EPIDEMIOLOGY AND CONTROL OF BANANA LEAF SPOT *(Mycosphaerella musicola* Leach*)* IN NORTH QUEENSLAND

By W. PONT, B.Sc.*

SUMMARY

The presence of Mycosphaerella musicola, **the ascigerous stage of** Cercospora musae **Zimm., in North Queensland is confirmed. Tip spotting is prominent on mother plants, while line spotting usually occurs on followers.**

The relationship of seasonal conditions and other factors to infection and duration of the incubation period is discussed in the light of field observations and experiments. Moisture and temperature have the most decisive influence during the epiphyllic stage and the greatest intensity of infection occurs during the wet summer months. The incidence is depressed during the winter when low temperatures and reduced humidity reduce sporulation and inhibit spore germination. Damage is at a minimum during the dry spring and early summer when copious dews permit a limited amount of infection only.

After penetration has taken place the interaction of temperature, intensity of infection and perhaps growth rate of the plant have to be considered. The period of latency before the streak stage appears is noticeably lengthened for infections which originate during the cooler and drier weather.

Two factors which are responsible for lengthening the apparent period of latency are spore dormancy and infection of older leaves.

The duration of the time taken for the streak stage to pass to the mature spot is dependent on intensity of infection and no effect of environment is clearly indicated.

Age of leaf has no influence on rate of spot development, at least while leaves are not apparently senescent.

Leaf spot control experiments conducted in North Queensland during the years 1951–1958 are described and discussed. The efficacy of white oil emulsion and malachite green mixed with either copper oxychloride, Bordeaux mixture or zineb is proved.

A limited amount of work with oil/copper mixtures for leaf spot control has indicated that when treatment with water-based mixtures is practicable it is preferable.

The role of white oil emulsion in the control of Mycosphaerella musicola **is considered. The striking effect produced appears to be due to a suppressive action linked with the physiology of the host. The oil is not fungicidal or fungistatic in the conventional sense.**

*Research Pathologist, Division of Plant Industry, Queensland Department of Agriculture and Stock.

I. INTRODUCTION

Cercospora musae, the conidial stage of *Mycosphaerella musicola,* was first recorded on *Musa sapientum* in Java (Zimmerman 1902) but did not attract attention as a major parasite of the banana until more than a decade later when it caused serious damage to plantations in the Sigatoka district in Fiji. It was probably present in Queensland in 1923 but the first authentic records from South Queensland are dated early in 1926. In December of that year it was reported in North Queensland. In 1933 it appeared in the West Indies and since then has been recorded successively from Central America, South America and Africa, so it can safely be said to be a threat to production in all major banana-producing areas.

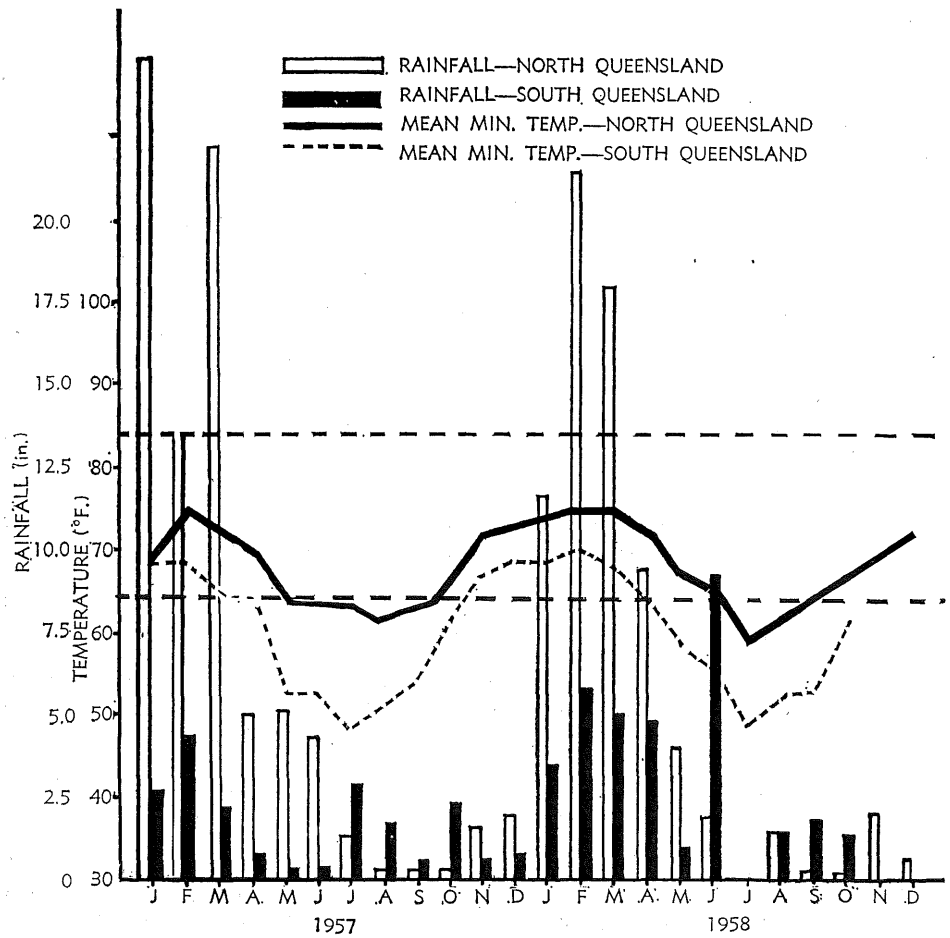

Fig. 1.—Climatic data for South Queensland (Brisbane) and North Queensland (Cairns), 1957-58. Horizontal broken lines mark off the temperature limits within which *M. musicola* is capable of 75 per cent. of its maximum growth.

Following the appearance of *Cercospora musae* in South Queensland, a considerable amount of work was done on the etiology of the disease and the effect of climatic factors on its development. The information gained was used to formulate a spraying schedule for that part of the State (Simmonds 1939). Because of the more favourable conditions, leaf spot proved to be a greater hazard in North Queensland banana plantations and in 1951 when investigations into its control were renewed it was decided to locate the experiments in the North. The major climatic factors contributing to differences in leaf spot incidence in South Queensland (Brisbane) and North Queensland (Cairns) are illustrated in Figure 1.

Concurrently with the control experiments, work was undertaken on the epidemiology of leaf spot under northern wet tropical conditions. The result of this work is discussed in the first part of the present article and a summary of the control experiments follows.

II. ECONOMIC IMPORTANCE AND HOST RANGE

Leaf spot infection first becomes apparent on affected foliage as minute chlorotic flecks which soon lengthen into yellow streaks 5 mm or more in length. These change colour to rusty brown, then broaden, become dark-brown in colour and develop a sunken centre which quickly dries out and becomes grey. The mature spots may be fusiform, elliptical or oval in shape. Their centres are studded with minute black sporodochia and they are encircled by dark-brown margins. On badly spotted leaves the majority of the spots do not reach a maximum size of 10 mm x 3 mm; however, isolated spots, particularly on water suckers, may be as large as 20 mm x 10 mm.

In North Queensland the streak stage may be first seen on the third, fourth or fifth fully expanded leaf, depending on plant growth and seasonal conditions. The rate of change from streak to spot is variable but mature spots are not often seen above the sixth leaf. Massed spotting in the mature stage quickly kills affected foliage, causing a progressive defoliation from the base of the plant upwards.

In this region of the State banana production is confined mainly to the coastal strip from Proserpine north to the Daintree River. Leaf spot is a major problem, however, only in the wet tropical zone which extends roughly from Ingham northwards. These coastal areas within the wet tropics are characterized by copious late-summer and autumn rains, giving a well-defined wet season early in the year. The wet season rains sometimes extend into the winter months but as a rule precipitation during the winter is much reduced and the spring and early summer are comparatively dry. Heavy dews are common during the drier part of the year.

Northern banana growers endeavour for economic reasons to manage their plantations so that the crop is harvested during the winter months. This means

that plants nearing the completion of their vegetative growth are subjected to the intense leaf spot infection which develops during and after the wet season rains. When the flower appears and leaf production ceases the plants are rapidly defoliated. Such a drastic reduction in the effective leaf area impairs the filling of the fingers, leading to reduced yields.

The bulk of the banana crop produced in the North is packed in cases as singles and consigned to southern markets by rail. A more insidious aspect of the leaf spot problem here is that consignments of fruit from plantations in which leaf spot damage is severe may arrive in the south in a "sprung" or mixed ripe condition and consequently be of little value. Exposure of the bunch following defoliation may also lead to premature ripening of the fruit while still on the plant. A bunch such as this is worthless and serves as a breeding ground for the banana fruit fly (*Strumeta musae* Tryon).

In new plantations, especially those with a reasonable degree of isolation, leaf spot may not become a problem until the plant crop and perhaps the first ratoon crop have been harvested. However, it is not uncommon to see severely damaged plant crops, particularly when these have been planted in proximity to sources of infection.

The varieties commonly grown commercially in North Queensland are the Cavendish and its mutants Mons Mari and Williams Hybrid. These are susceptible to leaf spot infection. The Lady Finger and Sugar varieties, which are grown on a comparatively minor scale, are also susceptible. The variety known in Queensland as Ducasses, which is of no commercial importance but is often grown in North Queensland and elsewhere as a windbreak, is immune. This plant is a natural hybrid which is believed to have arisen in Malaya, where it is still grown under the name of Awak Legor. It is a triploid made up of two genomes of *Musa acuminata* and one of *M. balbisiana* (F. W. Berrill, personal communication 1959).

The indigenous banana *Musa banksii* F. Muell., which is an inhabitant of rain-forests in the wet tropics, is susceptible but has only been observed to be infected when growing in the vicinity of infected plantations. The larger or yellow leaf spot (*Cordana musae* (Zimm.) von Hohn) is, however, commonly found on the wild banana and the native species is evidently an alternative host for this pathogen. At times first-year plantations established in rain-forest have been seen to be severely spotted by *Cordana musae* and it has been obvious that the infection originated from wild bananas nearby. It has been invariably noted that this parasite is quickly replaced by *Mycosphaerella musicola* in such plantations. In the first ratoon crop, or perhaps the second ratoon, *Cordana musae* plays a minor role only, being most often seen parasitizing the tissue around Cercospora lesions which have reached the self-limiting stage.

III. EPIDEMIOLOGY

(a) Spore Forms and their Importance

Mycospharella musicola Leach, the ascigerous stage of the banana leaf spot fungus, was first described from Jamaica (Leach 1941). Later the same author showed the difference in the manner of dissemination of the two spore

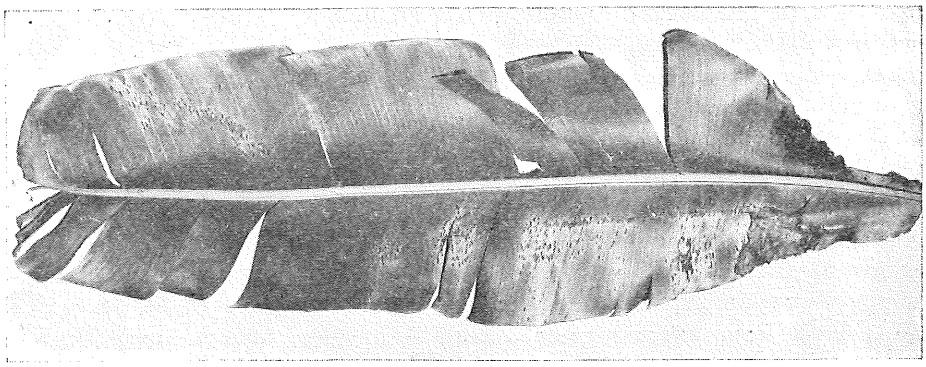

Fig. 2.—Line spotting on a leaf from a follower beneath a badly infected mother plant.

forms, conidium and ascospore, and its effect on the distribution of spots on affected leaves (Leach 1946). He demonstrated that conidial infection gave rise to line spotting (Figure 2) due to infection of the developing heart-leaf by the water-borne conidia, while infection of young fully expanded leaves by air-borne ascospores was responsible for tip spotting (Figure 3).

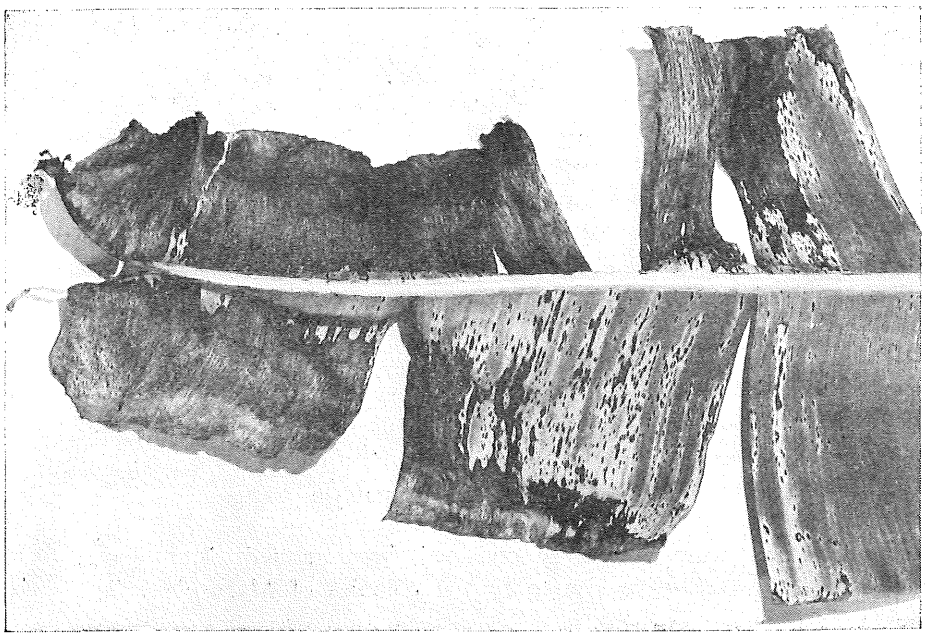

Fig. 3.—Tip spotting on a leaf from a mother plant.

Leach noted that with both types of spore secondary infection was rare. In Leach's work and in this paper also, the term secondary infection is intended to denote conidial infection of leaves other than the developing heart-leaf and ascospore infection of leaves older than the three youngest expanded leaves.

The isolation of the perfect stage of *Cercospora musae* has within recent years been confirmed in Cuba (Calpouzos 1955). Calpouzos found that the dimensions of the ascospores were greater than those described by Leach, viz. 18–20 x 4·5–5·6μ (Cuba) compared with 14–18·2 x 3–4μ (Jamaica). However, his measurements were made on germinated spores.

In North Queensland tip spotting as described by Leach is a common manifestation of leaf spot symptoms in banana plantations. In this region the ascospores of a related fungus (*Mycosphaerella musae* (Speg.) Syd.), the causal organism of a widespread and serious leaf speckle disease of bananas, are always present on the surface of banana leaves and it is easy to confuse the spores and infection processes of *M. musae* with those of *M. musicola* on leaf mounts from infected green leaves. However, after some practice the ascospores of the leaf spot fungus may be identified on such material because they are broader and their germ-tubes stouter than those of *M. musae*. The epiphyllic mycelium of the former is, in addition, less extensive, coarser and brown in colour. Penetration of the leaf in the case of *M. musicola* is quicker.

The isolation of spores of *M. musicola* for identification purposes is more difficult. The method employed to collect ascospores was to suspend spotted leaf pieces cut to fit the lids of 4 in. x 1 in. petri dishes over a water agar film in the bottom of the dishes or over agar-coated slides.

Most of the spores to reach the agar from spotted green leaf pieces of the Cavendish and Mons Mari varieties were those of another Ascomycete, *Leptosphaeria* sp. Limited numbers of ascospores of a species of *Mycosphaerella* were often seen in such preparations but it was impossible to isolate them from among the *Leptosphaeria* spores for purposes of identification. The association of *Leptosphaeria* with leaf spot lesions has been noted by previous authors (Simmonds 1933; Stahel 1937), and Calpouzos (1955) commented on the fact that the vast majority of ascospores obtained by the use of a similar technique from leaf-spotted material were multiseptate spores of this genus.

When spotted dead leaf pieces of the two varieties mentioned above were used, *Mycosphaerella*-type ascospores in addition to *Leptosphaeria* spores were obtained. However, perithecia of *M. musae* were present on this material and when the ascospores were plated out on potato dextrose agar the resulting colonies were invariably those of the leaf speckle organism.

Ascospores were eventually collected from spotted green leaves of the Sugar banana which gave rise to typical colonies of *Mycosphaerella musicola*

when transferred to potato dextrose agar. Measurement of these spores gave the following dimensions: range 14·4–19·2μ x 3·2–4·8μ; average of 50 spores 16·3 x 3.8μ. These agree well with those determined by Leach for *M. musicola* in Jamaica. It was found that in this work ascospores which had germinated on water agar were always considerably larger—up to 22·4μ x 6·4μ.

In Jamaica, Leach (1946) noted that ascospore infection is seasonal, occurring mainly in the autumn months. He observed that out-of-season tip spotting is common, however, on certain soils with "poor aeration, marked fluctuations in the oxidation/reduction conditions . . . and shallow tilth layers."

In North Queensland tip spotting (Figure 3) is prominent on mother plants, while line spotting (Figure 2) is usually found on followers as a result of heart-leaf infection by conidia washed from the overhanging leaves of the mother plants. Both types of infection are at a maximum during and following the wet season.

Commercial banana production here is mainly confined to flat or nearly flat land. Fertile alluvial soils or dark red loams derived from basalt are usually employed. There is no evidence that tip spotting is favoured by any one soil type but plantations on the deep, quick-drying basalt soils are generally considered to be more subject to leaf spot damage. This is probably because soil moisture drops quickly in these soils after the completion of the wet season rains and the reduced rate of leaf production due to this fact makes the disease more obvious.

(b) Seasonal Pattern of Disease Incidence

The seasonal variation in leaf spot incidence in North Queensland is shown in Figure 6 and is referred to again when discussing epidemiology and the results of control experiments. The data used were obtained from observations made throughout 1957 and 1958 on three unirrigated Cavendish stools in an isolated site at Kamerunga, in the Cairns district. As each new leaf emerged two rectangles were marked with a "Chinagraph" pencil on its lower surface. These were a 6 in. x 2 in. area in a central position on the left-hand side of the leaf (the side which unfurls first) and a 3 in. x 2 in. area in the region of the leaf tip, also on the left-hand side. The larger of the two rectangles was always situated so that its longest sides were at right angles to the midrib. All observations were then made within these marked areas. Readings were made each week when possible. Streaks and spots were marked as they appeared.

The monthly mean for intensity of infection plotted in Figure 6 was obtained by totalling the number of streaks which appeared within the marked areas on each plant during the month in question. The number of square inches within these datum areas was then calculated and the mean monthly figure, expressed as streaks per sq. in. was obtained by division. The numbers of

leaves involved each month in these calculations for each plant varied with the season and the growth rate of the plant and was greatest (maximum 7) during the first half of each year and least (minimum 3) during the second half.

It can be seen that there is a very noticeable lessening in leaf spot activity in the dry season. The seasonal pattern illustrated can be regarded as typical of the incidence of leaf spot throughout the banana-growing areas of North Queensland but the intensity of infection would be greater among large populations of plants, particularly on plantations in the wetter areas around Tully and Innisfail.

(c) Factors Influencing Infection

The effect of moisture and of the other factors listed below can be discussed in relation to germination and sporulation.

(i) Moisture

(1) *Effect on Germination.*—The effect of moisture on spore germination has been discussed by various authors. All agree that free moisture on the leaf surface is necessary for this process. In South Queensland, rainfall over a continuous 24 hr period is necessary to ensure abundant infection (Simmonds 1939). In the West Indies the important part played by dew as well as rainfall in the initiation of infection has been stressed (Stahel 1937; Leach 1946; Calpouzos 1955.)

In North Queensland, the wet season infection is undoubtedly initiated by rainfall. However, in the drier parts of the wet tropics, e.g. around Cairns, during the second half of each year periods of "effectual" rain are not common; the rainfall recorded is commonly in the form of storms or showers of much shorter duration, often during the daylight hours only. Such moisture quickly dries from the waxy banana foliage. On the other hand, heavy dews are usual during this dry period and it seemed probable that dew could provide the moisture needed to ensure infection at this time of the year.

In order to determine the effect of environment on infection during the dry part of the year a number of inoculations of Cavendish and Gros Michel plants was carried out during the second half of 1955. An aqueous suspension of conidia collected from leaf spots which had been incubated overnight in a humid atmosphere to induce sporulation was applied with a nebulizer to 3 in. x 2 in. rectangles marked on the lower surfaces of the leaves. Up to seven leaves on each plant were inoculated on occasions but usually five were used. Certain of the inoculated leaves were enclosed in plastic sleeves for 36 hr and the interiors of the covers and the leaves themselves were misted with water prior to enclosure. The results of some of these inoculations are summarized in Table 1. Rainfall and dates are given for the period during which wet weather could have influenced the particular inoculation.

TABLE 1

Results of Artificial Inoculation Experiments, Dry Season, 1955

Inoculated	Streaks Appeared	Incubation Period (days)	Wet Days and Rainfall (in.) to within 3 Weeks of Streak Appearance
July 18	Aug. 23	36	July 19 (0·02) ; 21 (0·05) ; 24 (0·02) ; 27 (0·03)
July 18*	Aug. 23	36	ditto
July 25	Aug. 29	35	27 (0·03)
Aug. 1	Sept. 13	43	Aug. 21 (0·04)
Aug. 1*	Aug. 29	28	Nil
Aug. 8	Sept. 13	36	Aug. 21 (0·04)
Aug. 8*	Sept. 13	36	ditto
Aug. 29	Oct. 7	39	Aug. 29 (0·14) ; 30 (0·20) ; 31 (0·86) ; Sept. 1 (0·18) ; 2 (0·24) ; 4 (0·05) ; 11 (0·14) ; 12 (0·52) ; 13 (0·13) ; 14 (0·04) ; 15 (0·14)
Sept. 13	Oct. 19	36	Sept. 13 (0·13) ; 14 (0·04) ; 15 (0·14) ; 18 (0·38) ; 19 (0·12) ; 22 (0·03)
Dec. 14	Jan. 16	33	Dec. 15 (0·03) ; 18 (0·80)

* Enclosed in plastic cover after inoculation.

It was obvious from the results of these experiments that infection could be independent of rainfall during these months. During the period from July 18 to August 8 four successful series of inoculations on uncovered leaves were accomplished. As the minimum observed period from germination to streak appearance is in the vicinity of four weeks, even during the wet season months, the only rain which could have affected the fate of these inoculations during the weeks from July 18 to September 13 occurred on five days with a maximum fall of only 0·05 in. Heavy dews were recorded during this dry period and in some cases dew was noted on inoculated foliage as late as 10 a.m. No consistent differences showed between covered and uncovered leaves.

In 1958 more information on the effect of dew on infection was obtained from two small experiments carried out at Kamerunga. In the first experiment two plants, one sheltered in a lath-house and the other in the open, were inoculated from the same stock spore suspension. The inoculum was applied within circles 4·5 cm in diameter marked on the lower surfaces of the three youngest fully expanded leaves. Three such circles were randomized on each leaf. The streaks were counted as they appeared. The following are the results expressed as mean number of streaks per circle:

Leaf position	1	2	3	Mean
Field plant	137	132	159	143
Sheltered plant	28	23	18	23

The plant in the open was exposed to only 0·04 in. of rainfall on 2 wet days from the date of inoculation (June 25) to July 29, when the first streaks were noted. Heavy dews were common during this dry period. Moisture visible to the naked eye was not at any time noticed on the lower surface of the inoculated foliage on the sheltered plant.

This evidence, however, does not exclude the possibility that some effect on the metabolism of the plant due to growth under conditions of partial shade could have contributed to the reduced infection on the sheltered plant.

In the second experiment the five youngest expanded leaves on a small plant in the open were inoculated with conidia on September 2, 1958. As before, the spores were applied within circles 4·5 cm in diameter, two of which were located on the lower surface of each leaf. The results of these inoculations first became evident as flecks in some of the inoculated circles on October 2. On October 9 an average of 80 streaks per circle was visible and by November 5, when the majority of streaks had become necrotic spots, an average of 86 lesions per circle had been counted.

From the date of inoculation until the time when the first streaks were counted the rainfall recorded was 0·20 in. on September 24, 0·15 in. on September 26 and 0·09 in. on October 2. None of these falls were early enough to have affected the issue.

A 7-day dew recorder (Theis and Calpouzos 1957) was used to record the occurrence of dews during the first fortnight after inoculation. This instrument recorded an average of 9 hr dew per night during this time. Evaporation of moisture from the glass plate of the instrument was complete on all mornings by 8 a.m. However, dew was seen on the protected lower surfaces of the inoculated leaves as late as 10 a.m.

These results suggest that dew can provide the free surface moisture which is needed to ensure infection by leaf spot spores. It must therefore be considered as a factor in the epidemiology of the disease, particularly in those banana growing areas in the North where long rainless periods are common during the dry season. In the wetter areas around Innisfail and Tully, rainfall in the second half of the year is greater and periods of "effectual" rain are more common. Nevertheless, in spring and early summer rainless periods of three weeks or more do occur during which copious dews are probably responsible for infection (Figure 1).

(2) *Effect on Sporulation.*—The part played by water—either rain or dew—in inducing the production of conidia has been well stressed (Stahel 1937; Simmonds 1939; Leach 1946). In addition, it has been demonstrated (Calpouzos 1955) that conidia are produced *in vitro* in atmospheres with relative humidities of 98 and 100 per cent. In the course of the work described in this paper conidia of *Mycosphaerella musicola* were produced, whenever required, by the exposure of leaf spots to a saturated atmosphere. It would be unusual if the incidence of

atmospheric humidities as high as those quoted above was not associated with either rainfall or condensation. Calpouzos (1955) has concluded therefore that, for all practical purposes, sporulation *in vivo* occurrs only when free moisture is present.

(ii) Temperature

(1) *Effect on Germination.*—The growth-temperature relationships of *Mycosphaerella musicola* have been investigated in detail (Simmonds 1933; Calpouzos 1955). Simmonds found that after incubation for 24 hr the optimum temperature for the germination of conidia was as high as 84°F. However, at lower temperatures germination increased with time and a fair percentage of spores could be expected to germinate even at temperatures as low as 59°F after exposure for 48 hr. Calpouzos determined 77°F to be the optimum for germination but also obtained excellent germination at 59°F after 48 hr.

According to Simmonds the optimum temperature for germ-tube growth was intermediate between that for growth of the fungus in culture (78°F) and that for germination (84°F).

A further study was made of the relationship of temperature to germination and germ-tube growth of conidia of *M. musicola* and the results are illustrated in Figure 4. Cercospora spores were collected from mature leaf spots which were exposed to a saturated atmosphere overnight; they were suspended in banana infusion broth and the suspension was used to prepare hanging-drop

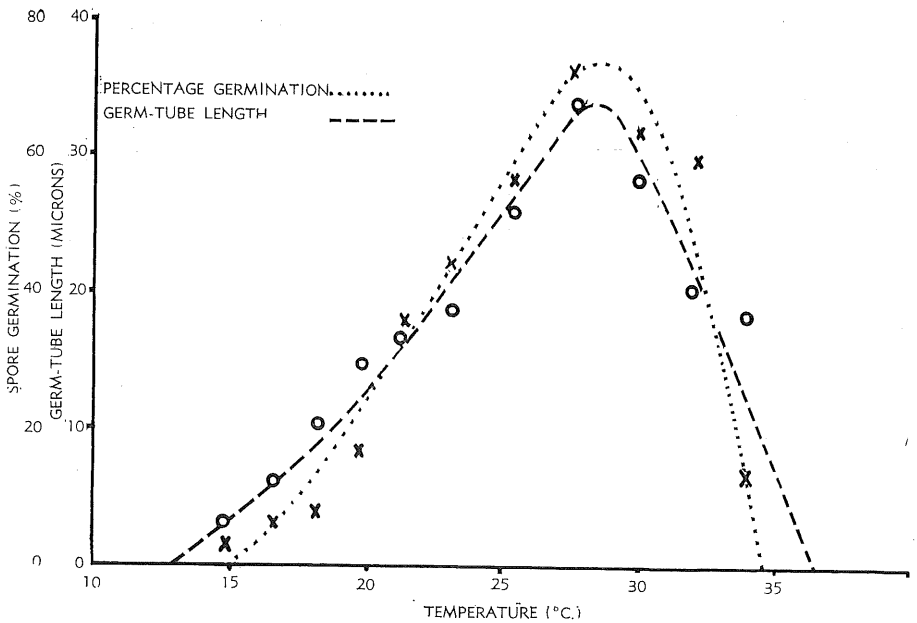

Fig. 4.—Relationship of germination and germ-tube growth (conidial) to temperature.

cultures in van Tieghem cells. The cells were then incubated in each of 15 compartments of a a multi-temperature incubator at temperatures ranging from 70°C to 34°C. After incubation for 24 hr they were removed and placed in a refrigerator overnight. Counts and measurements were made during the next day. Fifty spores were counted per hanging drop and measurements were made of the lengths of the longest germ-tubes on each of the germinated spores in these lots of 50. The figures plotted in the graph are means calculated from counts and measurements made by two observers.

It can been seen that the optimum temperature for both germination and germ-tube growth was 28°C (83°F) or a little higher. There was no germination below 15°C (59°F) in 24 hr.

In coastal areas of North Queensland the minimum temperature drops below 60°F usually only occasionally even in the coolest months of June, July, August and September (Table 2). Conditions are roughly similar to those in Jamaica, where "it is doubtful whether temperature ever falls low enough to prevent germination completely in any of the main banana growing areas . . ." (Leach 1946). However, in North Queensland the fact that the mean 10 p.m. to 6 a.m. temperatures do not exceed 70°F during these months indicates that low temperatures could be responsible for slowing up the processes of infection at this time of the year. The position is not comparable with that in South Queensland, where for long periods during the year nocturnal temperatures fall well below 60°F (Figure 1).

TABLE 2

Mean Monthly Night Temperatures (°F) for Cold Months, Kamerunga 1955–1957

(Thermohygrograph Readings)

Time of Reading	June	July	August	September
10 p.m.	67	66	67	69
2 a.m.	65	64	64	66
6 a.m.	64	63	62	68
Mean monthly minimum	63	62	62	64

(2) *Effect on Sporulation.*—Temperatures exert a marked effect on the production of conidia by *Mycosphaerella musicola.* In Jamaica the percentage of viable conidia formed on leaf spots each night is largely dependent on the prevailing night temperatures (Leach 1946). Leach considered that "the effect of low temperature on sporulation accounts for the consistent island-wide reduction in conidial infection which occurs every year in January and February."

In the work discussed here the variation in conidial sporulation with temperature was investigated in the following manner: Suitable spots were removed from leaves, placed in petri dishes in a saturated atmosphere and exposed to a range of temperatures in a multi-temperature incubator. Usually two dishes

each containing 4 or 5 spots were placed in each compartment. After 24 hr the spots were examined with a dissecting microscope and each was given a rating both for number of sporing sporodochia and for intensity of sporulation on the following scale: 0, nil; 1 trace to 25 per cent.; 2, 26–50 per cent.; 3, 51–75 per cent.; 4, 76–100 per cent. When the examinations commenced the dishes were removed from the compartments of the multi-temperature incubator and stood on an open bench at room temperature with the lids removed. The ratings were made by two observers on each occasion and the results were consistent.

In Figure 5 the results of two such experiments are used to illustrate the effect of temperature on sporulation. Means have been calculated for number of sporodochia and intensity of sporulation.

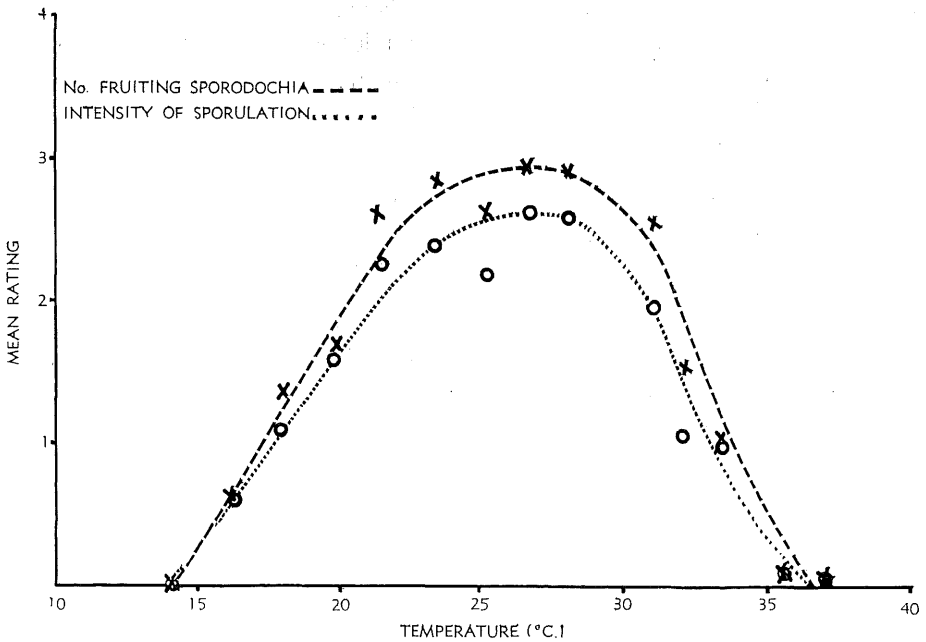

Fig. 5.—Relationship of conidial sporulation to temperature.

It can been seen that in a saturated atmosphere temperature affected both these variables. Both were negligible below 60°F and above 96°F. The temperatures most conducive to sporulation were in the range 70°F to 88°F and the optimum appeared to be around 83°F, i.e. very close to the optimum temperature for germination.

The mean monthly figures given in Table 2 indicate that the depressing effect of temperatures below 70°F on conidial sporulation could contribute materially to the reduced incidence of leaf spot in North Queensland during the second half of the year. It must be stressed, however, that in this part of the

State sporulation *in vivo* is not completely inhibited by exposure to night temperatures below 60°F. For example, during an unusual spell of cold weather in July 1959 which lasted for nine days the mean figures for the 10 p.m., 2 a.m. and 6 a.m. readings at Kamerunga were respectively 61, 57, and 52°F. On every morning during this period banana foliage was drenched with dew and it was possible to locate spots on which partial sporulation had occurred and from which mature conidia could be removed by stroking with a camel-hair brush.

(iii) Age of Leaf and Surface of Leaf

(1) *Effect on Infection.*—In Surinam, conidial infection of leaves older than the second fully expanded leaf was considered to be unimportant on the Congo variety (Stahel 1937). In Jamaica, the bulk of the conidial infection on the Gros Michel variety was shown to originate on the unfolding heart-leaf, while ascospore infection was unimportant on leaves older than the first three (Leach 1946). In South Queensland, however, quite extensive conidial infection of leaves other than heart-leaves was demonstrated on the Cavendish banana (Simmonds 1939).

During 1955 and again in 1959 a series of inoculation experiments on Gros Michel and Cavendish bananas was carried out to determine whether conidial infection of older leaves was possible under North Queensland conditions. The results of some of these inoculations are presented in Table 3. The conidia were collected from mature leaf spots and applied in aqueous suspension with a nebulizer to rectangles measuring 3 in. x 2 in. or to circles 4·5 cm in diameter on either the upper or the lower leaf surface. An attempt was made to ensure the application of approximately equal amounts of inoculum to each of the inoculated areas in any one experiment. The resulting intensity of infection is expressed in number of streaks per sq. in.

It can be seen that infection of older leaves on both Cavendish and Gros Michel varieties resulted from the application of conidia to the lower surfaces. On Plant 7, which was carrying a young bunch when it was inoculated, infection of the second and third leaves was obtained quite readily.

In Jamaica, the antibiotic effect exerted on leaf spot spores by miscellaneous epiphyllic mycelia has been considered responsible for the resistance of old leaves to infection (Leach 1946). In the tests described above no attempt was made to remove the mycelia except in the case of Plant 7 (upper surface).

There appears to be no reason why infection of older leaves by either conidia or ascospores should not be possible under plantation conditions in North Queensland. Some confirmation of this view has been provided by the fact that freshly germinated ascospores and new infections have been seen on leaf pieces taken from leaves in the sixth fully expanded leaf position showing both streak and spot stage lesions of the tip spotting type. Additional evidence of the existence

TABLE 3

Artificial Infection of Leaves of Gros Michel and Cavendish Banana
(Streaks/sq. in.)

Variety	Plant No.	Date of Inoculation	Surface Inoculated	Heart-leaf	Fully Expanded Leaves						
					1st	2nd	3rd	4th	5th	6th	7th
Gros Michel	1	18. vii. 1955	Lower ..	..	9	20	10	33	17	..	..
			Upper ..	..	0	1	1	4	1	..	..
,, ,,	2	29. viii. 1955	Lower ..	22	36	22	33	15	21	7	7
			Upper ..	..	1	0	1	0	0	2	0
Cavendish ..	3	18. vii. 1955	Lower ..	..	39	39	15	20	15	..	..
			Upper ..	..	7	1	1	1	0	..	..
,,	4	12. ix. 1955	Lower ..	..	6	13	9	4	6	..	..
,,	5	13. ix. 1955	Lower ..	14	15	20	7	10	4	..	..
,,	6	14. xii. 1955	Lower ..	..	13	10	11	10	5	..	..
,,	7	18. vii. 1955	Lower ..	..	..	24	21	..	..	..	..
			Upper ..	..	..	8*	0	..	..	..	..
,,	8	6. ii. 1959	Lower ..	..	68	33	36	20	..	..	..
,,	9	1. v. 1959	Lower ..	..	28	26	32	..	..	..	..
,,	10	1. v. 1959	Lower ..	..	11	22	16	9	9	..	..
,,	11	1. v. 1959	Lower ..	..	36	11	15	7	..	..	..

* Inoculated area rubbed well with damp cotton-wool pad prior to inoculation

of "secondary" infection was obtained in a leaf spot control experiment (Experiment 4, Stoney Creek) in which the incidence of the disease was significantly reduced by late cover sprays which were continued after the plants had flowered and leaf production had ceased.

The fact that leaf spot infection originates much more readily on the lower surface of a banana leaf than on the upper surface has been discussed by many authors. Stahel (1937) noted that the infection ratio between upper and lower leaf surfaces was much lower than one would expect from a comparison of numbers of stomata on these two surfaces. Leach (1946) suggested that there was a hydrotropic response by germ-tubes towards open stomata and concluded that the higher rate of infection of stomata on the lower surface could be attributed to the facts that stomata on this surface opened earlier in the morning and that the microclimate of the lower leaf surface was favourable to germ-tube growth for longer periods.

The results shown in Table 3 indicate that infection through the upper leaf surface is unimportant in North Queensland. The inoculations made on Plants 1, 2, 3 and 7 in 1955 resulted in an average intensity of infection of 21·2 streaks per sq. in. on the lower surface, compared with only 1·5 on the upper.

(2) *Effect on Sporulation.*—In North Queensland, conidia have been collected from the dead trash which hangs about the base of an affected plant. However, sporulation is most prolific on spots on living leaves. Although the production of conidia does not cease immediately a leaf dies, the capacity to produce these is apparently lost fairly soon after death, for they are usually not found in quantity on any except the two or three leaves last to die. An alternative possibility is that these spores are produced before the leaf dies but have not been disseminated.

Sporodochia develop on both upper and lower surfaces of spots. Those on the upper surface are the first to become evident and on narrow linear spots these appear to be more numerous. On broader spots the sporodochia on the lower surface are smaller and more numerous than those on the upper surface. Conidia are produced freely on both surfaces when environmental conditions are favourable.

The results reported here relating to the effect of certain variables on infection by the banana leaf spot fungus have been derived from experiments and observations involving only the asexual spores of the pathogen. Because of the difficulties, mentioned previously, involved in isolating the ascospores of *Mycosphaerella musicola* from the spores of other Ascomycetes commonly found on banana leaves in North Queensland, it has been impossible to obtain any precise information of a similar nature about these spores. However, it is assumed that the moisture and temperature requirements for sporulation, germination and infection are closely similar for the two spore types.

(d) The Developmental Periods

The manner in which the leaf spot fungus infects a leaf was first described by Stahel (1937). The essential processes involved in the establishment of infection may be briefly described as follows: Following on the germination of the spores and after a certain period of epiphyllic existence, said by Stahel to last 4–8 days, the germ-tubes form appressoria over stomata and penetration of the leaf takes place through the stomatal pores to the air chambers. The infection hyphae eventually spread to neighbouring air chambers and travel through the spongy parenchyma to the palisade tissue. About this time the streaks become visible.

As the streaks age, epiphyllic mycelia of the fungus emerge from stomata on both surfaces, extending for some distance around the streak. Infiltration of the tissue around the streak then ensues and the fungus re-enters the tissue through the stomatal pores. Necrosis then takes place, the sporodochia are initiated below stomata in the brown area and the typical sporing spot appears.

There are thus two obvious stages in the development of a typical leaf spot, viz. the streak and the sporing spot. Associated with these are two critical periods of development—firstly, the incubation period, which is the time that elapses between germination of a spore and the appearance of a streak; and secondly, the period during which the change from streak to sporing spot takes place.

The effect of environment on the duration of development was first investigated by Simmonds (1939) in South Queensland. He found: "The effect of temperature is fairly well defined. The length of the incubation period required before streaks develop increases with decrease in temperature." The incubation period in South Queensland was calculated to vary from a minimum of approximately a month in summer to a maximum of 3½ months in winter. No attempt was made to effect a correlation of incubation period with plant growth rate, which in South Queensland is bound up with seasonal temperature. In his observations this worker found that streaks appeared in peaks of development subsequent to the first appearance. He noted that the length of the incubation period did not in many cases allow this phenomenon to be attributed to germination in a second period of more suitable weather and suggested as a probable explanation that "the fungus is present within the tissues in an inactive or latent condition, and requires a lowering of resistance on the part of the host such as might occur with the ageing of the leaf or as a result of a sudden drop in temperature." Simmonds noted that the time taken for the streak to pass into the brown stage is not so definitely related to seasonal temperature. This period was usually relatively short compared with the incubation period. He did, however, suggest that the transition from the streak to the brown stage is stimulated by a sudden fall in temperature.

In Jamaica, where winter temperatures are above those recorded in South Queensland and the range in temperatures is far less, Leach (1946) found that temperature alone had no effect on development but that "fast growing plants developed streaks and spots significantly faster than slow growing plants, the effect being most marked in the case of the development of the spots." His main finding, however, was that spot development was closely correlated with the number of infections per unit area of leaf surface. He noted that "the degree of latency, inherent in spot development, is closely correlated with certain anatomical features of the leaf and with the number of spots which finally develop on a leaf." The results of his work "definitely supported the hypothesis that the rate of development of streaks and spots is dependent upon the number of spores infecting a unit area of leaf surface, also . . . that spots appear first in those areas where the greatest number of streaks and spots develop and that the percentage of streaks which turn into spots is highest where the number of streaks is greatest." Leach postulated the secretion of some toxin by the hyphae and stated: "The greater the number of stomata that become infected per unit area of leaf, the greater will be the growth of internal hyphae and consequently the greater will be the quantity of toxin produced so that the more stomata infected per unit area the quicker will be the loss of tissue resistance and the faster will be the rate of spot development."

In North Queensland there is a seasonal variation in the rate of development of the leaf spot fungus. The observations on disease incidence made at Kamerunga during 1957 and 1958, using the technique described when discussing the seasonal pattern of disease incidence, have been used to illustrate this seasonal fluctuation (Figure 6).

The various data used were arrived at as follows:

Approximate minimum incubation periods for the infections appearing within the marked areas on each leaf were derived by assuming that the infections originated on the expanding heart-leaf. When the appearance of streaks was first recorded the approximate incubation period was obtained by calculating the time that had elapsed since the particular leaf was in the heart-leaf stage.

The period from streak to spot was determined by using a separate symbol for each fresh batch of streaks which was recorded within any one observation area each week. As streaks changed to spots the time involved was calculated back.

For determining growth rate, leaves were numbered as they appeared and it was known from these observations how many leaves were produced in a given month. These figures were used to calculate the number of days between successive leaves. The reciprocal of this value was termed the growth rate. The figure plotted for each month is the average growth rate of the three plants.

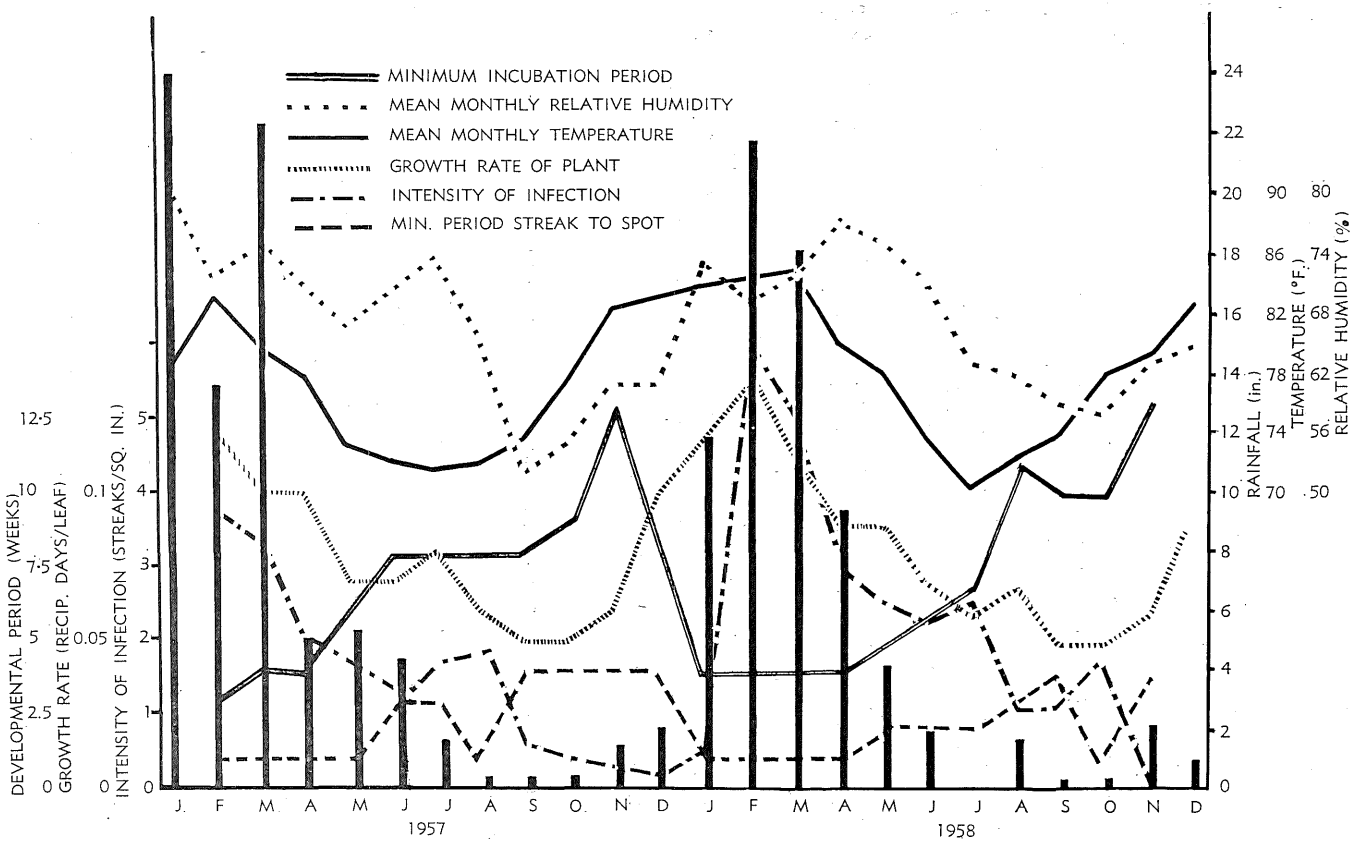

Fig. 6.—Seasonal variation in rate of development of leaf spot. Kamerunga, 1957-58.

Intensity of infection is expressed as streaks per sq. in. and the values were determined by the method described on page 217. The arrangement of those values into monthly groups was accomplished by assigning to each month those values for areas in which streaks first became evident during that month. The monthly figures plotted in Figure 6 comprise the minimum incubation period from each monthly group together with the associated intensity of infection and minimum period from streak to sporing spot.

To facilitate the plotting all developmental periods which were originally recorded in days have been expressed as weeks by using the following conversion scale: 0–10 days, 1 week; 11–17 days, 2 weeks; 18–24 days, 3 weeks; and so on.

It is not intended that the times quoted for incubation and streak to spot be construed as accurate estimates of their duration but rather that they be regarded as indices of rate of development.

The values given for humidity are the averages of the mean monthly 9 a.m. and 3 p.m. relative humidities. The total rainfall for each month is plotted.

These observations on natural infection as well as the results of some inoculation experiments (Table 4) are used below to investigate the effect of environment, intensity of infection, growth rate and age of leaf on leaf spot development. The periods covering incubation and the change from streak to mature spot are considered separately.

(e) Influence of Specific Factors on the Incubation Period

(i) Moisture

From Figure 6 it will be seen that in both 1957 and 1958 the combined effect of high rainfall and high daytime humidity was apparent in the short incubation periods for those infections which appeared during January, February, March and April. In 1957 lower rainfall and reduced humidity in April could have been responsible for the lengthening of the incubation period for infections which appeared in May and June, The mean humidity rose again in June and July and this was associated with the flattening of the incubation curve, which extended to include infections appearing during September. The drop in humidity and scant rainfall in August, September and October can be related to the peak in the incubation curve in November.

In 1958 humidity fell steadily from May through to July and rainfall was negligible in July. This was apparently responsible for the sharp rise in incubation for infections which appeared in August. The flattening of the incubation curve for infections appearing during September and October was related to the rain group in August, and the peak in incubation during November was apparently due to the low humidities and negligible rainfall during September and October.

TABLE 4

Developmental Data from Artificial Inoculation Experiments and the Associated Environmental Conditions

Plant No.	Date Inoculated	Date Streaks Appeared	Growth Rate	Intensity of Infection (streaks/ sq. in.)	Developmental Periods (days)		Mean Daily Temperature (°F)		Mean Daily Relative Humidity (%)		Rainfall† and Date
					Incubation	Streak to spot	Incubation	Streak to spot	Incubation	Streak to spot	
11	6 [illegible] 195	2. iii. 1959	·14	58	24–31 (23·5)	7–21	74–96 (85)	74–86 (80)	79	87	Rgs. 13/2–16/2, 3·23 in.; 24/2–2/3, 9·46 in.
12	[illegible] 195	18. v. 1959	·08	3	40–70 (55)	6–60	69–84 (76·5)	62–82 (72)	78·5	77·5	0·09 in. on 3 wet days to 14/4 ; Rg. 14/4–16/4, 0·95 in.
13	1. v. 1959	9. vi. 1959	·07	29	39–59 (49)	5–27	64–79 (71·5)	61–80 (70·5)	80·5	76·2	0·02 in. on 1/5 ; Rgs. 5/5–18/5, 4·19 in. ; 20/5–28/5, 1·29 in. ; 1/6–8/6, 1·23 in.
14	1. v. 1959	17. vi. 1959	·07	15	47–66 (56·5)	5–33	65–79 (72)	63–79 (71)	79·5	76·2	As Plant 13
15	1. v. 1959	17. vi. 1959	·07	15	47–66 (56·5)	5–33	65–79 (72)	63–79 (71)	79·5	76·2	As Plant 13, plus 11/6–16/6, 0·27 in.
6*	25. vi. 1958	28. viii. 1958	·06	10	64–120 (92)	7–70	59–85 (72)	65–88 (76·5)	68·75	65·75	0·23 in. on 3 wet days to 2/7 ; Nil 2/7–10/8 ; Rg. 11/8–18/8, 1·58 in.
7	25. vi. 1958	30. vii. 1958	·07	56	35–56 (45·5)	7–30	61–83 (72)	62–83 (72·5)	68·5	69	0·23 in. on 3 wet days to 2/7
1	18. vii. 1955	24. viii. 1955	·06	20	37–70 (53·5)	7–44	62–83 (72·5)	65–83 (74)	73·75	74·2	0·16 in. on 5 wet days
2	25. vii. 1955	31. viii. 1955	Flowered	22	36–60 (48)	10–31	62–83 (72·5)	64–83 (74)	73·25	75·5	0·11 in. on 4 wet days to 26/8 ; Rg. 29/8–31/8, 0·71 in.
8	31. vii. 1958	4. ix. 1958	·06	40	35–76 (55·5)	7–42	59–85 (72)	66–89 (77·5)	68·75	67·75	Rg. 11/8–18/8, 1·58in.
9	31. vii. 1958	28. viii. 1958	·1	36	28–68 (48)	7–42	63–84 (73·5)	64–86 (75)	69·0	66·5	Rg. 11/8–18/8, 1·58in.
3	29. viii. 1955	7. x. 1955	·07	23	39–71 (55)	7–39	64–85 (74·5)	70–91 (80·5)	74·75	74·25	Rg. 29/8–5/9, 2·02 in.; 12/9–16/9, 0·97 in.; 19/9–22/9, 0·53 in.; 5/10–7/10, 0·38 in.
10	2. ix. 1958	9. x. 1958	·06	34	37–51 (44)	7–21	65–86 (75·5)	67–89 (78)	64	68·5	25/9–26/9, 0·35 in. ; 2/10, 0·09 in.
4	13. ix. 1955	20. x. 1955	·07	12	37–56 (46·5)	7–34	65–86 (75·5)	70–91 (80·5)	75·5	72·75	Rg. 13/9–22/9, 1·36 in. ; 5/10–10/10, 0·43 in.
5	14. xii. 1955	16. i. 1956	·09	10	33–63 (48)	7–35	74–95 (84·5)	74–91 (82·5)	77	80·25	16/12, 0·03 in. ; 19/12, 0·80 in. Rg. 8/1–13/1, 3·40 in.

* Plant 6 grown in shelter of lath-house.

† Rg. = Rain group.

Another illustration of the effect of moisture on the incubation period is provided by a comparison between Plants 6 and 7 in the inoculation experiments summarized in Table 4. These plants were inoculated on the same day from the same stock spore suspension. Plant 6 (minimum incubation period 64 days) was grown in the shelter of a lath-house, while Plant 7 (minimum incubation period 35 days) was in the open and exposed to dews, which were very frequent at that time of the year. However, as mentioned previously, this evidence is not positive because of the possibility of some metabolic disturbance to the plant due to the partial shading. An additional illustration of the effect of rainfall on intensity of infection will be found in Figure 7.

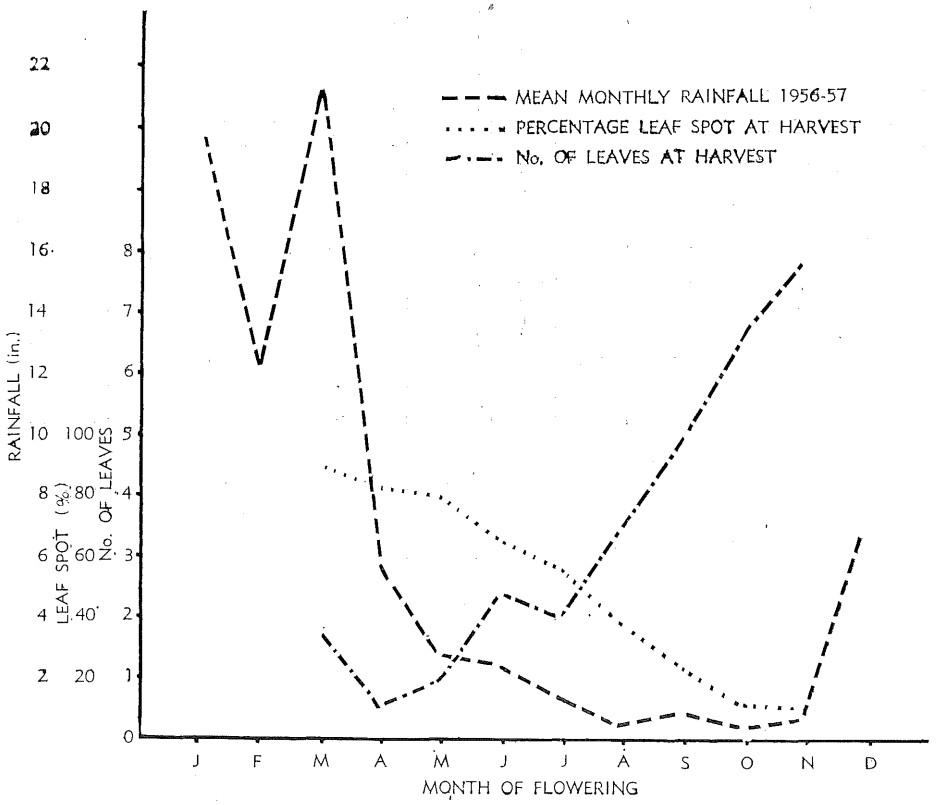

Fig. 7.—Effect of time of flowering on leaf spot percentage at harvest. Combined data from unsprayed plants in Trial 5 and 6. Stoney Creek, 1956 and 1957.

(ii) Temperature

The variation in minimum incubation period (Figure 6) can be related to temperature as well as to moisture. In both 1957 and 1958 incubation period lengthened as temperature decreased. The maximum incubation period in each year was recorded for those few streaks which appeared in November from infections which apparently originated some three months earlier in August at a time when the daily minimum temperature was reaching very low levels. December

infection is presumed to have originated in October and the higher minimum temperatures recorded in this month and November were associated with a marked reduction in the incubation period.

(iii) Intensity of Infection

In Figure 6 the minimum incubation period is inversely proportional to intensity of infection, which was highest during the wet season and lowest at the dry season in both years. Intensity of infection itself broadly follows the growth rate and both are probably related to the same general meteorological features.

When the incubation periods for the artificial inoculations (Table 4) on Plants 8 and 9 (intensity of infection 36-40 streaks/sq. in., minimum incubation period 4-5 weeks) are compared with those calculated for natural infections which appeared during the same period of the year (August intensity of infection 1·2–1·8 streaks/sq. in., minimum incubation period 8–11 weeks), the reduction in incubation associated with higher intensities of infection is quite apparent.

The difference in incubation period for the inoculations on Plants 13 (intensity of infection 29 streaks/sq. in., minimum incubation period 39 days) and 14 or 15 (intensity of infection 15 streaks/sq. in., minimum incubation period 47 days) also points to a relationship between these two factors.

Further evidence is furnished in Table 5, which summarizes the results of the inoculations on Plant 13 in April 1959.

TABLE 5

Effect of Intensity of Infection on Development (Artificial Inoculations Plant 13, 8. iv. 1959)

Intensity of Infection (streaks/sq. in.)	Developmental Period (Days)	
	Incubation	Streak to Sporing Spot
9·6	40–48	5–60
9·2	40–61	9–30
7·6	40–70	5–27
2·8	53–70	5–30
1·2	53–70	19–37
0·4	70	19

(iv) Growth Rate

In Figure 6 it can be seen that incubation is least for those infections which occurred when growth rate was highest. Conversely, it is at a maximum for those infections that took place when the plants were growing very slowly. Growth rate itself appears to be closely related to both temperature and rainfall. In the field, therefore, any association of growth rate with rate of development of leaf spot would be secondary, the primary influences being temperature and rainfall.

A further indication appears in the inoculation experiments (Table 4). Plant 9 (growth rate 0·1, minimum incubation period 28 days) was a sucker from the same stool as Plant 8 (growth rate 0·06, minimum incubation period 35 days). They were inoculated on the same day from the same stock spore suspension. The shorter incubation period is apparently related to the higher growth rate for the intensities of infection were very similar.

(v) Age of Leaf

When the results of various inoculation experiments were examined to provide information on the effect of age of leaf on rate of spot development, the data presented in Table 6 were obtained. It can be seen that there is no indication of any variation in incubation period when rate of development on the youngest leaves is compared with that on leaves as old as the seventh. However, it must be admitted that the seventh leaf on a banana plant is, to the eye at least, far from being senescent. This evidence fails to support the suggestion that the long incubation period following heart-leaf inoculation is due to some essential maturation changes in the leaf itself.

Before concluding this section it should be mentioned that maximum periods of incubation as long as 22 weeks have been calculated for some of the dry season infections recorded in 1957 and 1958 at Kamerunga using the method described above and assuming that the infection processes were initiated on the unfolding heart-leaf. Incubation periods of this duration are obviously apparent rather than real.

The factors which are involved in the prolongation of the apparent period of incubation are infection of leaves when they are older than the heart-leaf stage and dormancy of the spores.

The latter has been demonstrated experimentally in North Queensland. Microscopic examination of banana foliage inoculated with conidia during the dry season has shown that these spores may remain dormant on the leaf surface for a considerable time before the infection processes are initiated. In addition, there can be a considerable variation in the rate of germination of spores from the same suspension applied to the same leaf and exposed to the same environment. The reason for this variation is not obvious.

Simmonds (1939) quoted strong circumstantial evidence to indicate that in South Queensland conidia of *M. musicola* remained ungerminated on the leaf surface for periods up to 22 days in the absence of "effectual" rain.

(f) Factors Influencing the Change from Streak to Sporing Spot

(i) Moisture

It is clear from Figure 6 that the minimum period streak to spot was least during the wet halves of both years. The steep drop in this curve in January

TABLE 6

Effect of Age of Leaf on Rate of Leaf Spot Development (in weeks)

Leaf Position When Inoculated (Numbered from top down)	1		2		3		4		5		6		7	
Date Inoculated	Incubation	Streak to Spot	Incubation	Streak to Spot	Incubation	Streak to Spot	Incubation	Streak to Spot	Incubation	Streak to Spot	Incubation	Streak to Spot	Incubation	Streak to Spot
18. vii. 1955	5– 9	1–4	5–10	1–4	5–10	1–6	5–7	1–5	6–7	2–5	..	..	..	..
18. vii. 1955	5– 8	2–4	5– 8	2–4	5–10	2–4	5–8	2–5	..	..	..	..	..	..
29. viii. 1955	6– 9	1–4	6– 9	1–5	6– 9	3–6	6–9	1–4	6–9	2–5	6–9	2–5	6	1–2
13. ix. 1955	5– 8	2–4	5– 8	1–5	5– 8	2–5	6–9	1–4	5–9	1–5	..	..	..	..
14. xii. 1955	5– 8	1–4	5– 9	1–4	5– 7	1–3	5–8	1–3	5–8	1–3	..	..	..	..
25. vi. 1958	9–17	1–8	9–17	1–6	9–12	2–5	..	..	..	..	..	..	..	..
25. vi. 1958	5– 8	1–4	5– 8	1–4	5– 8	1–4	..	..	..	..	..	..	..	..
31. vii. 1958	4–11	1–6	4–10	1–4	4–11	1–4	..	..	..	..	..	..	..	..
31. vii. 1958	5–12	1–6	5–11	1–6	5–10	1–5	..	..	..	..	..	..	..	..
8. iv. 1959	8–10	3–5	6– 9	1–9	8–10	1–4	6–10	1–6	..	..	..	..	..	..
1. v. 1959	7–10	1–5	7– 9	1–4	7– 9	1–3	7–9	1–3	7–9	1–3	..	..	..	..
1. v. 1959	7–10	1–5	7–10	1–5	7– 9	1–5	7–9	1–3	7–9	1–3	..	..	..	..
1. v. 1959	6–10	1–4	6– 9	1–3	6– 8	1–9	..	..	..	..	..	..	..	..

1958 could be associated with the higher humidities and rainfall during this month. However, there is no evidence in Table 4 to indicate any relation between the duration of this stage of development and atmospheric moisture.

(ii) Temperature

A direct relationship between temperature and period streak to sporing spot is not obvious from an examination of either Figure 6 or Table 4. The minimum occurs in the months of January to May with high to moderate temperatures but this is more obviously related to heavy rainfall of these months. The maximum occurs during the drier months with low to moderate temperatures.

(iii) Intensity of Infection

The seasonal variation in minimum period streak to spot illustrated in Figure 6 can be more closely correlated with intensity of infection than with any other factor. The reduced values in August 1957 and October 1958 can be related to the increased intensities in those months. When the results of the inoculation experiments (Table 4) are compared with the field observations it can be seen that the much higher intensities of infection resulting from the inoculations have exerted a marked influence on the duration of this period of development, for the highest minimum value recorded is 10 days. The effect of reduced intensity of infection in lengthening the time taken for streaks to change to sporing spots is also illustrated in Table 5. The apparent relationship between the period under discussion and high rainfall could well be due to the effect of the latter on intensity of infection.

(iv) Growth Rate

The relationship between period streak to spot and growth rate, if any, is of an inverse nature as in the case of incubation period. This probably indicates a general link with the principal meterological features.

(g) Discussion

In summing up the implications discussed above, it is evident that atmospheric moisture, temperature, intensity of infection and growth rate of the plant may influence the duration of incubation, i.e. of latency. The latent period during which infection is present but not obvious can be subdivided into the period when the fungus is epiphyllic, from germination of the spore to penetration of the leaf by the infection hypha, and the endogenous period which precedes the appearance of the streak stage lesion.

Moisture and temperature, the effect of which on germination and germ-tube growth has already been discussed, would, undoubtedly, be the decisive influence on development until the fungus reaches the inside of the leaf. Once penetration has taken place the interaction of temperature, intensity of infection and perhaps the growth rate of the plant have to be considered.

The duration of the period from streak to sporing spot is largely dependent on the number of infections per unit leaf area and no effect of environment is clearly indicated in this case.

In this experimental work it was noted that the first streaks to appear are not necessarily the first to change to spots; although usually if a large number of streaks were recorded in the first batch in a particular observation area, some of these would be included among the first to change to spots. The streaks which changed in the shortest time were very often those which appeared last. This could be interpreted as meaning that the spores from which these late streaks arose germinated later so that penetration took place later, by which time the earlier infections had rendered the substrate less resistant or more suitable for development.

As stated above, Leach (1946) considered that increased production of toxin by the fungus and loss of resistance of the leaf tissue was responsible for the acceleration in rate of development of leaf spot with increase in intensity of infection.

It can also be regarded as being due to an increase in the intensity of enzymatic reactions due to increased concentration of the enzymes released by the fungus consequent on the increased amount of mycelium present. By the same reasoning the effect of temperature can be regarded as an effect on reaction rate of the enzymes as well as on the rate of respiration and vegetative growth of the fungus.

Similarly, the effect of increased growth rate of the plant, i.e. increased plant vigour, can be credited to an increased reaction rate consequent on increased hydration and increased concentration of the substrate, two factors which increase the rate of enzymatic reactions (Meyer and Anderson 1956).

Other variations in composition between the leaves of a slowly and a quickly developing plant could alter the situation through the enzyme complex or fungal nutrition.

The lack of evidence of any effect of growth rate on the change from streak to spot could be attributed to the fact that at this stage in leaf spot development enzyme activity becomes very intense, as evidenced by the rapid infiltration of the tissue surrounding the streak which precedes the actual necrosis. This high concentration is evidently sufficient to overcome the normal host resistance which is adequate to restrain the fungus in the earlier stages.

IV. CONTROL EXPERIMENTS IN NORTH QUEENSLAND

(a) Historical Summary

Work on the control of banana leaf spot was carried out in South Queensland during the years 1928–1930. Simmonds (1933) investigated the effect of various practices, including dusting with copper fungicides and stripping of infected

foliage. No definite conclusions were derived from these experiments and it was considered doubtful whether the control exercised by the standard fungicides then available was sufficient to justify regular treatment under South Queensland conditions.

In 1937 it was reported from Surinam that spraying plantations two or three times a year with Bordeaux mixture or Bayer fungicide had given good results (Stahel 1937).

In New South Wales it was demonstrated that summer treatment of plantations with fungicides gave promising results and that excellent control was obtained by applying Bordeaux (4-4-40) each month from mid-December to mid-April (Magee and Foster 1938).

A study of the influence of weather on leaf spot development made by Simmonds in South Queensland about this time also stressed the importance of summer infections and enabled recommendations to be made regarding the timing of sprays in a control programme (Simmonds 1939).

Meanwhile the spread of the disease in the Caribbean region led to investigations into leaf spot control in Jamaica. Here the seasonal effect of the disease was less marked and spraying had to be continued throughout the year. It was found that spraying with "Perenox" ($\frac{1}{4}$–$\frac{1}{2}$ per cent.) at intervals of either 2 or 3 weeks using 100-200 gal per ac gave promising results. Bordeaux mixture (4–4–40) also gave control but the cuprous oxide preparation was superior (Ward 1938). Later work in Jamaica demonstrated the importance of the ascigerous stage of the leaf spot fungus and showed that spraying effectively controlled infection by conidia but not by ascospores (Leach 1946).

In the large banana plantations in Central America, where the disease was well established by the early 1940s, the threat to production was met by the adoption of a costly control programme. Leaf spot was controlled by spraying in cycles of 2–3 weeks with copper sprays, employing stationary spraying apparatus. The mixture was delivered from central pumping stations through permanent pipelines reticulated throughout the plantations. From these the spray was distributed by hoses (Wardlaw 1941).

Continued investigations into the control of leaf spot and speckle in New South Wales resulted in the adoption of control measures consisting of spray applications commencing in early December and repeated each month until March. The mixture recommended was copper oxychloride ($1\frac{1}{2}$ lb to 40 gal) and colloidal sulphur (1 lb to 40 gal) plus a wetting agent. It was stressed that the spray should be directed at the heart-leaf and at the under-sides of the three youngest fully opened leaves (Magee 1945). Application was achieved by the use of portable power pumps or by the installation of permanent pipelines radiating from a central pumping station. For various reasons only a limited number of growers adopted these control measures.

More recent investigators in Jamaica gave attention to improving the performance of leaf spot fungicides by the use of better wetting agents and it was found that the use of a new wetter, sodium succinate, with copper carbonate, Bordeaux mixture or "Perenox" gave good control (Anon. 1953, 1954). The benefits of using suitable wetting agents with banana leaf spot sprays have since been confirmed at Long Ashton (Kearns and Martin 1957).

In 1953 an important development in leaf spot control took place in Guadeloupe. French workers began experimenting with low-volume techniques and a radical departure from conventional low-volume methods was made by the substitution of mineral oil for water as the carrier for the fungicide.

In the initial experiments it was found that fogging with zineb or copper oxychloride, both suspended in oil, gave better leaf spot control than the customary copper oxychloride spray (Guyot 1953). Guyot later showed that two thermo-aerosol treatments in five months were more efficient than fortnightly knapsack spraying with aqueous zineb/copper oxychloride suspensions during the same period. He attributed this result to the fortuitous timing of the fog applications. It was also demonstrated that a fine oily mist applied with a "Minimicron" shoulder-mounted mister gave better control than did a fog applied with a "Swingfog" (Cuillé and Guyot 1954).

When misting and fogging were again compared it was found that misting with zineb/oil was superior to fogging with the same mixture when the output was the same, i.e. 2 gal per ac. Misting with zineb/oil was superior to misting with oil alone but the beneficial effect of the oil alone treatment was undeniable (Guyot and Cuillé 1955).

The low-volume treatment with oily mixtures was quickly adopted elsewhere and has been responsible for a very high degree of leaf spot control. In the Cameroons a widespread outbreak of Cercospora leaf spot was arrested by misting with copper/oil mixture applied either as a mist by knapsack misters or by high-altitude "fogging" from fixed-wing light aircraft. The mixture was applied at intervals of either 2 weeks or 3 weeks (Merle, Cuillé, and De Laroussilhe 1958).

Phytotoxicity from continued application of oil is proving to be a problem, however, and it appears that this treatment can cause premature death of foliage and a reduction in fruit weight (Anon. 1957). In addition, it has been reported that continued use of oil causes a reduction in plant growth and fruit yield (W. A. Brun, personal communication August 1959). F. J. D. Thomas (personal communication July 1959) stated: "It is said that the liability of the banana stem to bark or sheath splitting is increased where oil is used. Continuation with oil spraying, during periods of drought, or in plantations where root efficiency is weakened due to eelworm attack, makes the plants turn yellow and take on a distinctly unhealthy appearance."

The low-volume technique has been used also for the application of a water-based fungicide for leaf spot control. In Ecuador good control of the disease was obtained by misting bananas with Banacobre-Sandoz (a cuprous oxide containing a special waxy sticker) suspended in water. The output was 10 gal per ac and the treatments were repeated at intervals of 3–4 weeks (Tollenaar 1955).

It has been reported from that country that the control of leaf spot obtained by low-volume spraying was equal to or better than that obtained with earlier methods. At 3 gal per ac, "agricultural oil" in water with various fungicides gave good control of the disease and results improved as the oil was increased from 20 per cent. to 60 per cent. "Agricultural oil" alone at 3 gal per ac was almost equally good (Desrosiers and Ampuero 1957).

While the copper fungicides and zineb appear to be the only compounds which have been used in large-scale leaf spot control experiments, many others have been tested (Merny 1954, 1955). Merny, in his first tests of eight fungicides, found that only zineb was better than copper oxychloride. He later tested another 24 compounds for toxicity against *Cercospora musae,* using copper oxychloride as a standard. Copper oxide and zinc coposil (both in oil) were superior to copper oxychloride.

(b) High-volume Spraying with Water-based Fungicides

North Queensland banana plantations are either planted on arable land or are accessible to motor vehicles and hence are suitable for spray application by means of high-pressure spray outfits. Water supply is no problem in the coastal areas, where there is an abundance of fresh running water handy to most plantations at all times of the year. For this reason the main purpose of the work reported here has been an investigation of control measures using high-volume methods.

In the high-volume experiments a series of six major spray trials was carried out during the years 1951-1958. Trials 1 and 2 were located at Mission Beach, near Tully; Trials 3 to 6 on a plantation at Stoney Creek, near Cairns.

Trials 1 to 4 were designed to investigate the economics of protectant spraying for leaf spot control with the standard fungicides available at that time. In the discussion which follows these trials are considered conjointly and the results summarized.

An important development was evident in the results of Trials 5 and 6, viz. the improvement in leaf spot control due to the suppressive effect of mixtures containing white oil emulsion, and these two trials are therefore considered separately in more detail.

(i) Procedure

In all these trials the variety sprayed was either Cavendish or its tall mutant Mons Mari. The work was carried out with a small spray plant consisting

of a twin-cylinder pump powered by a 98 c.c. 4-stroke engine. Working pressures of up to 250 lb per sq. in. were used. Delivery was by high-pressure spray hosing through an adjustable swirling-type nozzle. Under normal working conditions the spray plant was transported through the plantations on a motor truck.

Output per acre varied considerably according to size of plant, planting distance and method of spraying used. An average dosage for a heart-leaf spray on a plant nearing flowering was half-a-gallon. A cover spray required considerably more.

In this work heart-leaf spraying was regarded as treatment of the unfolding heart-leaf and all the leaves which had emerged since the previous spray round, the spray being directed particularly at the lower surfaces of the leaves. Cover spraying was regarded as treatment of all the leaves on the plant on both upper and lower leaf surfaces.

In all experiments a randomized block layout was used. Single-row plots were employed and the number of plants used varied from 7 to 10 per plot in individual experiments.

The assessment of results was achieved in the following manner:

(1) *Infection.*—When a plant flowered and again when its bunch was harvested a count was made of live leaves and each leaf was given a percentage rating for leaf spot infection based on a visual estimate of the damage. From these data an average figure for amount of infection per leaf per plant at each of these critical stages was calculated.

(2) *Yield.*—In the Mission Beach experiments the bunches were weighed when they were cut each week. The yield data were then expressed as a mean weight of marketable fruit per plant. In arriving at this figure the grower's practice of discarding any bunches which ripened prematurely was followed.

In the experiments at Stoney Creek the bunches were harvested once or twice each week. Each bunch was weighed immediately it was cut. If a bunch carried any prematurely ripened fingers the weight of the fruit thus involved was estimated and expressed as fruit wastage. In addition, three fingers were chosen at random on the third hand of each bunch. The length (L) and girth (G) of each of these fingers was measured (in in.). As a convenient index of fruit size the quantity $L \times G^2$, which has the dimensions of volume, was used.

Butt circumference is not regarded in this work as being related to spray treatment but rather to plant vigour. Butt circumference measurements were made in these experiments in order to make allowance for the large variations in stool vigour which are usually evident in a banana plantation. Bunch size in relation to number of fruit appeared to be proportional to butt circumference, and bunch weight is dependent on bunch size as well as on other factors such as fruit size which definitely may be related to spray treatment.

The yields were then assessed on adjusted bunch weights, which were estimates of the bunch weights adjusted by co-variance analysis for variations in butt circumference.

(3) *Economic Returns.*—Plant density in the Stoney Creek trials was approximately 500 stools per acre. The prices which the grower received for fruit harvested from the experimental blocks during the period of the experiments ranged from 6d. to 1s. 3d. per lb. For the purposes of this experimental work a figure for estimated gross return per plant due to spraying was arrived at by allowing a price of 7d. per lb (£2 10s. per standard banana case). This was calculated for those adjusted mean bunch weights which were significantly greater than the mean bunch weight for the unsprayed treatment. The gross return per acre was then assessed on the basis of a harvest of 500 bunches per acre.

(ii) Trials 1 to 4, 1951–1955

In these experiments copper oxychloride was compared with copper oxychloride plus wettable sulphur. Copper oxychloride, Bordeaux mixture (3-2-40) and home-made cuprous oxide (Mandelson 1933) were compared with the organic fungicides thiram, ziram and zineb. The only additive used was a wetting agent, which was varied in the different experiments. "Lissapol N300" (0·1 per cent.), "Comprox" (0·3 per cent.) and soap powder (1½ lb-40 gal) were all tried. Soap powder was incompatible with Bordeaux mixture.

In addition, various schedules were tested, the chief purpose being to determine the minimum number of heart-leaf sprays which was necessary to provide satisfactory control under North Queensland conditions. A schedule involving cover spraying commencing late and carried through until the bunch was harvested was also investigated.

The information gained from this phase of the work can be summarized as follows:

(1) Copper oxychloride and copper oxychloride plus wettable sulphur were equal in efficiency.

(2) The copper fungicides as a group were superior to the organic fungicides, and copper oxychloride (1½ lb-40 gal) and Bordeaux mixture (3-2-40) were more efficient than home-made cuprous oxide (3 gal-40 gal).

(3) The effects of leaf spot spraying were cumulative and progressive reduction in inoculum potential resulted in much better control in the second crop when two successive ratoons were treated with the same materials.

(4) The control of the disease obtained with a schedule of heart-leaf sprays at intervals of four weeks which commenced prior to the wet season (November) and continued until the plants flowered was equal to that which resulted from a similar schedule which commenced some months earlier (July). However, some

degree of control was obtained by a schedule in which heart-leaf spraying was commenced in March and was repeated at intervals of four weeks until the plants flowered. The importance of fungicidal cover during the late summer and autumn was thus emphasized.

(5) An interesting point was that some protection was given by late cover sprays which were first applied when the plants were close to flowering and were continued after the bunches were thrown. This indicated that fresh infection during the period between flowering and harvest could be an important cause of damage.

(6) No significant differences either in bunch weight or in amount of fruit wastage due to premature ripening resulted from the use of brown paper or hessian bunch covers.

(7) Increments in gross monetary return per acre of up to £187 10s. were estimated for the best treatments, which were copper oxychloride (1½ lb-40 gal) and Bordeaux mixture (3-2-40).

(iii) Trial 5, 1956–1957

This experiment was designed to determine whether the efficacy of a leaf spot fungicide, in this case Bordeaux mixture (3-2-40), could be improved by the use of a sticker. White oil (1-160) was chosen because this adhesive had given good results when used with Bordeaux mixture for citrus black spot control in New South Wales (Kiely 1950) and also when used with cuprous oxide mixture for the control of the same disease in North Queensland. This is the common agricultural white oil prepared for use as an insecticide. The second sticker used was "Calspred", a proprietary product containing calcium caseinate. In addition, in an attempt to improve the antisporulant properties of the mixture, malachite green, which has been mentioned as a possible antisporulant (Horsfall 1945), was included. Urea was likewise used as an additive, mainly because it had been suggested to the author as a depressor of sporulation (G. D. Bowen, personal communication 1955).

Treatments were as follows:

A. Bordeaux mixture (3-2-40) plus "Calspred".

B. Bordeaux mixture (3-2-40) plus urea (1 per cent.) plus glucose (·25 per cent.) plus wetting agent ("Lissapol N300" 0·03 per cent.).

C. Bordeaux mixture (3-2-40) plus wetting agent ("Lissapol N300" 0·03 per cent.).

D. Bordeaux mixture (3-2-40) plus white oil (1-160) plus malachite green (1-10,000).

E1. Unsprayed. Bagged.

E2. Unsprayed.

All bunches on sprayed plants were bagged (enclosed in brown paper or hessian covers). Two schedules were used:

Schedule 1.—Cover spray in January followed by heart-leaf sprays repeated at intervals of four weeks until the plants flowered.

Schedule 2.—Late cover sprays commenced in May and repeated at intervals of four weeks until the bunches were reaped.

The results are summarized in Tables 7, 8, 9 and 10.

TABLE 7

Mean Values for Number of Leaves at Flowering and Harvest, Trial 5, 1956–1957

	Flowering		Harvest	
	1 Heart-leaf	2 Cover	1 Heart-leaf	2 Cover
A. Bordeaux mixture + "Calspred" ..	11·0	10·0	5·3	4·6
B. Bordeaux mixture + Urea	11·2	10·1	6·0	4·0
C. Bordeaux mixture + "Lissapol N300"	11·0	10·0	6·4	4·6
D. Bordeaux mixture + White oil + Malachite green	13·4	10·7	6·7	5·4
E1. Unsprayed. Bagged	9·0	..	1·9	..
E2. Unsprayed. Not bagged	9·7	..	3·6	..
	s.e. = ·38	..	s.e. = ·44	..
Necessary differences for significance 5%	1·1		1·3	
Necessary differences for significance 1%	1·5		1·7	
	D1 >> all others B1, C1, A1, D2 >> E1 B1 > A2, B2, C2, E2 C1, A1 > E2 B2 > E1		D1, C1 >> A2, B2, C2, E1, E2 B1 >> B2, E1, E2 D2 >> E1, E2 A1, A2, C2, B2 >> E1 E2 > E1 D1 > A1 B1 > A2, C2 D2 > B2 A1 > E2	

TABLE 8

Leaf Spot Infection—Equivalent Percentages, Trial 5, 1956–1957

	Flowering		Harvest	
	1 Heart-leaf	2 Cover	1 Heart-leaf	2 Cover
A. Bordeaux mixture + "Calspred" ..	14·0	15·0	18·7	26·4
B. Bordeaux mixture + Urea	15·0	14·4	22·2	21·6
C. Bordeaux mixture + "Lissapol N300"	16·3	14·4	20·1	21·5
D. Bordeaux mixture + White oil + Malachite green	9·1	13·5	11·0	18·7
E1. Unsprayed. Bagged	17·7	..	76·4	..
E2. Unsprayed. Not bagged	14·2	..	40·6	..
	No significant differences		All others << E1 D1 << E2	

TABLE 9

Mean Values for Bunch Weights and Butt Circumferences, Trial 5, 1956–1957

—	Observed Bunch Weight		Butt Circumference		Adjusted Bunch Weight		Estimated Increased Gross Return (per plant)	
	1 Heart-leaf	2 Cover	1 Heart-leaf	2 Cover	1 Heart-leaf	2 Cover	1 Heart-leaf	2 Cover
							s. d.	*s. d.*
A. Bordeaux mixture + "Calspred" ..	35·7	37·0	24·1	24·9	37·7	35·9	2 10	1 9
B. Bordeaux mixture + Urea	36·8	34·1	24·2	23·8	38·6	37·1	3 4	2 5
C. Bordeaux mixture + "Lissapol N300"	42·0	36·0	25·8	24·3	37·6	37·3	2 9	2 6
D. Bordeaux mixture + White oil + Malachite green	40·1	36·7	24·3	24·8	41·3	36·3	4 11	2 0
E1. Unsprayed. Bagged	30·4	..	24·7	..	30·2	..	..	..
E2. Unsprayed. Not bagged	36·0	..	25·4	..	32·9	..	..	..
	s.e. = 2·45				s.e. = 1·52			
	No significant differences				D1 >> E1, E2 A1, B1, C1, C2, B2, D2 >> E1 A2 > E1 D1 > A2, D2 B1, A1, C1 > E2			

TABLE 10

Mean Values for Fruit Size (L × G²), Trial 5, 1956–1957

	1 Heart-leaf	2 Cover
A. Bordeaux mixture + " Calspred "	199·8	184·7
B. Bordeaux mixture + Urea	201·1	176·6
C. Bordeaux mixture + " Lissapol N300 "	211·8	189·5
D. Bordeaux mixture + White oil + Malachite green	208·8	188·5
E1. Unsprayed. Bagged	154·2	..
E2. Unsprayed. Not bagged	183·5	..
	s.e. = 8·71	
	C1, D1, B1, A1, C2, D2 >> E1 C1 >> B2 C1 > A2, E2 D1 > B2 A2, E2 > E1	

When the cover sprays (Schedule 2) commenced in May all plants in these plots except a very few backward stools were treated. Spraying was initiated on these odd plants when they reached an appropriate size. Some of the Schedule 2 plants had flowered when they received their first treatment in May but the majority were still producing leaves.

It is apparent from the results that the outstanding feature of this experiment was the success of Bordeaux mixture plus white oil plus malachite green, Schedule 1. The efficacy of this mixture was also evident when it was used as a late cover spray.

Observations made during the course of this trial showed that the increased efficacy of Bordeaux mixture (3-2-40) when it was used with malachite green and white oil was not due to any superior sticking qualities but to the fact that it suppressed or delayed leaf spot development, i.e. it was a potent fungistatic agent. This phenomenon will be discussed in detail in a later section of this article.

There was little difference between the other treatments. The slight differences in performance between Treatments B (Bordeaux mixture/urea/"Lissapol N300") and C (Bordeaux mixture/"Lissapol N300") could have been due to the fact that the 0·25 per cent. glucose included in the urea formula brought about a partial reduction of the basic copper sulphate to cuprous oxide. Schedule 2 treatments were consistently inferior to their Schedule 1 counterparts but in most cases were not significantly so.

The estimated increment in gross monetary return per acre ranged from £123 (Bordeaux mixture/white oil/malachite green, Schedule 1) to £43 15s. (Bordeaux mixture/"Calspred", Schedule 2).

The seasonal variation in disease intensity which has been discussed previously had obvious effects on the results of this trial. In Figure 7 leaf counts and leaf spot appraisals made for the 1956 and 1957 trials on the unsprayed plants when their bunches were cut have been related to time of flowering and mean monthly rainfall. There was a definite relationship between the time of flowering and the amount of leaf spot at harvest. Those plants which flowered late in the year exhibited a much reduced amount of leaf spot damage.

The apparently anomalous results in Tables 7 and 10 showing Treatment E1 (unsprayed, bagged) to be significantly worse than E2 (unsprayed, not bagged) are better understood when it is considered that 35 per cent. of the E1 plants flowered in the April-May period compared with 16 per cent. of the E2 plants during the same critical period. Nevertheless, the results indicated that in this experiment bagging bunches on unsprayed plants was of no benefit.

(iv) Trial 6, 1956-1958

Because of the success of the Bordeaux mixture/white oil/malachite green formula, the use of these two additives with other fungicides was investigated. It was also necessary to know whether the increased efficacy gained by the use of white oil plus malachite green in the spray mix could be equalled by the addition of white oil alone.

The stools used in the experiment were first ratoons, the mother plants of which had comprised portion of the experimental block used in Trial 5.

Treatments were as follows:

A. Bordeaux mixture (3-2-40) plus white oil (1-160).

B. Copper oxychloride (1½-40) plus white oil (1-160) plus malachite green (1-10,000).

C. Zineb (1½-40) plus white oil (1-160) plus malachite green (1-10,000).

D. Bordeaux mixture (3-2-40) plus white oil (1-160) plus malachite green (1-10,000).

E. Unsprayed.

Spraying commenced in December 1956, when most plants were spear suckers or suckers with first broad leaves commencing to unfurl. Subsequent applications were heart-leaf sprays repeated at intervals of four weeks until the plants flowered.

TABLE 11

Number of Leaves, Percentage Leaf Spot, and Fruit Size (L × G²), Trial 6, 1956–1958

—	No. of Leaves		Percentage Leaf Spot (Equiv. %)		Fruit Size
	Flowering	Harvest	Flowering	Harvest	
A. Bordeaux mixture + White oil ..	13·0	7·4	2·4	6·4	218·0
B. Copper oxychloride + White oil + Malachite green	12·8	6·7	1·7	7·8	220·2
C. Zineb + White oil + Malachite green	14·0	8·3	3·4	9·7	224·5
D. Bordeaux mixture + White oil + Malachite green	13·4	7·5	2·7	7·7	216·6
E. Unsprayed	10·0	4·0	14·4	51·2	189·1
s.e.	·41	·43	1·14	3·92	5·62
	A, B, C, D >> E		A, B, C, D << E		A, B, C, D >> E

The results are given in Tables 11 and 12 and illustrated in Figures 8 and 9.

All the spray treatments were significantly better than the controls, and Bordeaux mixture (3-2-40)/white oil was equal in efficacy to Bordeaux (3-2-40)/white oil/malachite green.

The increment in estimated gross monetary return per acre ranged from £122 18s. 4d. (zineb/white oil/malachite green) to £87 10s. (Bordeaux mixture/white oil).

The effect of the dry season on leaf spot incidence in the block was once more particularly noticeable (Figure 7). It was apparent in this experiment that plants which flowered later than July retained sufficient foliage to allow the fruit to reach the desired state of fullness without the benefit of spray treatment.

TABLE 12

Mean Values for Bunch Weight and Butt Circumference, Trial 6, 1956–1958

—	Observed Bunch Weight	Butt Circum-ference	Adjusted Bunch Weight	Estimated Increased Gross Return (per plant)
				s. d.
A. Bordeaux mixture + White oil	46·8	28·5	45·5	3 6
B. Copper oxychloride + White oil + Malachite green	47·0	27·9	47·3	4 8
C. Zineb + White oil + Malachite green	48·4	28·2	47·9	4 11
D. Bordeaux mixture + White oil + Malachite green	43·9	27·1	46·3	4 0
E. Unsprayed	40·2	28·3	39·4	..
s.e.	1·93	..	1·57	..
	A, B, C > E		C, B >> E D, A > E	

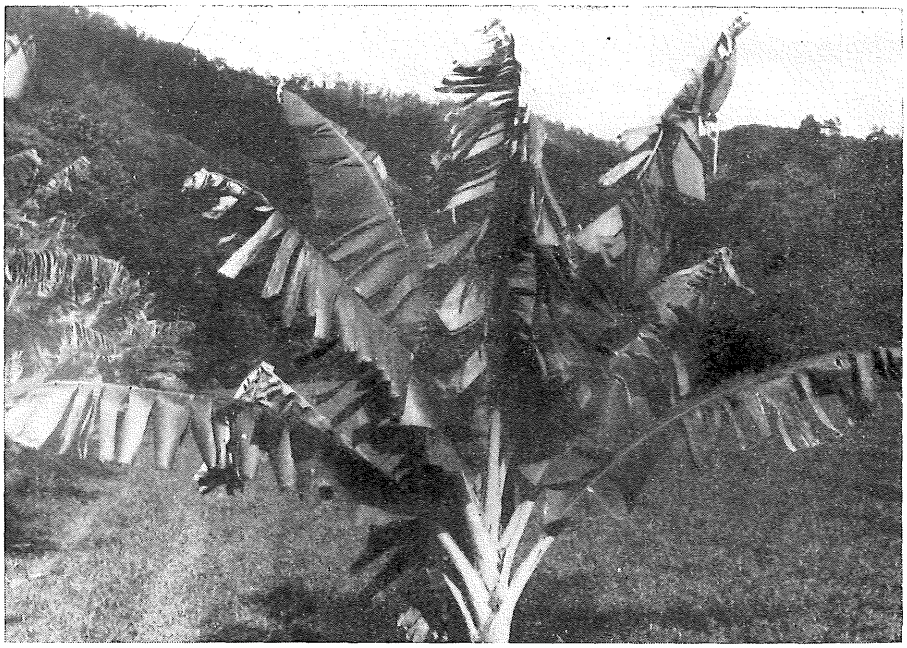

Fig. 8.—Appearance at time of harvest of a plant sprayed with zineb + white oil + malachite green. Trial 6, Stoney Creek, July 1957.

Fig. 9.—Appearance at time of harvest of an unsprayed plant. Trial 6, Stoney Creek, July 1957.

(c) Low-volume Treatment with an Oil-based Fungicide

When details of the low-volume technique employing oils and oil/fungicide mixtures developed by Guyot and Cuillé in the Antilles became known a limited amount of work was done on this aspect of the leaf spot problem. This was carried out in the hope that the technique would prove suitable for use in South Queensland. Here the majority of plantations are on hillside sites and motor vehicles cannot be used for transport. Water supplies are either absent or deficient on most farms.

(i) Procedure

The machine used was a "Swingfog SN6." This was the only low-volume machine available in Queensland at the time. This machine could be fitted with an atomizer for misting or a fogging attachment as desired. A 1·1 mm flow control jet was generally used for misting and a 1·0 mm jet for fogging. The mixtures employed were slight modifications of those tested by the French workers in their early trials, namely:

Misting mixture:	Copper oxychloride	6½ lb
	Oil (4½ gal "Vacuum EF 217S"; 1 gal Dieselene)	5½ gal
Fogging mixture:	Copper oxychloride	12 lb
	Oil (2 gal lubricating oil SAE 30; 2 gal Dieselene)	4 gal

The treatments were applied at a slow walking pace. The operator walked along each row, the direction of travel being at right angles to the wind, with the mist or fog discharging in the direction of the wind. The output in the case of misting was 1½-2 gal per ac and for fogging ¾-1 gal per ac.

The method of assessing the results was as follows: The centre row in each plot was regarded as the datum row. Leaf spot damage on each plant in this row was assessed when it flowered by making leaf counts and leaf spot appraisals as in the experiments described above. The same procedure was employed to assess leaf spot damage on the followers when the treatments were terminated in September 1957.

The bunch harvested from each plant in the datum row was weighed and the data were used to calculate an average bunch weight for each treatment.

(ii) Trial 1, 1956-1957

The plants treated were first ratoon Mons Mari bananas which commenced to flower in April 1957. Treatments commenced on December 24, 1956, and continued until September 1957, by which time the bunches on the first ratoon plants had been harvested.

A randomized layout was not used. Two reasonably uniform blocks of plants were selected in a plantation near the Barron River outside Cairns. Each block consisted of 15 rows each containing 21 plants.

Three treatments were located in each block, 5 rows of 21 plants being allotted per treatment. These were as follows:

Block 1: A. Misting at weekly intervals.

B. Fogging at weekly intervals.

C. Untreated.

Block 2: D. Misting at fortnightly intervals.

E. Fogging at fortnightly intervals.

F. Untreated.

The results are summarized in Table 13.

TABLE 13

Mean Values for Number of Leaves, Percentage Leaf Spot and Bunch Weight, Trial 1, 1956–1957

Treatment	No. of Leaves		Percentage Leaf Spot		Average Bunch Weight (lb)
	Parent	Follower	Parent	Follower	
Block 1—					
Misting—weekly ..	10·2	8·7	8·4	11·1	38·9
Fogging—weekly ..	10·2	8·4	16·2	14·1	37·1
Unsprayed	8·3	6·6	18·5	15·3	29·0
Block 2—					
Misting—fortnightly ..	8·7	7·1	19·1	16·2	29·6
Fogging—fortnightly ..	8·5	6·8	21·4	15·8	31·4
Unsprayed	7·7	6·5	19·6	18·0	30·1

Unusually heavy rains in December 1956 encouraged an early build-up of leaf spot in the experimental blocks and the benefits of oil treatment were plainly visible by March 1957. The weekly treatments were of much more benefit than those applied at fortnightly intervals. This is reflected in the means shown in Table 13 for number of leaves and percentage leaf spot at flowering. The yield figures show that both misting and fogging at weekly intervals gave substantial increases in mean bunch weight. The fortnightly treatments were definitely not economical.

The control of the disease obtained on the followers was disappointing, however, and the cumulative effects noted from spray treatment of successive crops was not evident here.

Control of leaf spot was actually better than the figures show, since the oil treatments were not effective against speckle (*Mycosphaerella musae* (Speg.) Syd.) and damage from this disease contributed considerably to premature death of foliage. Also, particularly on those plants which were misted weekly, oil toxicity to the lower leaves detracted from the performance of the mixture.

(d) Phytotoxicity

In the course of the field experimental work described above notes were made on the type and severity of injury to the plant associated with the various treatments. The important features of these observations are discussed below.

(i) Water-based Mixtures

Leaf spot sprays containing white oil and malachite green have been observed to be phytotoxic under certain conditions, in particular during the wet season months when temperature and atmospheric humidity are high and overcast skies often retard the drying of the spray on the leaf surface. Three leaf symptoms are commonly seen:

(1) Tan or brown speckled patches, spots or blotches on the lower surface of the leaves. These blemishes appear to be mainly superficial and apparently are not harmful. It is thought that they are occasioned by the oil emulsion.

(2) Watersoaked areas on the older leaves which eventually become evident as large necrotic spots or dark-brown to black specks on the upper surface of the leaves. These symptoms are encountered when mixtures containing copper are used—particularly copper oxychloride. The condition was often seen when copper oxychloride alone was used for leaf spot control. This type of damage is restricted to the older foliage and becomes more evident when treatment is continued after a plant has flowered. This might be due to the accumulation of toxic residues on the tops of these leaves; again, it might be occasioned by a loss of resistance as the leaf ages, perhaps due to the gradual loss of the protective waxy coating.

(3) A bright yellowing and leaf drop of the oldest leaf or leaves. This is seen only on obviously unthrifty plants.

In addition, some fruit pitting and oil staining are likely when a coarse spray is applied to young fruit. This is not usual when plants are hand-sprayed, for care can be taken to ensure that the jet is not directed at the young fruit. It has been seen when fixed nozzles are employed, e.g. on vertical boom spray rigs. It can be corrected by using higher pressures and small nozzle orifices.

(ii) Oil-based Mixtures

Two types of symptoms have been observed on plants treated with oily mixtures, both usually on lower leaves:

(1) *Acute.*—This eventuates when a leaf receives a direct blast of the concentrate at short range. The damage quickly becomes evident as a large necrotic patch.

(2) *Chronic.*—This is a result of continued treatment with oily mixtures and is manifested by a bright chlorosis, sometimes tending towards a bronzing, of the oldest leaves. It causes premature death of this basal foliage.

The fruit are particularly prone to oil staining and marking when the misting technique is employed and great care has to be taken to ensure that the oily mist is not directed towards the bunches.

(e) Effect of the Individual Spray Constituents

The excellent control of conidial infection obtained in Jamacia by spraying every third week with either cuprous oxide or Bordeaux mixture was attributed by Leach (1946) firstly to reduction in the number of conidia produced on sprayed leaf spots and secondly to the toxic effect, on any conidia which were produced, of dew which had been in contact with spray residues. The efficacy of these two sprays in reducing conidial sporulation was demonstrated by this author, who also showed that the viability of conidia of *Mycosphaerella musicola* was much reduced after they had been suspended in dew taken from the upper surface of sprayed leaves. He concluded, therefore, that leaf spot control was "not obtained in the usually accepted manner", i.e. by the fungicidal action of the spray residue which adheres to the susceptible surfaces of young leaves.

The antisporulant effects of Bordeaux mixture were also noted by Calpouzos (1955), who, however, qualified his observations by the statement that "the length of time that the spray application exerts its inhibiting properties is closely related to prevailing weather conditions".

In North Queensland the superiority of mixtures containing white oil and malachite green over those previously tested for efficiency in leaf spot control led to an investigation of the properties of these mixtures. The spray selected for testing was copper oxychloride (1½ lb-40 gal) plus white oil (1-160) plus malachite green (1-10,000). This mixture had given results equally as good as those obtained with the other fungicides used in the final field trial. In the experiments described below the three ingredients were used singly or in pairs so that their fungitoxic and antisporulant properties could be compared with those of the complete mixture.

The white oil emulsion used in the field trials, viz. a proprietary mayonnaise type formulation containing 80 per cent. by weight refined paraffin oils, was employed in these tests.

(i) Fungitoxicity

In Experiments 1 and 2 each spray treatment was applied with a "Rega" continuous atomizer to the upper and lower surfaces of the four youngest fully opened leaves on each of three plants. A plot thus comprised one plant. There

were two control plots in Experiment 1. The leaves were sprayed once only. The observations were made within a 6 in. x 2 in. rectangle marked with a "Chinagraph" pencil in a central position on the left-hand side (i.e. the first side to unfurl) of each leaf. Before the leaves were sprayed any lesions present within these areas were marked, and at regular intervals from the date of spraying, notes were made of the numbers of fresh streaks which had appeared and of the streaks which had changed to spots.

The treatments and results are given in Table 14, where the data are expressed as mean numbers of streaks per marked rectangle for the three plants. In Figure 10 a histogram illustrates the extent and the rate of change from streak to spot associated with the various treatments in Experiment 1. The columns represent weekly counts over a period of six weeks.

TABLE 14

Toxicity Experiments 1 and 2—Total Number of Streak Lesions Appearing within 6 × 2 in. Rectangles (Expressed as Means of Results from 3 Plants)

Leaf Position (from top)	1		2		3		4	
Treatment	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
Copper oxychloride (1½–40)	0	117	54	112	66	149	110	124
Malachite green (1–10,000)	95	31	87	90	83	187	89	110
Malachite green (1–5,000)	..	136	..	81	..	102	..	167
White oil emulsion (1–160)	0	27	3	134	125	133	71	81
White oil emulsion (1–160) + Malachite green (1–10,000)	0	0	26	53	159	50	120	146
Copper oxychloride (1½–40) + Malachite green (1–10,000)	16	85	41	144	46	25	63	101
Copper oxychloride (1½–40) + White oil emulsion (1–160)	0	9	38	124	34	75	91	174
Copper oxychloride (1½–40) + White oil emulsion (1–160) + Malachite green (1–10,000)	3	0	7	70	45	78	39	107
Unsprayed	161	172	140	173	136	180	89	89

The lesion counts in these experiments were very variable and the degree of control obtained, except in the case of those sprays having a suppressive action, varied with the amount of infection which had been initiated prior to the spray application. For example, the apparent anomaly in Experiment 2 where malachite green (1-10,000) gave better results than either copper oxychloride (1½-40) or malachite green (1-5,000) was quite obviously related to the length of time the first leaves had been unfolded when the treatments were applied. This was easy to determine by comparing the states of development of the unfolding heart-leaves on the various plants.

In order to reduce or eliminate this variation it was necessary to adopt an experimental design in which the treatments to be compared were randomized

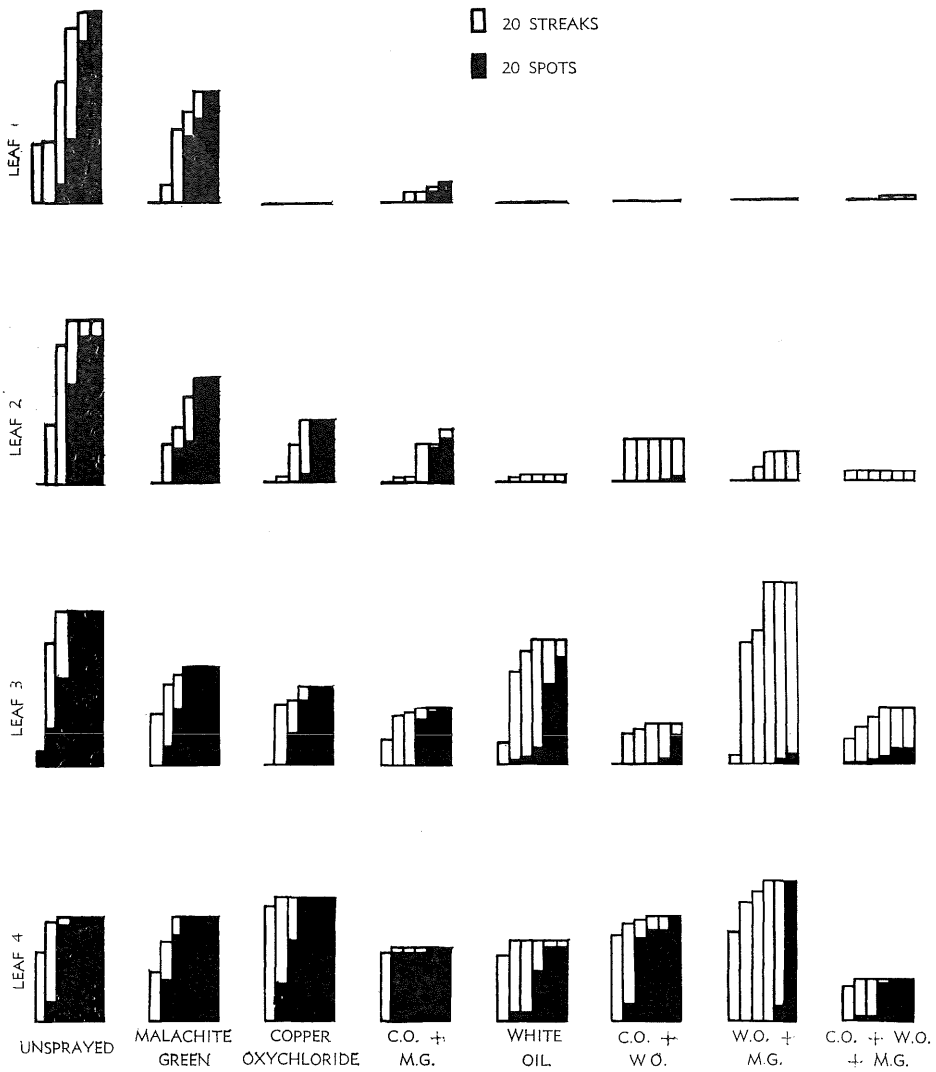

Fig. 10.—Toxicity Experiment No. 1. Mean number of streaks (white) and spots (black) per marked leaf area in weekly counts over a period of six weeks.

on a single leaf. A modification of a method used elsewhere for testing banana leaf spot fungicides (Merny 1955) was therefore used in Experiments 3, 4, 5 and 6. The fungicides were applied with a nebulizer to circles 4·5 cm in diameter drawn on the lower surfaces of the treated leaves. A single application was made when the trial commenced. Usually four treatments—three sprays and one unsprayed check—were included in each trial (Figure 11). The four circles were randomized in each of four positions on the leaf in question and this was repeated on each of four plants. Sixteen replicates of each treatment were thus provided. The four youngest fully opened leaves on each plant were treated.

In each case copper oxychloride was used at a strength of 1½ lb to 40 gal and white oil emulsion at 1-160 by volume.

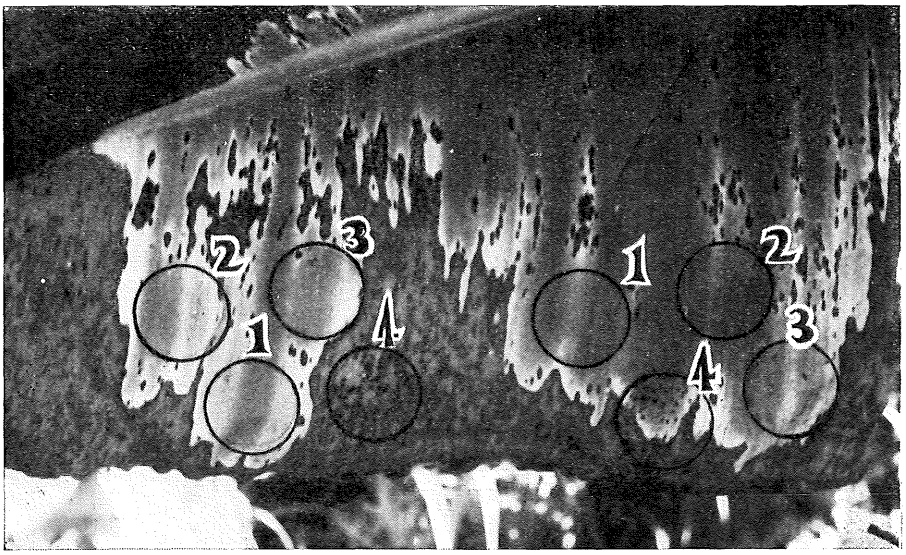

Fig. 11.—The replicated circle method, Toxicity Experiment No. 6. Control obtained by one application to first leaf. Treatments: 1, Copper oxychloride + white oil + malachite green. 2, White oil + malachite green. 3, White oil. 4, Unsprayed.

A proprietary wax emulsion at a strength of 1 in 80 was included in Experiment 3 in order to determine whether this type of paraffin compound possessed properties similar to those of the white oil emulsion.

Results were assessed by making counts at regular intervals of the numbers of streaks and spots in the treated circles. The data are detailed in Tables 15, 16, 17 and 18.

TABLE 15

Toxicity Experiment 3. Equivalent Means of Numbers of Streaks

Treatment	Leaf No.				Mean
	1	2	3	4	
1. Copper oxychloride	3·3	40·3	40·9	48·3	23·6
2. Wax emulsion (1–80)	14·7	47·5	54·6	49·0	37·2
3. Copper oxychloride + Malachite green (1–5,000)	4·7	42·0	47·5	46·8	26·5
4. Unsprayed	29·5	50·6	50·8	57·5	45·8
Means	9·4	44·9	48·2	50·3	32·1
	1, 3 << 2 2 << 4	No significant differences			

TABLE 16

Toxicity Experiment 4. Equivalent Means of Numbers of Streaks

Treatment	Leaf No.				Mean
	1	2	3	4	
1. Copper oxychloride + White oil emulsion	·3	5·4	17·6	9·4	5·3
2. White oil emulsion	2·8	5·9	11·6	21·4	8·2
3. Unsprayed	35·1	40·5	66·9	47·1	46·0
Mean	4·6	11·2	24·2	21·3	13·0
	1<<2<<3	1, 2<<3	1, 2<<3	1<<2<<3	

TABLE 17

Toxicity Experiment 5. Equivalent Means of Numbers of Streaks

Treatment	Leaf No.				Mean
	1	2	3	4	
1. Copper oxychloride + White oil emulsion + Malachite green (1–5,000)	2·1	13·9	16·5	16·3	9·9
2. White oil emulsion + Malachite green (1–5,000)	0·6	3·6	9·9	15·4	5·1
3. Malachite green (1–5,000) ..	23·9	43·1	53·1	33·1	36·7
4. Unsprayed	41·8	41·4	53·8	31·8	41·6
Mean	7·6	17·9	26·5	22·7	17·0
	2<1<<3 3<4	2<<1 1<<3, 4	1, 2<<3, 4	1, 2<<3 2<<4 1<4	

TABLE 18

Toxicity Experiment 6. Mean Numbers of Streaks

Treatment	Leaf No.		
	1	2	3
1. Copper oxychloride + White oil emulsion + Malachite green (1–10,000)	0·1	0·6	13·4
2. White oil emulsion + Malachite green (1–10,000)	0·	0·1	13·9
3. White oil emulsion	0·	0·5	16·1
4. Unsprayed	22·2	35·1	30·8

These data were not analysed, the differences being obvious for Leaves 1 and 2.

Fungicidal and/or fungistatic properties conventionally are evaluated by ability to inhibit spore germination. It was necessary to know the toxicity to leaf spot spores of each of the three constituents of the leaf spot spray formula. This was therefore investigated in a series of *in vitro* spore germination tests.

The conidia employed in these experiments were removed from mature leaf spots which had been excised from spotted leaves and incubated overnight in a saturated atmosphere at 81°F. The following technique was used to prepare the spore suspension: A drop of the liquid in which the spores were to be suspended was placed on each spot. These drops were then sucked up with a 1 ml pipette and the washings from at least six spots were transferred first to an excavated block and thence to sterile glass slides, coated with a film of plain agar (2 per cent. water agar) or with a 2 per cent. agar prepared from white oil emulsion (1-160). These slides were enclosed in petri dishes on damp cotton wool. Usually three drops were placed on each slide.

Germination counts were made after incubation at 81°F for at least 48 hr. Three hundred spores per treatment were counted.

TABLE 19

Toxicity Experiments 7, 8, 9. Effect of White Oil Emulsion, Malachite Green and Copper Oxychloride on Germination of Conidia of Mycosphaerella musicola

Spore Suspension	Germination Surface	Germination (%)
Experiment 7—		
White oil emulsion (1–160)	2% plain agar	55
Malachite green (1–10,000)	„	3
Sterile water	„	94
Sterile water	2% plain agar + white oil emulsion (1–160)	70
Experiment 8—		
Sterile water	2% plain agar	60
"Agrimycin" solution 200 p.p.m.	„	100
"Agrimycin" solution + white oil emulsion (1–160)	„	100
Experiment 9—		
"Agrimycin" solution 200 p.p.m.	2% plain agar	100
"Agrimycin" solution + Malachite green (1–10,000)	„	0
"Agrimycin" solution + White oil emulsion (1–160)	„	100
"Agrimycin" solution + Copper oxychloride (1½–40)	„	0
"Agrimycin" solution 200 p.p.m.	2% plain agar + white oil emulsion (1–160)	100

In preliminary spore germination tests a great variation due to bacterial contamination had been noted in percentage germination of spores removed from spots in sterile water and it had been found that this could be avoided by washing the spots lightly prior to incubation in "Agrimycin" (15 per cent. streptomycin; 1·5 per cent. oxytetracycline) 200 p.p.m. or by using "Agrimycin" solution 200 p.p.m. as the germinating medium.

The series comprised three experiments. In the third experiment the spots were washed lightly in "Agrimycin" solution prior to incubation. The treatments and results are summarized in Table 19.

(ii) Antisporulant Action

In the first series of experiments four trials were conducted. In three of these the sprays were applied to the upper surfaces of spots which had been cut from the leaves, washed in water, dried and pinned on heavy cardboard so that they could be sprayed collectively. After spraying, the spots were dried and then incubated overnight at 81°F on damp cotton wool in 4 in. petri dishes. Six spots were exposed in each dish. In the remaining trial individual leaves were treated and the required number of spots was excised as soon as the spray had dried. The spots were then incubated as above. In each of these trials 18 spots per treatment were examined.

In a second series of two trials the effect of weathering on antisporulant properties was investigated. The treatments used were those combinations which had been shown to give the best control of leaf spot. The sprays were applied to the upper surfaces of spotted leaves. One plant was sprayed with each treatment and the results were assessed by examining 36 spots at a similar stage of development from each of these sprayed plants. The conditions in these two trials were: (1) exposure for 3 days, no rain during this period, heavy dews each night; and (2) exposure for 6 days, 0·81 in. of rain on one night during the 6-day period, heavy dews on the other nights.

The method of assessment in both series was as follows: Each spot was examined with a dissecting microscope (x10 eyepiece, x4 objective) and assigned a rating for number of sporing sporodochia and intensity of sporulation on these sporodochia on the following scales:

Number		Intensity	
	Nil	0	Nil
1	1– 25%	1	Trace
2	26– 50%	2	No more than 25%
3	51– 75%	3	26– 50%
4	76–100%	4	51– 75%
		5	76–100%

These ratings were used to derive a figure for percentage sporulation for each treatment. This figure was obtained by calculating the product (mean number x mean intensity) in each trial; this was then expressed as a percentage of the maximum possible product (20) and the mean percentage sporulation for the series (in the case of the first series) or for each of the two trials (in the case of the second series) was calculated.

The results are shown in Tables 20 and 21.

TABLE 20

Antisporulant Experiments. First Series. Mean Percentage Conidial Sporulation on Sprayed Leaf Spots

Treatment	Sporulation
Copper oxychloride (1½–40)	20·4
Malachite green (1–10,000)	12·8
Malachite green (1–5,000)	0·6
White oil emulsion (1–160)	39·15
Copper oxychloride + Malachite green (1–10,000)	2·0
Copper oxychloride + White oil emulsion	22·4
White oil emulsion + Malachite green (1–10,000)	15·0
Copper oxychloride + White oil emulsion + Malachite green (1–10,000)	6·0
Unsprayed	47·0

TABLE 21

Antisporulant Experiments. Second Series. Mean Percentage Conidial Sporulation on Sprayed Leaf Spots after Exposure

Treatment	Period of Exposure	
	3 days	6 days
Copper oxychloride (1½–40) + White oil emulsion (1–160) + Malachite green (1–10,000)	6	31
White oil emulsion + Malachite green	31	58
White oil emulsion	33	60
Unsprayed	52	60

(iii) Discussion

Analyses of the data from toxicity Experiments 3, 4 and 5 were carried out in terms of the variable log (1 + x), where x is the number of streaks. In all analyses the term treatment x leaves was highly significant, indicating that the differences between treatments varied from leaf to leaf. In the tables showing mean values, differences between treatments for each leaf are shown.

The results of Experiments 1 and 2, though variable, indicated that all the fungicides tested, whether used singly or in any of the various combinations

possible, reduced leaf spot infection on the first leaf. Similarly, in Experiments 3, 4 and 5 all the sprays, including the wax emulsion, significantly reduced leaf spot infection on this leaf. The outstanding feature of these experiments, however, was the fact that sprays containing white oil emulsion alone, or in its various combinations with copper oxychloride and malachite green, significantly reduced infection on leaves which received a single application when they were respectively second, third or fourth from the top of the plant. This finding indicated that these sprays were strongly fungicidal and/or fungistatic. Evidence of a suppressive effect was provided by the observations made on rate of leaf spot development within the treated areas which are illustrated in Figures 10 and 11. White oil emulsion, white oil emulsion plus malachite green, and copper oxychloride plus white oil emulsion plus malachite green, plainly were the most potent fungistatic agents.

The results of Experiment 6 showed that there was little to choose between the complete mixture and either white oil emulsion or white oil emulsion plus malachite green for fungicidal and/or fungistatic properties. White oil plus malachite green appeared, if anything, to be slightly superior.

The effect of these mixtures in preventing or delaying the change from streak to spot stage lesions which was so pronounced in Experiments 1 and 2 was not equally evident in Experiments 3, 4, 5 and 6. This appeared to be due to the fact that in the early experiments whole leaves were treated, whereas in the later trials the sprays were applied to small circles. These small treated areas were eventually encompassed by necrotic tissue and the consequent disturbance to the physiology of the leaf apparently counteracted the suppressive action of the sprays.

The *in vitro* germination tests (Table 19) demonstrated that, contrary to expectations, white oil emulsion did not hinder germination and indicated that this additive was not fungicidal at the strength used. Moreover, it was not fungistatic in the conventional sense. Copper oxychloride (1½-40) and malachite green (1-10,000) were strongly fungitoxic.

The trials designed to investigate the extent and duration of the antisporulant effect of a leaf spot spray formula and its constituents were somewhat limited in scope. Nevertheless it was apparent that copper oxychloride (1½-40) and malachite green (1-10,000) when used alone or together caused an appreciable reduction in conidial sporulation on the upper surface of treated leaf spots which were not subjected to weathering (Tables 20 and 21). The effect of malachite green was more pronounced when the concentration was increased to 1-5,000. The effect of white oil emulsion alone was indefinite and the efficacy of mixtures containing white oil emulsion was approximately equivalent to the fungicidal ingredient or ingredients in the mixtures. A sharp reduction in the antisporulant properties of the complete mixture was associated with weathering of the spray residues on the upper surfaces of treated leaves for six days, during which period

they were exposed only to 0·81 in. of rain and to heavy dews. It is concluded, therefore, that in North Queensland plantations reduced sporulation must contribute little to the control of leaf spot obtained with this mixture. This would be particularly so during the wet season months, when spraying is often interrupted by heavy rain and it is not uncommon to have a majority of wet days in the period of four weeks between sprays.

The implications of the above work are that the efficacy of this spray mixture is due to its fungicidal and suppressive effects. The suppressive effect is dependent on the inclusion of white oil emulsion, which is plainly not fungistatic in the usual fashion.

(f) Commercial Application of Results of Control Experiments

The addition of white oil emulsion to any one of the fungicides copper oxychloride, Bordeaux mixture and zineb greatly improved its efficacy due to the suppressive action of the mixture in addition to its fungicidal properties. Malachite green was shown to be fungicidal and to have an antisporulant action, even though limited. In addition, it is a cheap ingredient. It was therefore thought preferable to retain this additive in the mixtures recommended to growers, which are as follows:

(1) Copper oxychloride (1½-40) + white oil emulsion (1-160) + malachite green (1-10,000).

(2) Zineb (1½-40) + white oil emulsion + malachite green.

(3) Bordeaux mixture (3-2-40) + white oil emulsion + malachite green.

In the control experiments the disease was checked by heart-leaf spraying at intervals of four weeks throughout the year. However, the seasonal variation in disease incidence was found to have a great bearing on the efficacy of the spray programme.

Due to the rapid build-up of infection during late summer and autumn, spraying should commence before the onset of the wet season, preferably in late November or early December, and should be continued at intervals of four weeks during the first half of the year. In the dry season, i.e. the greater part of the second half of the year, leaf spot is less damaging and unsprayed plants which flower later than July may retain sufficient foliage to enable them to fill their fruit to marketable size. It appears, therefore, that the interval between sprays can be considerably lengthened during this period.

Growers have quickly adapted their methods of plantation management to include leaf spot control measures. Most large plantations now are established on arable land and the use of tractors in leaf spot control programmes as well as for other cultural purposes has led to the adoption of a modified planting arrangement whereby the plants are spaced 5 ft apart in rows 13 ft apart.

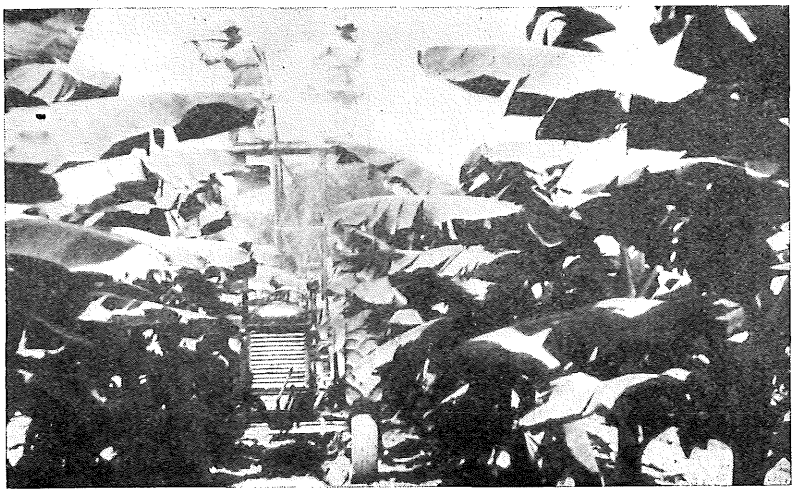

Fig. 12.—Spraying from an overhead tower on Mr. S. J. Mackay's plantation, Tully. Note complete absence of leaf spot as a result of routine operations.

The method of application on small farms is manual, using small, portable, high-pressure spray plants, pressure hosing and spray lances. Plants receive individual treatment. This method is time-consuming; 1½ ac treated per day would be a maximum. Dosages of up to 200 gal per acre may be used depending on the size of the plants.

On large plantations quicker methods of application are essential and two methods are currently in use:

(1) Overhead manual application of a spray from a tower mounted behind a tractor. The spray liquid is delivered by short lengths of pressure hosing leading to lances and nozzles held and directed by men on the tower (Figure 12).

(2) Misting row by row with air-blast misting machines towed behind tractors. This method is automatic and the spray is delivered from an arc of fixed nozzles (Figure 13).

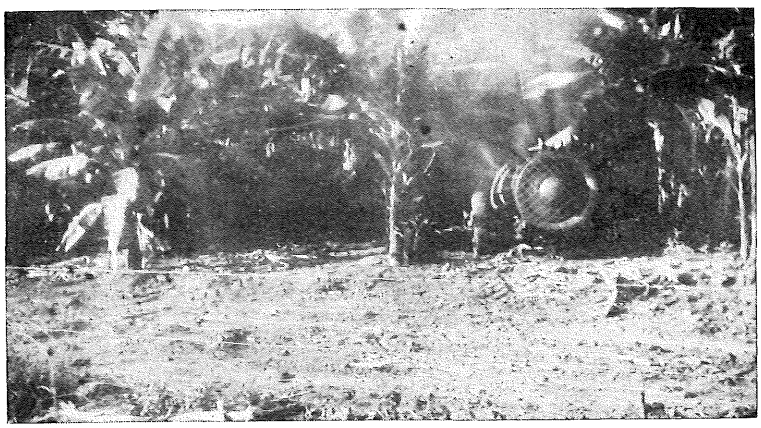

Fig. 13.—Airblast machine operating in Mr. S. J. Mackay's plantation.

Both these methods employ reduced volumes of spray mixture. Dosages of 50-60 gal per ac are commonly used. The original formula is not altered, i.e. approximately 2 lb of fungicide and 3 pt of oil are applied per acre.

The use of air-blast machines in particular has made the treatment of banana plantations for leaf spot control most economical. The cost of materials is reduced to 18s. 6d. per acre. The estimated cost of treatment, including material, labour and depreciation, is £1 10s. per acre.

Misting or fogging with oil-based mixtures gave reasonable control of leaf spot. However, no cumulative effects were noted from continuous treatment and damage from leaf speckle disease and leaf loss due to phytotoxicity detracted from the performance of the treatments. It is considered that this method of treatment should not be adopted on plantations where water-based mixtures can conveniently be used.

No consistent advantages were gained from the use of brown paper or hessian bunch covers on sprayed or unsprayed plants. This may not apply in South Queensland, where colder conditions are experienced.

V. MODE OF ACTION OF WHITE OIL EMULSION

(a) Historical Summary

The "fungicidal" properties of mineral oils were demonstrated a long time ago (Halsted and Kelsey 1903), but until comparatively recently, and despite its widespread use as a scalicide and a sticker, oil emulsion has rarely been mentioned as a specific for plant diseases.

An oil emulsion (1 in 4 dilution) was used to control powdery mildew of rose (McWhorter 1927). Improved control of citrus black spot was obtained by the use of white oil emulsion (1-160) with Bordeaux mixture and copper oxychloride (Kiely 1950). Recently the use of one or two oil sprays at scalicidal strength in addition to copper sprays has been found to give better control of fig rust and leaf fall in South Queensland orchards (Brimblecombe 1959).

Following the discovery by Guyot and Cuillé in 1955 that straight oil misted onto bananas reduced damage by *Mycosphaerella musicola,* the role of oil in the control of this pathogen was investigated in some detail. Merny (1955) found that copper oxide and zinc coposil both suspended in oil and also oil with or without copper oxychloride in suspension gave better disease prevention than that obtained with copper oxychloride in water. All the oil treatments suppressed infection on the fourth leaf. Whether used for prevention or suppression they were most effective when applied to the lower surfaces of the leaves.

The superior effect of oil and oil/copper mixtures was later attributed by Brun (1958) to a combination of the following factors: (1) reduction in conidial sporulation; (2) reduction in spore germination on treated leaves to a point approaching zero; and (3) a check in the development of infection already established in the leaves.

There is ample evidence available in the literature to prove that fungi can remain in intimate contact with paraffin oil for considerable periods and retain their viability. Pure mineral oil is now employed as a culture preservative, this use stemming from early work which demonstrated that *Fusarium* spp. and *Alternaria* sp. remained viable under oil for six months (Sherf 1943). Since then it has been shown that many fungi can be stored by immersion in oil without fear of affecting viability (Buell and Weston 1947).

Price (1958) assessed the effects of liquid paraffin and various banana leaf spot spray oils on fungus growth by their ability to depress growth of *Fusarium oxysporum* f. *cubense* in culture. He considered the effect of spray oils on the leaf spot fungus to be partly physical and partly chemical due to the chemical nature of the unsaturated fraction. All the spray oils he tested gave a greater percentage retardation of growth than did liquid paraffin, which retarded growth by 8 per cent. compared with the control. He assumed that the effect of the same oil on a germinating *M. musicola* spore would be at least as great.

Other workers (Calpouzos, Theis, Rivera, and Colberg 1955) showed that petroleum oils which are used for leaf spot control did not reduce ascospore discharge or conidial production, nor did they inhibit germination of either conidia or ascospores; and stomatal penetration was not affected to an extent sufficient to account for the disease control obtained. These workers suggested that the oils inhibited the fungus at some stage after stomatal penetration and before the appearance of symptoms.

In a continuation of his work on the mode of action of oil in leaf spot control, Brun (1959) found that oil treatment hindered germination, germ-tube development and penetration of ascospores applied to oil-treated leaves of *Musa sinensis*. He considered it possible that the oil had a double effect, viz. (1) action on the fungus itself by which it limits its development, and (2) action on the leaf, which becomes unfit for the development of the parasite.

(b) Experimental Evidence

Some additional investigations in North Queensland on the role of white oil emulsion in leaf spot control are reported below.

(i) Effect of Time of Treatment and Leaf Surface Treated

This experiment was designed not only to provide a comparison of the relative effects of applying white oil emulsion (1-160) to the upper as opposed

to the lower leaf surface, but also to illustrate the effect on the degree of control obtained by treatment at various intervals before and after inoculation.

The emulsion was applied at the stipulated times with a nebulizer to either upper or lower surfaces in circles 4·5 cm in diameter marked on the three youngest leaves of a single plant. An aqueous suspension of conidia was atomized onto the lower surface of each circle at the required time. The results are presented in Table 22.

TABLE 22

Effect of Leaf Surface Sprayed and Time of Application of White Oil Emulsion expressed as Mean Number of Lesions per Treatment

Leaf Surface Sprayed	Weeks before Inoculation			Weeks after Inoculation		
	3	2	0	1	2	3
Lower	9	15	9	1	5	0
Upper	42	106	75	51	48	49
Nil	63	106	89	87	85	58

Clear-cut reductions in infection were obtained by oil emulsion treatments applied to the lower leaf surfaces at intervals ranging from three weeks before inoculation to three weeks after inoculation. The effect of applying the spray at similar times to the upper surface was indefinite.

This finding could have meant that the oil applied to the lower surface, i.e. the surface onto which the conidia had previously been atomized, had affected germination and penetration, whereas oil applied to the upper surface could not have had a similar effect for it was not in contact with the spores. However, oil may affect the growth of the fungus after it has entered the leaf, for oil absorption is rapid and oil soaking is evident immediately the water in the emulsion has evaporated from the leaf surface. Despite this fact, treatment three weeks prior to inoculation was as effective as treatment and inoculation on the same day. Likewise, treatment three weeks after inoculation, by which time some infection must have resulted, was as efficient as treatment and inoculation on the same day.

(ii) Effect on Spore Germination

In the course of this work it was often noted when leaf mounts were made from inoculated areas on treated and untreated leaves that spore germination and appressoria formation on the former were not appreciably different from that on the latter. One such case is shown below. The mounts were made, using the method previously described, at intervals of two and three weeks from the

date of spraying. The following were the totals for percentage germinated spores and percentage germ tubes with appressoria:

—	Percentage Germination	Percentage Appressoria
White oil treated ..	76	22
Untreated	79	28

(iii) Viability of the Fungus in Treated Leaves

The fungus remains viable in lesions which have been treated with white oil emulsion. This fact was verified by many isolations from treated tissue. One such experiment is described below.

Leaves showing early (yellow) streak stage infections were treated on upper and lower surfaces with white oil emulsion applied with a "Rega" continuous atomizer. Five weeks later, during which period no further development of the streaks ensued, isolations were made from these lesions. Streaks from untreated leaves on a nearby plant were plated out also as checks.

The infected tissue was cut into strips, dipped in mercuric chloride/hydrochloric acid solution (0·5 per cent.) for 5 min, washed well in 70 per cent. alcohol and dried. The streaks were cut out, crushed with a sterile scalpel and plated. Three culture media were employed, viz. standard potato dextrose agar, 2 per cent. plain agar and acidified potato dextrose agar (2 large drops of lactic acid to 70 c.c. of medium).

The results after three weeks' incubation were:

(1) Streaks treated with oil emulsion—96 plantings, 40 *Cercospora* cultures.

(2) Untreated streaks—92 plantings, 32 *Cercospora* cultures.

(c) Discussion

The implications of the above work when considered in conjunction with that described in the section dealing with the effect of individual spray constituents are that an emulsifiable-type paraffin oil when diluted 1–160 with water and used with or without a fungicide reduced visible leaf spot infection and suppressed disease development on leaves 1, 2, 3 and 4. The emulsion did not reduce conidial sporulation appreciably, nor did it hinder germination *in vitro*. Percentage spore germination and appressoria formation on sprayed leaves were apparently not depressed. A single application of oil emulsion was sufficient to suppress growth of the leaf spot fungus up to three weeks after it had been applied and it was able to exert its effect when applied three weeks

after the spores reached the leaf. In addition, white oil emulsion, while suppressing the further development of established infections, did not affect the viability of the fungus in the treated leaf tissue.

The above evidence suggests that the suppressive action of the oil emulsion on banana leaf spot is not due to its being fungistatic in the conventional sense.

It is also of interest to note here that an emulsion of a vegetable oil, viz. cottonseed oil, has given somewhat analogous results in the control of another fungous disease, tobacco blue mould. The superior efficiency of a cuprous oxide/cottonseed oil emulsion spray for blue mould control was attributed to the action of the oil, which, in spore germination tests, was definitely not fungicidal (Clayton *et al.* 1943). These authors stated that when oil-sprayed leaves were infected "the mycelium spreads for a short time then ceases to grow."

There appear to be three possible methods of action. These are:

(1) *A purely physical effect whereby contact of the oil with the fungus inhibits the growth of the latter.* However, it has been shown in this work that spores will germinate and germ tubes grow quite readily in white oil emulsion. In addition, the leaf spot fungus grew quite readily on agar prepared with white oil emulsion as a base. Calpauzos and his collaborators (1959) also found that oils which gave complete disease control when sprayed on leaves did not inhibit growth in culture.

(2) *An inhibition by the oil of the activity of enzymes secreted by the fungus.* If there is an action of this nature then it can only function when the enzymatic activity of the fungus is at a minimum. White oil treatment does not suppress infections which have progressed to a stage of development beyond the yellow streak stage, when, presumably, enzyme secretion by the fungus is active. Also, white oil fails to prevent or delay the change of yellow streaks to spots where infected treated areas are encompassed by heavily infected untreated areas.

(3) *An effect on the physiology of the leaf tissue which results in an inhibition of fungus growth within the leaf.* White oil emulsion was noted by Kiely (1950) to delay the appearance of lesions from latent mycelia of the citrus black spot fungus. He attributed this largely to the effects of the oil in slowing down the processes of fruit maturity.

As a result of their wide use as insecticides and weedicides, some attention has been paid to the effects of oils on the physiology of plants.

Spuler, Overley, and Green (1931) commented: "Highly refined oils are not toxic to foliage in the sense that leaf tissue is destroyed, but they do under certain conditions interfere with leaf metabolism."

A number of authors have shown the oil to affect the respiration and photosynthesis of the treated plant. However, the nature of this effect varies considerably with type of oil, species of plant and method of investigation (Wedding, Riehl, and Rhoads 1952).

Most recent workers on the subject agree that photosynthesis, respiration and transpiration are inhibited after the application of oil sprays. These effects are more usually associated with oils such as citrus spray oils, which cause slow-developing chronic injury, than with the weedicide oils which cause rapid-acting, acute injury (Currier and Peoples 1954). These relatively non-toxic spray oils enter entirely or largely through stomata and usually spread widely throughout the plant (Dallyn 1953).

There are differences of opinion as to the manner in which oil effects photosynthesis. Dallyn (1953) considered that a mechanical interference by the oil with gaseous exchange or water supply is the best explanation for any interference with photosynthesis. van Overbeek and Blondeau (1954), however, suggested that the effect might be due to solvent action on the lipoid phase of the chloroplasts and that the action of oils in plants is apparently primarily a physical-chemical effect of solubilization influencing the semipermeability of the plasma membrane. Riehl and Wedding (1959) found a definite relation between inhibition of photosynthesis and increasing oil deposit. The inhibition appeared to be the result of interference with gaseous exchange caused by the presence of the spray oil. The theory of solubilization expounded by van Overbeek and Blondeau seemed to agree with experience with oil sprays on citrus in California.

Riehl *et al.* (1958) found that a single application of 1·75 per cent. oil emulsion to citrus reduced transpiration by two-thirds within 24 hr. Recovery of transpiration occurred with the dissipation of oil from the leaves, which in this case took 3–5 weeks. These authors considered that reduction in transpiration in citrus foliage after application of an oil spray was due to physical interference by the spray oil on or in the leaf tissue.

Wedding and Riehl (1958) were able to demonstrate that petroleum oil of the commercial "emulsive" spray formulation, in amounts approximating those deposited by the usual insecticidal operations, inhibits the transport of phosphate into leaves to which it has been applied. They consider the inhibiting effects of oil on translocation are probably general and non-specific and come about through physical interference with the transport mechanism either by actual obstruction of the vessels with oil or by the solubilization reaction.

It is evident, therefore, that the physiological processes could be materially affected following the application of oil emulsion to banana leaves. The disturbance to the metabolism of the leaf tissue may be responsible for the prolongation of the latency of the leaf spot fungus. The manner in which the

growth of the fungus could be affected is not known. However, the inhibiting effect of oil on photosynthesis and translocation and its influence on the semi-permeability of the plasma membrane indicates that an alteration in the carbohydrate balance of the host which possibly deprives the fungus of its favoured carbohydrate substratum could be involved. Alternatively it might mean that the action of the oil increases the resistance of the host tissue to the enzymes or toxins secreted by the fungus.

The fact that oil has little or no effect on lesions which have developed beyond yellow streaks to the stage where the fungus is vigorously parasitic indicates that the activities of the fungus have disorganised the functioning of the parasitised leaf tissue to the extent where photosynthesis and other normal physiologic processes have ceased.

In infected treated areas encompassed by heavily infected untreated areas, the failure of white oil to prevent or delay the change of yellow streaks to spots while preventing the development of streaks is of interest but without adequate explanation.

Some preliminary work attempting to control the physiological aging of leaf tissue by growth regulators has been carried out but no positive results have yet been obtained.

VI. ACKNOWLEDGEMENTS

The author wishes to acknowledge the assistance of Mr. Rex Grattidge (Assistant Experimentalist in the Department) with all aspects of the experimental work reported here. Thanks are due to Mr. S. J. Mackay and Mr. C. Harm on whose plantations at Mission Beach and Stoney Creek respectively the field trials were carried out. The department's Senior Biometrician (Mr. P. B. McGovern) was responsible for all statistical analyses. Mr. H. A. Burkitt (Commercial Photographer of Cairns), provided the photographs used in Plates 2, 3, 8, 9 and 11.

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(Received for publication July 6, 1960)