyllic mycelium and appressoria. Thirty-eight days after inoculating the fifth leaf (X384).

the stomatal pores. At this stage necrosis of the stomatal guard cells commences and they assume a light-brown colour. Stout brown infection tubes 3μ or more in diameter arise beneath the appressoria and penetrate through the pores into the substomatal chambers (Figure 18). The terminal cells of the infection tubes are often rounded or campanulate. From the bases of these infection tubes coarse, hyaline, intercellular hyphae up to 3μ thick grow through the mesophyll and across or along the walls of the air chambers to the palisade tissue. These intercellular hyphae branch as they grow and develop finger-like processes, presumably absorptive in function. The lateral hyphae are finer and may not exceed 1μ in thickness.

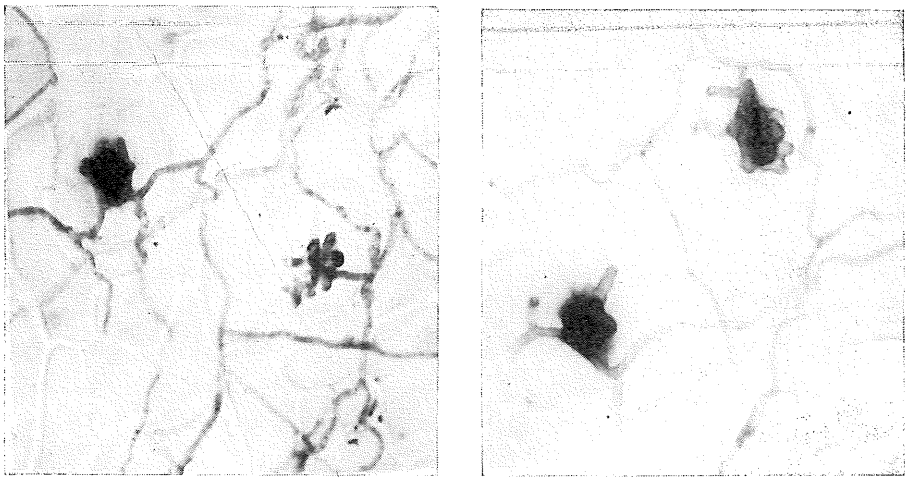

Fig. 17.—Natural infection with *M. musae*. Mount of leaf showing first visible signs of infection (X384).

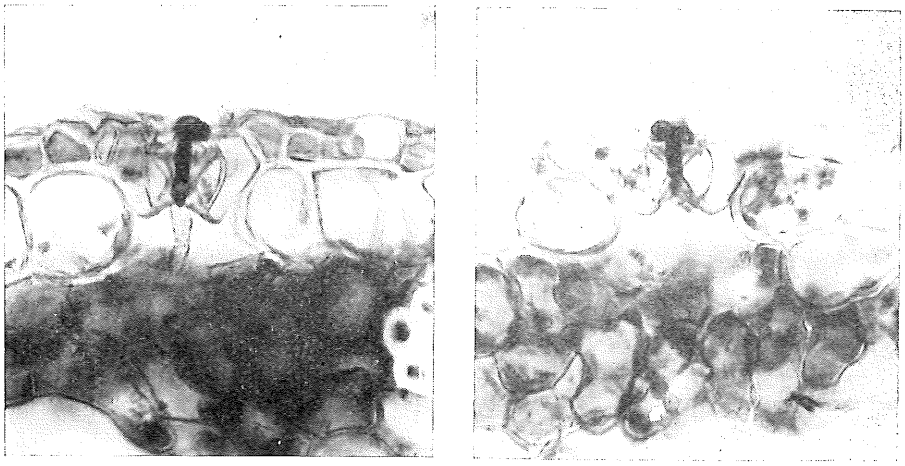

Fig. 18.—Freehand sections of banana leaf showing first stage of penetration by *M. musae* through stomatal pore (X384).

With the formation of the appressoria the epiphyllic hyphae become thicker and darker in colour. They may measure up to 3μ dia., especially close to the appressoria.

(h) Growth-Temperature Relationships

In addition to observations on growth-temperature relationships in culture made according to the method previously described and graphed in Figure 4, the effect of temperature on germ-tube growth was investigated. Using the

technique described above, leaf discs containing perithecial material were suspended above slides coated with plain agar. Single petri dishes were then placed in each of a number of chambers in the multi-temperature range incubator to give a number of temperature regimens ranging from 12°C to 37°C. The dishes were incubated for three days and at the end of this period the slides were flooded with cotton blue, covered with oblong coverslips and examined. Fifty germ-tubes were measured on each slide. The length was expressed in microns.

Two such experiments were conducted and the results are shown in graph form in Figure 19.

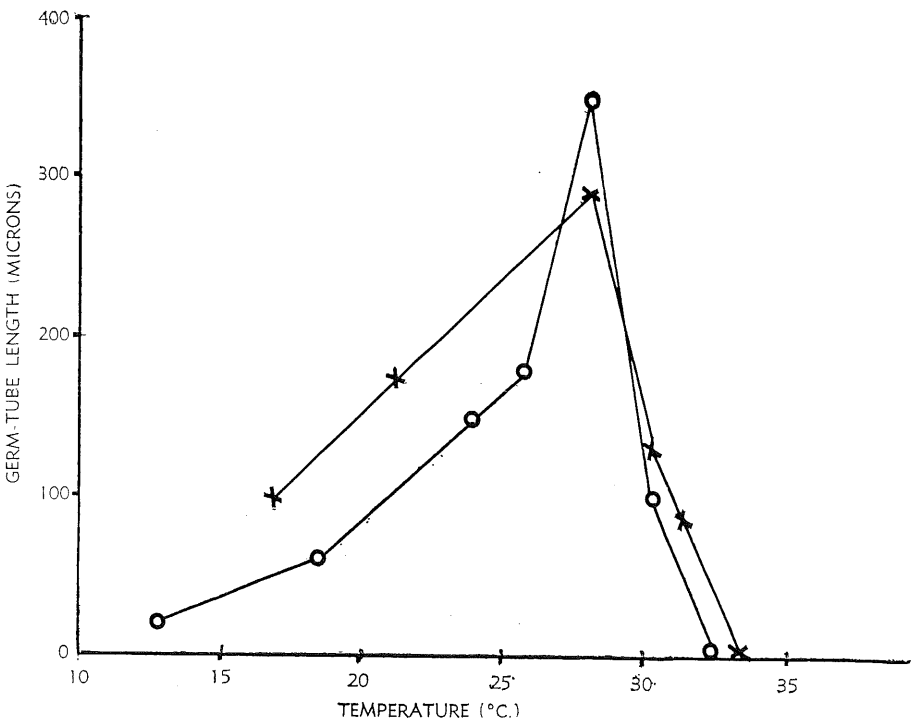

Fig. 19.—*Mycosphaerella musae.* Effect of temperature on germ-tube development. Three days' incubation on potato dextrose agar.

Examination of Figure 4 shows that while the optimum temperature was close to 26°C (79°F) the fungus grew well over the range 20°–30°C (68°–86°F). Growth was negligible above 33°C and below 10°C. The optimum temperature for germ-tube growth was in the vicinity of 27°C. There was no spore germination below 12°C or above 32°C, although ascospores were ejected from some of the leaf discs incubated at temperatures beyond these limits. The lack of germination in these cases may, however, have been due to belated discharge of the spores.

A comparison of the growth-temperature curve for *Mycosphaerella musae* (Speg.) Syd. with that for *M. musicola* Leach as determined by Simmonds (1933) (Figure 4) shows that the temperature requirements of these two species are very similar.

(i) Identity of the Speckle Fungus

The immersed, aparaphysate perithecia and the 2-celled, hyaline, cylindrical or obtuse fusoid ascospores place the banana leaf speckle fungus within the genus *Mycosphaerella.*

Several Mycosphaerella species have been recorded on the banana plant. *Sphaerella musae* Speg. was first described by Spegazzini (1909, p. 354). associated with an indefinite spotting on decaying foliage of *Musa sapientum* in Argentine. Spegazzini's description of the symptoms—"maculae quandoque plane nullae quandoque subparvae cinerascentes indeterminatae"—is significant in the present connexion.

H. & P. Sydow (1914) recorded *Mycosphaerella musae* (Speg.) Syd. on dying leaves of *Musa sapientum* in the Philippines. They were apparently somewhat doubtful of the identity of the Philippine material with Spegazzini's species, as the asci and ascospores were both considerably broader. (Measurements kindly made by E. W. Mason from one collection of Philippine material held by the Commonwealth Mycological Institute were similar.) Later, however, Saccardo (1917) described *Sphaerella musae* Sacc. on dying stems of *Musa coccinea* from the Philippines. The spots were "vagis, expallescentibus, indeterminatis" and the asci and ascospore measurements more closely resembled Spegazzini's description.

Reinking (1918), in discussing Philippine plant diseases, described and illustrated a leaf spot on *Musa sapientum* and *Musa textilis* Nee ascribed to *Mycosphaerella musae* Speg. No spore measurements were given and the illustration suggests a circumscribed spotting only rarely seen in the Queensland disease.

Sphaerella musae Speg. was recorded again from South America on living banana leaves by Ciferri and Fragoso (1927).

Stahel (1937) found perithecia of a fungus he described as *Mycosphaerella minima* in association with other fungal flora on the dry centres of Cercospora leaf spots (*Cercospora musae* Zimm.) on bananas in Surinam. *Mycosphaerella musicola* Leach, the perfect stage of *Cercospora musae* Zimm., was later identified in Jamaica (Leach 1941) and has since been found in other countries, including Queensland (Figure 15).

In Table 3 the dimensions recorded for fruiting structures of the various species of *Mycosphaerella* mentioned above are presented. When these comparative data are considered it appears that the Queensland organism could well be referred to Spegazzini's species. This evidence is substantiated further by the fact that the Queensland *Mycosphaerella* is not associated with margined leaf spots and in this agrees with Spegazzini's description. In this these two fungi differ from the other *Mycosphaerella* species recorded on banana with the exception of *Sphaerella musae* Sacc. and there appears to be justification for assuming that *Sphaerella musae* Sacc. is synonymous with *Sphaerella musae* Speg. There is still uncertainty regarding some of the Philippine records of this species.

The tentative identification of the Queensland speckle fungus was confirmed by E. W. Mason of the Commonwealth Mycological Institute, who advised that as the data established for the Queensland species completely covered Spegazzini's original description he was disposing of the fungus as *Mycosphaerella musae* (Speg.) Syd.

The conidial fungus *Phyllosticta musa-sapientii* Frag. et Cif. was recorded in association with *Sphaerella musae* Speg. on flaccid or dry leaves of *Musa sapientum* in Santo Domingo (Ciferri and Fragoso 1927). The spores of this fungus were described as hyaline, cylindrical, sub-bacillar and 3–4.5μ, x 1–1.3μ. The pycnidia were 60–110μ in diameter.

During the investigations reported herein it was found that dense clusters of small spore-like bodies could always be obtained together with *Mycosphaerella* ascospores from dead speckle-infected banana leaves (Figure 20). The shape and size of these bodies were variable. They ranged from obtuse oblong rods measuring 4–5μ x 1–2μ to sub-globose spores with a diameter of 5–6μ. The rod-shaped cells were biguttulate. On odd occasions small flask-shaped or depressed globose sacs of the same approximate size as perithecia (approx. 80μ dia.) were seen in sections of diseased leaves, but only on the upper surfaces. These contained dense masses of irregularly oblong bodies measuring no more than 4μ in length. While these structures bore some resemblance to the pycnidia and conidia of *Phyllosticta musa-sapientii* Frag. et Cif., germination was never observed on plain agar under conditions which resulted in good germination and germ-tube growth of ascospores. It has therefore been assumed that they were spermatia and spermogonia.

Some basis for this assumption has been provided by the remarks of Wolf and Wolf (1947): "Some species do not possess conidial stages, for example,

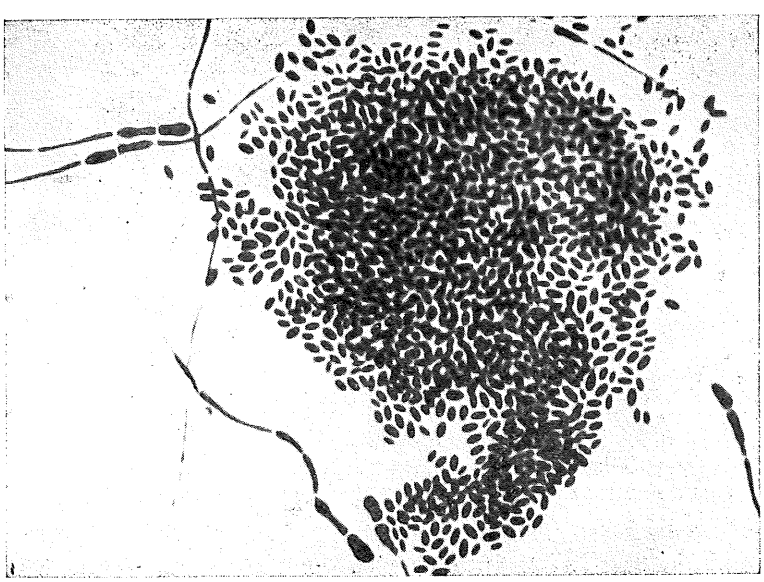

Fig. 20.—Group of spermatia and germinating ascospores on plain agar. (X384).

Mycosphaerella fraxinicola [Wolf (1939)] and *M. nyssaecola* [Wolf (1940, 1940a)]. *Mycosphaerella fraxinicola* is associated with *Phyllosticta viridis* and *M. nyssaecola* with *P. nyssae;* both of these species of Phyllosticta proved to be not conidial stages but spermogonial stages".

(j) Dissemination and Infection Site

The ascospores are airborne and are emitted when leaf tissue containing perithecia is wetted. It has been proved by inoculations (see above) and by experiments with fungicides (see below), in which comparisons were made of the control obtained by spray treatment of upper and lower leaf surfaces, that infection originates on the under-side of a leaf. Inoculations have shown that leaves older than the first expanded leaf can be infected and this is evidently quite common under natural conditions. However, examination of leaves on plants exhibiting speckle lesions on living and dead lower leaves always shows a large number of spores of *M. musae,* germinated or otherwise, on the first leaf and it has been assumed that some of the infection on a plant can originate from spores which alighted on this leaf.

(k) Epidemiology

Unlike Cercospora leaf spot, the development of this disease is not inhibited by shading. In North Queensland it is the usual case that if leaf spot is present in a plantation Mycosphaerella speckle is relatively inconspicuous. This is due to the prolonged incubation period of the latter disease and the masking of its symptoms by those of the more virulent leaf spot. However, when plantations are situated within rain-forest, Mycosphaerella speckle, together with those caused by *Chloridium musae* and *Ramichloridium musae,* is often quite noticeable on plants around the edges in the shelter of the surrounding trees, where *Mycosphaerella musicola* does not thrive. As a general rule the speckle diseases are always more prevalent in sheltered spots or in hollows or wet spots in a plantation, that is, where air movement is at a minimum and humidity is high. Mycosphaerella speckle has been observed to behave similarly in South Queensland (Simmonds 1933). In contrast to the tropical speckles, however, *M. musae* can develop quite well in exposed situations.

The factors which appear to influence speckle development are considered below.

(i) Humidity

The influence of high atmospheric humidity is quite evident under natural conditions during rain periods or early in the morning following heavy dews when a rapid watersoaking of infected tissue precedes the appearance of new lesions or extension of existing lesions. The effect of favourable humidity was shown experimentally by the results of the infection experiment (inoculated March 22, 1960) summarized in Table 2. Typical speckle lesions were reproduced within 45 days on two of the leaves inoculated on the lower surface

and enclosed each night for the duration of the experiment in a saturated atmosphere. This is much quicker development than has been observed to occur without saturation, e.g. speckle lesions from the successful inoculation made on November 7, 1956, did not show until 102 days later; while the minimum incubation period recorded (inoculated December 13, 1956) was 80 days.

(ii) Temperature

The temperature relationships of the causal fungus have been previously commented on. It is plain that as temperatures fall below 70° F there is an increasing retarding effect on the development of the disease.

(iii) Plant Vigour

Leaf damage is always more severe on backward plants. This may be because the growth rate of the plant as measured by the rate of extrusion of leaves determines the relative amount of damage on a plant. While the host is growing quickly, leaf development keeps ahead of the disease. When the growth rate is slow, the effects of speckle infection are more evident because the ratio of healthy leaf surface to speckled leaf surface is decreased due to the slower rate of leaf extrusion. However, there may also be physiological differences in backward plants which accelerate the rate of development of the disease.

(iv) Leaf Senescence

When the senescence of a leaf is accelerated by removing it from the plant, speckle development is also accelerated. This is particularly noticeable if a young leaf not showing visible speckle symptoms is removed from a speckle-infected plant and kept indoors for several days. The growth of the fungus is expedited so much under these circumstances that it is usual for lesions to appear within two or three days of removal. On vigorous plants speckle damage is confined to the basal leaves and serious defoliation does not occur until after vegetative growth has ceased. In the course of the inoculation experiments it was noted that when young leaves were inoculated the disease developed within about six weeks to the brown blotch stage; development was then apparently suspended until the inoculated leaf was well down the plant.

(v) Injury

Speckle development on a leaf often shows first around an injury, e.g. where the lamina has been torn by wind or some other agency. The results of the inoculations made on January 29, 1960 (Table 2) suggest that damage to the inoculation site in the form of pin pricks has resulted in quicker development of speckle. It seems possible, therefore, that injury resulting from red spider infestation may expedite the development of speckle lesions.

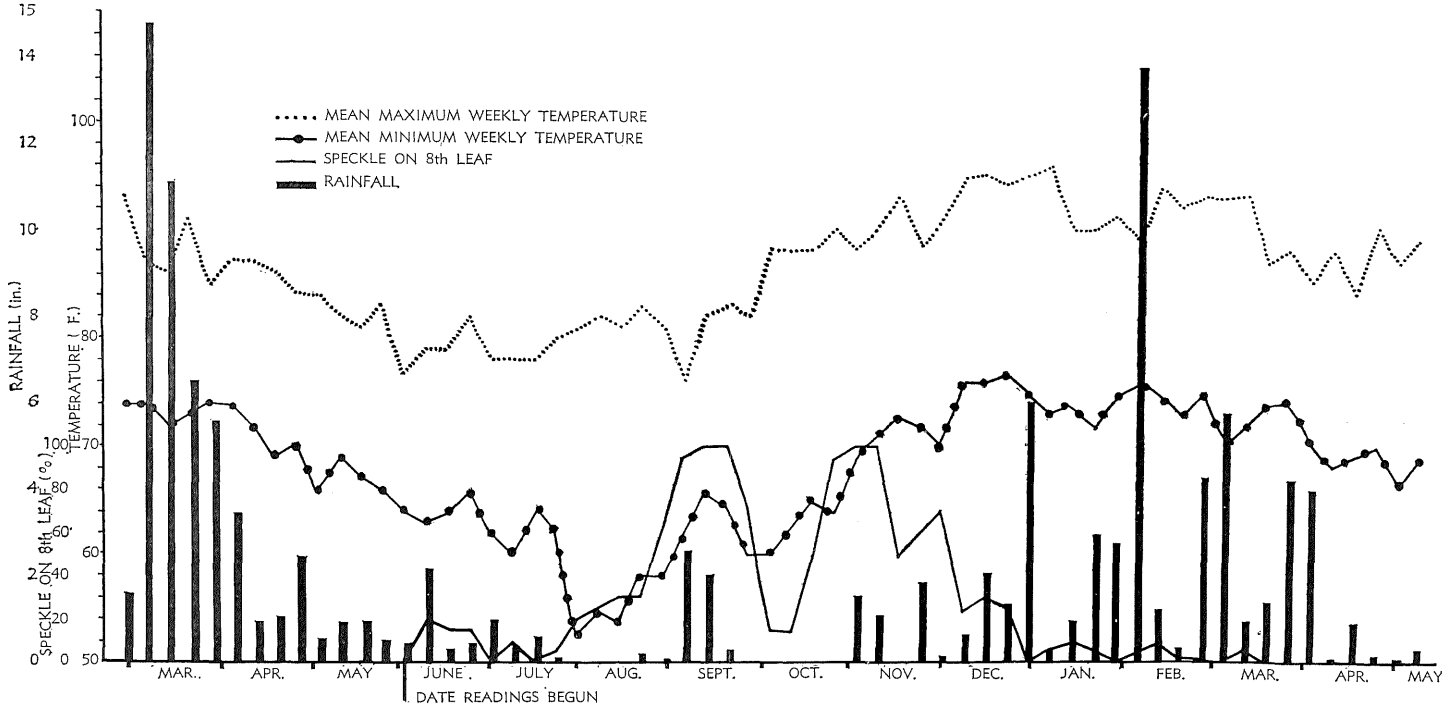

Fig. 21.—*Mycosphaerella musae*. Weekly record of amount of infection on the eighth expanded leaf of Ducasses plant, Kamerunga, 1959-60.

(vi) Interaction

In North Queensland, Mycosphaerella speckle is present and causes leaf loss throughout the year. There is, as would be expected, a noticeable build-up during the wet season in the late summer and autumn. However, growth rate of the plant is also at a maximum at this time of the year due to the favourable environment, and damage is usually relatively no greater than during the dry cool weather in late winter and spring when the plants are growing very slowly.

This is illustrated in Figure 21 by plotting the percentage leaf surface affected by speckle each week on the 8th fully expanded leaf at that time on a Ducasses plant growing in the open at Kamerunga, near Cairns. Mean weekly maximum and minimum temperatures and the weekly rainfalls during the period are also plotted.

The growth rate of the observation plant was very slow during late July, August and early September due to cool, dry weather during July and August. The amount of speckle showing on the 8th leaf rose from July onwards and reached a maximum in September. Due to the September rain and higher temperatures the growth rate increased and the amount of speckle damage on the 8th leaf dropped rapidly. It rose again in late October and early November when the growth of the plant once again slackened following dry, cool weather in late September and October. In December, plant growth quickened due to higher temperatures and improved rainfall and the speckle damage on the 8th leaf at that time was considerably reduced. A low incidence of speckle on the 8th leaf was sustained during January, February, March and April while the plant was putting on new leaves at a much faster rate.

These observations indicate that the inhibiting effects of low temperatures and dry weather on speckle development are offset during the North Queensland dry season by the increased susceptibility of the senescent basal leaves.

(l) Control

(i) General

In New South Wales, spray treatment each month from early December till March with copper oxychloride (1½ lb to 40 gal) plus wettable sulphur (1 lb to 40 gal) was recommended for the control of leaf spot and speckle (Anon. 1945). The sulphur was presumably included for the control of the red spider mite.

Experimental work in North Queensland later showed that spraying with copper oxychloride gave control of both diseases equal to that obtained with copper plus sulphur and a control schedule consisting of monthly applications of copper oxychloride (1½ lb to 40 gal) plus a wetting agent was recommended (Anon. 1953).

Further work in North Queensland led to the recommendation of either copper fungicides such as Bordeaux mixture (3-2-40) and copper oxychloride (1½ lb-40 gal) or zineb (1½ lb-40 gal) plus white oil (1-160) and malachite green (1-10,000) for leaf spot control (Pont 1960). In experimental work these mixtures were applied at intervals of four weeks as high-volume sprays (pressure 250 lb/sq. in.; dosage up to 200 gal/ac) to the lower surfaces of the young foliage and controlled speckle in addition to leaf spot.

While the Departmental recommendations for leaf spot control were being adopted by North Queensland growers, a modified method of application, employing reduced volume (dosage 50–60 gal/ac) without increased concentration (that is, 2 lb fungicide per acre), was introduced in many cases in order to expedite treatment, particularly on large plantations. In this method the mixture was misted down on to the plants from towers towed behind tractors. Good leaf spot control was obtained in this fashion but control of Mycosphaerella speckle was far from satisfactory.

Experiments in North Queensland with low-volume application (2 gal/ac or less) of copper oxychloride/oil mixtures to bananas showed that reasonable control of leaf spot could be obtained with this method of treatment but here again speckle control was inadequate (Pont 1960).

In New South Wales, where low-volume misting of bananas with oils is recommended for leaf spot and speckle control, oils alone were reported to be ineffective in the control of speckle (Anon. 1958, p. 21) and the inclusion of oil-miscible copper fungicides or zineb with oils also failed to give consistent control of the disease (Anon. 1959, p. 26). Recent New South Wales recommendations advocate the use of fungicide/oil mixtures in districts where speckle is usually a serious problem but some uncertainty evidently exists as to the degree of control which can be expected (Blake 1960).

(ii) Copper Residues in Relation to Speckle Control

The failure of overhead spraying and misting or fogging with copper oxychloride/oil mixtures to control Mycosphaerella speckle in North Queensland was considered to be due to an insufficient deposit of copper on the lower surfaces of older, still susceptible foliage. The following experiment was therefore carried out to compare the copper deposits immediately after the application of these various treatments. The residues after a period of weathering were also determined. The high-volume treatment of the lower surfaces of young foliage

which had been shown in experimental work to control speckle was included as a standard. The methods of application, dosage and formulae were as follows:

Treatment	Gal/Ac	Copper Oxychloride	Water	Oil
		(lb)	(gal)	(gal)
(*a*) High-pressure, high-volume spraying of undersides of heart-leaves. ("Edgell" adjustable nozzle)	200	1½	40	..
(*b*) Overhead spraying (Twin "Rega Cyclone" nozzle)	50–60	1½	40	..
(*c*) Low-volume misting ("Swingfog S.N.6." Misting attachment, 1·1 Flow Control Jet) ..	2	6½	..	5½
(*d*) Low-volume fogging ("Swingfog S.N.6." Fogging attachment, 1·0 Flow Control Jet) ..	¾–1	12	..	4

The residual copper on the leaf was analysed by the Chemical Laboratory of the Division of Plant Industry, using the following series:

(1) First expanded leaf; sampled immediately after treatment. Air-dried.

(2) Fifth expanded leaf; sampled immediately after treatment. Air-dried.

(3) First expanded leaf at time of spraying, sampled later when in fifth leaf position. Air-dried.

(4) Similar to (3), only prepared so that the deposits on upper and lower leaf surfaces could be analysed separately. Green leaf samples. Surfaces washed with 2N H_2SO_4 and worked with a small brush to remove deposited copper.

In all cases the samples were obtained by cutting the leaves into discs 9·2 cm in diameter. Treatments were replicated six times. Each plot consisted of a single leaf.

The spray deposit on leaves in Series 3 and 4 was exposed to weathering for 8 weeks, during which time 0·69 in. of rain fell on 7 wet days. Heavy dews were frequent.

The results are summarized in Table 4.

TABLE 4

Copper Residues Analyses (Mean Values)

Treatment	Total Leaf Cu (p.p.m.)			μg Cu per sq. cm leaf	
	Series 1 Leaf 1. Immediate	Series 2 Leaf 5. Immediate	Series 3 Leaf 1. After 8 weeks	Series 4 Leaf 1. After 8 weeks	
				Upper	Lower
(*a*) High-volume, Heart-leaf	2,117	563	997	2·98	10·18
(*b*) Reduced Volume, Overhead ..	711	853	247	1·13	0·45
(*c*) Low-volume, Misting	217	179	50	0·12	0·08
(*d*) Low-volume, Fogging	82	108	60	0·08	0·03

The far greater fungitoxic residue provided by high-pressure spraying with the jet directed at the lower surfaces of the youngest leaves was apparent in the means for leaf copper for Series 1. The Series 2 figures showed that overhead spraying gave a greater residue on what was the fifth leaf at time of spraying. However, this would have been almost entirely located on the upper surface of the leaf, where its value for speckle control would have been negligible. The value of 563 p.p.m. leaf copper for high-volume heart-leaf spraying in this series indicated that fall-out during spraying deposited more copper on the leaf than did either misting or fogging with oil-based concentrate.

The amount of copper remaining on the first leaf after 8 weeks' exposure (Series 3) was far greater for the high-volume treatment than for any of the other three and the detailed analyses (Series 4) revealed that over three times as much copper remained on the lower leaf surface as on the upper. The amount of copper recovered from the lower surfaces of leaves which had been overhead sprayed, misted or fogged and similarly exposed for 8 weeks was negligible in comparison. In the case of these three treatments there was more residue on the upper surface of the leaf than on the lower.

It appears therefore that the unsatisfactory control of speckle obtained with the last three methods of application may be due to the fact that the amount of fungicide deposited on the under-sides of the older leaves is negligible. In addition, the fungitoxic residues on the lower surfaces of the youngest leaves are reduced by weathering to such an extent that infection by the speckle fungus at a later date when the leaf is further down the plant is not prevented.

(iii) Experiment with Protectants

The success of a mixture containing copper oxychloride, white oil emulsion and malachite green for banana leaf spot control led to an investigation of the control of *Mycosphaerella musicola* obtained with the ingredients of this mixture

used either singly or in pairs. This work showed that white oil emulsion alone and white oil emulsion/malachite green controlled the disease equally as well as the complete mixture (Pont 1960). It was necessary to know the efficacy of these sprays against *Mycosphaerella musae* and this aspect of their fungitoxicity was investigated in a series of screening trials conducted during 1958 and 1959. The fungicides zineb and phenyl mercuric chloride and the misting oil "Mobil Orchard Oil No. 1," which is typical of a large number of similar products with high unsulphonatable residue being used in Australia and overseas for leaf spot control, were also screened in these trials.

Plants of the variety Ducasses were employed in these tests because of their immunity to leaf spot (*M. musicola*). Details of procedure are given below.

(1) *Trial 1.*—The youngest fully expanded leaf on each plant was sprayed and each week for 10 weeks any freshly expanded leaves were treated. The results were based on the amount of infection which subsequently appeared on these leaves until the time of their death. Single-plant plots were used and there were three replications of four treatments.

(2) *Trial 2.*—The four youngest fully expanded leaves on each of two plants were treated. The treatments were applied with a nebulizer to circles 4·5 cm in diameter marked on the leaf surface with a "Chinagraph" pencil. The five treatments employed were replicated five times on each of the four treated leaves on each plant, giving 10 replications for each leaf position.

(3) *Trial 3.*—The sprays were applied to circles as before located on the five youngest expanded leaves on each of four plants. The treatments were replicated twice on each leaf to give 8 replications of 6 treatments for each leaf position.

(4) *Trial 4.*—The treated circles were randomized on each of the six youngest expanded leaves of four plants. Treatments were again applied with a nebulizer and were replicated twice on each leaf to give 8 replications of 4 treatments for each leaf position.

The efficacy of the treatments was assessed by a visual appraisal of the percentage of the surface infected with speckle on a sprayed leaf or within a treated circle and from these data means for each treatment were calculated. The results are summarized in Table 5.

A single application of either copper oxychloride or copper oxychloride/white oil emulsion/malachite green on the lower surface gave good control of speckle, and copper oxychloride was efficacious even when the fifth and sixth leaves were treated. Zineb also was very effective. White oil emulsion was slightly inferior to these two fungicides. Phenyl mercuric chloride was inferior to copper oxychloride and was roughly as effective as "Mobil

TABLE 5

Combined Results from Fungicide Screening Trials
(Mean Percentage Speckle Infection)

Trial No.	Treatment	Leaf Position when Treated and Surface											
		1		2		3		4		5		6	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
1	Copper oxychloride (1½–40)	0·6	74										
	White oil emulsion (1–160)/malachite green (1–10,000)	9·3											
	Untreated	94	94										
2	Copper oxychloride (1½–40)/white oil emulsion (1–160)/ malachite green (1–10,000)	1		0		0		10					
	White oil emulsion (1–160)/malachite green (1–10,000)	2		0		1		22					
	White oil emulsion (1–160)	1·5	25	3	18	9	53	38	71				
	Untreated	26	26	16	16	51	51	83	83				
3	Copper oxychloride (1½–40)	0		0·7		0·2		8·3		36·2			
	Zineb (1½–40)	4·3		7·1		0·1		7·8		47·5			
	White oil emulsion (1–160)	2·8	43	12·7	45·1	1·6	54·3	20·7	90·6	76·2	86·2		
	Malachite green (1–10,000)	28·1		27·1		8·3		50·0		87·5			
	White oil emulsion (1–160)/malachite green (1–10,000)	3·4		13·8		1·5		25·6		75·6			
	Untreated	57·5	57·5	62·0	62·0	77·5	77·5	90	90	91·8	91·8		
4	Phenyl mercuric chloride (0·1%)	27·5		25·7		1·3		15·8		14·5		10	
	Copper oxychloride (1½–40)	0		0		0		0·7		2·1		2·7	
	" Mobil Orchard Oil No. 1 "	5		25·6		4·8		22·6		30·8		27·5	
	Untreated	59·3		58·1		93·1		80·6		90·6		90·6	

Orchard Oil No. 1," Malachite green was worse than any of the above sprays and the efficacy of a white oil emulsion/malachite green mixture was equivalent to that of the white oil alone.

Both copper oxychloride and white oil emulsion were ineffective when applied to the upper surface.

The efficacy of copper oxychloride when applied to the fourth, fifth and sixth leaves is of particular interest as it indicates that effective penetration of the fungus had not been obtained at this stage.

(iv) Experiments with Eradicants

Phenyl mercuric chloride (0·1 per cent.) was shown to prevent the development of perithecia in apple leaves infected with *Venturia inaequalis* (Hutton 1954) and a pre-leaf-fall eradicant spray with this chemical was later found to control the disease (Anon. 1956).

Pentachlorphenol (0·75—0·2 per cent.) has recently been found to prevent ascospore discharge from mature perithecia of *Mycosphaerella pinodes* on pea straw (Carter 1959). The contents of the perithecia were completely destroyed by this treatment. Phenyl mercuric chloride (0·1 per cent.) failed to destroy mature perithecia of this fungus.

The evergreen habit of the banana plant when considered in relation to the fact that the leaf speckle disease is active throughout the year places limits on the practicability of eradicant spraying for the control of *Mycosphaerella musae*. Nevertheless, two possibilities appear to exist, viz. (1) regular spraying with a fungicide which has eradicant as well as protectant properties, and (2) regular detrashing followed by ground spraying as an adjunct to the usual schedule of protectant sprays. In many instances normal plantation management calls for the regular application of weedicides and it would be preferable if the chemical employed as a ground spray had eradicant as well as weedicidal properties.

Some preliminary trials were undertaken as a first step in the assessment of the feasibility of eradicant spraying for banana leaf speckle control. These are discussed below.

(1) *Trial* 1.—This was designed to investigate the effect of phenyl mercuric chloride on the development of perithecia. Heavily infected leaves on Ducasses plants were sprayed on both surfaces with phenyl mercuric chloride (0·1 per cent.) while still alive and prior to the formation of perithecia. When the leaves were dead, leaf discs were cut from them and from comparable untreated leaves. These discs were placed on damp cotton wool in the lids of 4 in. petri dishes and exposed for three days at 80°F over ten $\frac{7}{8}$ in. cover slips located in the bottom of the dishes. Seven dishes were allowed for each treatment. Three cover slips were selected at random from each of the seven dishes for examination. They

were mounted on slides and scanned with the low-power objective of a microscope. Ratings were given for density of ascospore discharge using the following scale:

1—Spores in scattered groups with a density not exceeding 10 spores within the area bounded by the length and breadth of a micrometer eyepiece scale (approx. 0·18 sq. mm).

2—Scattered groups. Density 11—20 spores.

3—Scattered groups. Density 21—30 spores.

4—Spores in aggregated groups. Density 31—40 spores.

5—Aggregated groups. Density 41—50 spores.

The mean values for the ratings for the two treatments were phenyl mercuric chloride (0·1 per cent.) 0·4, untreated 1·5.

(2) *Trial* 2.—The object of this experiment was to determine the effect of chemical treatment on ascospore discharge from mature perithecia. Dead leaves from Ducasses plants were sprayed thoroughly on their lower surfaces. Selected circular areas smaller in diameter than a $\frac{7}{8}$ in. coverslip and containing dense aggregations of perithecia were excised, pinned to small pieces of blotting paper and fixed in position on moist cotton wool in the lids of 4 in. petri dishes so that each leaf circle was directly over a coverslip in the bottom of the dish. The five treatments were randomized in each of five dishes. After three days' incubation at 80°F the coverslips were removed and rated for ascospore density as above.

Treatments and results were as follows:

Treatment	Mean Rating
Copper oxychloride (1½–40)	3·2
Phenyl mercuric chloride (0·1 per cent.)	3·2
Malachite green (1–10,000)	2·4
White oil emulsion (1–160)	2·8
Unsprayed	3·2

(3) *Trial* 3.—The main purpose of this trial was an investigation of the eradicant properties of sodium pentachlorphenate, which is a widely used weedicide. Phenyl mercuric chloride was included for comparison purposes and a new eradicant spray Cyanamid "Cyprex" (65 per cent. n-dodecyl guanidine acetate) was also tested.

Leaf discs containing mature perithecia were selected from dead leaves on Ducasses plants, sprayed on their lower surfaces, dried and then fitted into the lids of petri dishes on damp cotton wool with their lower surfaces exposed. There were six replications of each treatment.

The plates were incubated for three days at 80°F. At the conclusion of this period the bottoms of the dishes were examined with the low-power objective of a microscope and spore counts were made. In doing this two traverses of each plate were made along diameters at right angles. At seven points along each traverse the numbers of spores contained within the length and breadth of a micrometer eyepiece scale were counted. Treatments and results were as follows:—

Treatment	Mean Spore Count
Sodium pentachlorphenate (0·2 per cent.) ..	0
"Cyprex" (8 lb–100 gal)	1
Phenyl mercuric chloride (0·1 per cent.)	7·2
Unsprayed	12·8

(4) *Trial* 4.—In this experiment the eradicant properties of sodium pentachlorphenate were compared with those of an arsenical weedicide (56 per cent. w/v As_2O_3) commonly employed in banana plantations in Queensland. The effect of treatment of upper *v.* lower leaf surface was also investigated. The procedure followed was similar to that used in Trial 3. Leaf discs from dead speckled leaves of the Cavendish variety were treated. The lower surfaces of the treated leaf discs were exposed in all cases. There were four replications of each treatment. The petri dishes containing the leaf discs were incubated for 24 hr at 80°F.

This experiment was repeated in Trial 5, using five replications of the same treatments. The results of both trials are given below:

Treatment	Mean Spore Count	
	Trial 4	Trial 5
Sodium pentachlorphenate (0·2 per cent.)—		
Lower surface	0·1	0
Upper surface	11·8	0·8
Arsenical weedicide (1 gal–50 gal)—		
Lower surface	3·3	0·4
Upper surface	5·0	1·7
Unsprayed	13·2	6·8

The indications from these trials were that phenyl mercuric chloride inhibited subsequent ascospore discharge when leaves in which the fruiting structures had not developed were treated, but had much less effect on spore discharge from leaves in which the perithecia were mature when the spray was applied. Sodium pentachlorphenate almost completely inhibited ascospore discharge from mature perithecia when sprayed onto the lower leaf surface. "Cyprex" was also effective in this respect but it should be borne

in mind that the concentration employed, viz. 0·52 per cent. active ingredient, was 10 times as strong as that normally employed as an eradicant spray for apple black spot control.

Treatment of the upper leaf surface with sodium pentachlorphenate was not so effective. The majority of the perithecia of *Mycosphaerella musae* open to the lower leaf surface. These results suggest, therefore, that the chemical or its vapour penetrates into the perithecia through the ostioles.

An indefinite result was obtained from treatment of either leaf surface with are arsenical weedicide at the concentration employed, i.e. normal weedicidal strength.

(v) Conclusions

In this experimental work a single application of a standard fungicide to the under-side of a leaf gave complete and lasting freedom from speckle attack to that leaf. This treatment was effective on all leaves not showing visible infection when the spray was applied. The dosage used would approximate to that employed when high-volume spray treatment of a banana plantation is practised, i.e. around 6 lb fungicide per acre on plants nearing flowering. These results suggest that the method of overhead spraying at intervals of four weeks with copper oxychloride/white oil/malachite green mixture, which has failed to give effective control of speckle in North Queensland plantations, can be modified by alternating at intervals of four weeks an overhead spray for leaf spot control and a combined leaf spot and speckle control schedule in the form of high-volume treatment directed at the under-sides of the leaves.

Manual overhead spraying for leaf spot control is, however, now being displaced in North Queensland by automatic treatment, employing air-blast misting machines towed behind tractors. Dosage approximates 60 gal per ac. With this method, coverage of the under-sides of all leaves is possible and the amount of fungicide applied—2 lb per ac—appears to persist sufficiently long to permit of good speckle control if the treatment is repeated at intervals of four weeks. Some growers aim to treat their plantations at intervals of three weeks during the wet season. Treatment of a plant has to be continued until the bunch is taken off.

The fact that the disease is disseminated by ascospores ejected from dead leaves indicates that detrashing should play a big part in the control of speckle. Periodic removal and destruction of the dead leaves draped around the base of a stalk would undoubtedly drastically reduce the amount of inoculum available and increase the efficacy of the spray programme.

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