STUDIES ON THE BLACK SPOT DISEASE OF CITRUS CAUSED BY *GUIGNARDIA CITRICARPA* KIELY, WITH PARTICULAR REFERENCE TO ITS EPIPHYTOLOGY AND CONTROL AT LETABA.

by

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F O R E W O R D

The author was seconded by African Explosives and Chemical Industries Limited to Consolidated Citrus Estates Limited (Schlesinger Organisation) in July 1959 to investigate the citrus black spot problem at Letaba Estates. When investigations commenced losses due to black spot were close to R200,000 per annum. Research was therefore aimed at a practical solution. For this reason particular attention was paid to control measures and certain aspects on the epidemiology of the disease.

I declare that the thesis submitted herewith for the degree of Doctor of Science to the University of Pretoria has never before been submitted for a degree at any other University.

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1. INTRODUCTION

A. GENERAL

Letaba Estates is the second biggest Citrus Company in the Republic of South Africa and is expanding rapidly. The present total tree population is close to 320,000 of which approximately 200,000 trees are in various stages of production. All commercial plantings are on rough lemon rootstock. Slightly more than 50% of the bearing trees are of the Valencia orange variety. Black spot is particularly troublesome on Valencia oranges, mainly because ripening of this variety coincides with the onset of warmer weather conditions which favour black spot lesion development.

B. LOCALITY

Letaba Estates is situated in low lying bushveld of the Northern Transvaal. It is 15 miles North-East of Tzaneen, at latitude 30 19' and longitude 23 52'. The altitude varies between 1,690 feet and 1,900 feet.

C. CLIMATIC CONDITIONS

The climate is subtropical. In Table 1 the average monthly rainfall, minimum and maximum temperatures, taken from 1945 to 1962 are presented. The temperatures were determined by means of a recording thermograph in a Stevenson screen at a height of about 4 feet above ground.

TABLE 1.

Summary of the monthly mean rainfall in millimeters, and minimum and maximum temperatures in degrees Fahrenheit as recorded at Letaba, 1945-1962.

Month	Rainfall (mm)	Temperature (degrees Fahrenheit)	
		Minimum	Maximum
September	17.0	53.6	81.6
October	46.2	60.4	84.3
November	84.6	63.8	84.5
December	137.7	65.5	84.9
January	168.1	67.0	84.5
February	146.8	67.0	84.4
March	117.3	65.1	83.5
April	50.3	60.2	82.0
May	24.9	51.2	78.6
June	8.4	44.5	74.7
July	7.4	44.6	74.6
August	5.3	47.3	78.4
Total	814.0	-	-

From Table 1 it is evident that the summers are very hot and the winters mild. Daily maximum temperatures during the summer months often go well over 100°F in the shade. Frost is uncommon but does occur occasionally in a mild form during July and August.

Rains may come through thunderstorms, but soft penetrating rains are often experienced from November onwards.

D. HISTORICAL REVIEW.

The Black Spot disease of citrus, caused by *Guignardia citricarpa* Kiely (*Phoma citricarpa* Mc Alp.) was first noted and described in Australia nearly 70 years ago. It became a major problem to citrus growers on the central coast of New South Wales and Queensland, but is absent in the inland citrus-growing areas (Kiely,1957). The disease has inflicted the greatest damage in Australia and South Africa (Calavan 1960). The fungus is known to occur also in Argentine, Brazil, West Indies, Portugal, Mozambique, U.S.S.R., India, Indo-China, China, Formosa, Japan, Philippines, Hawaii and New Zealand, according to Lee (1920), Kiely (1948), Wager (1952) and Calavan (1960). The disease has not yet appeared in the continental part of the United States of America and has not become

very important in the Western portion of any continent. (Calavan, 1960)

In South Africa, Doidge (1929) identified the disease near Pietermaritzburg, Natal and Wager (1952) reported that "the disease spread slowly in the area, fluctuating from season to season". Wager stated further that "considerable damage" was caused in 1940 in that area. In 1944 "alarming" losses were caused near Pietermaritsburg and the disease appeared in relatively dry areas. After an intensive survey of the occurrance of black spot throughout South Africa, Wager (1952) reported that "the disease is causing heavy losses in all citrus orchards in Natal and certain areas of the Eastern and Northern Transvaal". He also found the fungus on dead citrus leaves and latently in green leaves in all parts of Eastern, Northern and Western Transvaal, throughout the Eastern Cape and in one district in the Western Cape.

According to Wager (1952), "a few infected fruits on smooth lemon trees" were found at Letaba Estates in 1946. No indication could be found in the record books at Letaba Estates or elsewhere that black spot appeared at Letaba Estates prior to 1946.

The smooth lemon trees that Wager referred to were pulled up that same year and burned. These trees were grown for domestic purposes in the residential area at "Leeufontein" in the centre of Letaba Estates. It is known that lemons are very susceptible to black spot and it is possible that the causal organism, Guignardia citricarpa, was unknowingly introduced to these lemon trees by the residents of "Leeufontein", who brought in infected plant materials from another area.

If black spot at Letaba Estates originated through natural spread of airborne spores it is a strange phenomenon that the disease started in the centre of a relatively big block of citrus trees. The results of Buchanan and Kimmey (1938), Bateman (1947) and Gregory (1952) on spore dispersal, and the data of Van der Plank (1949), suggest that the disease should first have been noticed around the borders of the citrus estates. On the other hand, it may be argued that the disease was first observed on the lemon trees because of their greater susceptibility. Such reasoning would not be strictly valid because old lemon and grapefruit trees were scattered over the entire estates and some of these trees were close to the borders.

Although the infected lemon trees were burned, it is extremely doubtful whether the infected dead leaves on the ground were destroyed. It is doubtful because the importance of dead leaves in the epidemiology of black spot was not fully appreciated at that time.

After the destruction of the lemon trees no black spot was observed until 1949. Wager (1952) recorded that "a few infected fruits were found on the grading table" in 1949. During the 1953 picking season a considerable percentage of fruit from old Valencia trees at "Leeufontein " showed black spot and during the 1953 - 1954 season 19.7 acres in this area were sprayed once only for the control of this disease. During the 1954 - 1955 season the same acreage were sprayed, but the number of applications were increased to three. In 1955 and 1956 these old Valencia trees were pulled up and burned, but the epidemic was already on such a firm footing that large scale spraying was carried out during the 1957 - 1958 season. Since then, spraying against black spot became a practice and a spray programme was introduced. In desperation all big trees were sprayed 4 times during the 1959 - 1960 season. The following year some of the Navel trees received only three sprays. As this proved to be a safe procedure, most of the bearing Navel trees received only three sprays, during the 1961 - 1962 season.

TABLE: 2.

Annual acreages treated in controlling the black spot disease of citrus at Letaba Estates, 1953 - 1962.

Season	Acreage receiving			
	1 Spray	2 Sprays	3 Sprays	4 Sprays
1953 -54	19.7	0	0	0
1954 -55	0	0	19.7	0
1955 -56	No spraying		Infected trees burned.	
1956 -57	No spraying	-		
1957 -58	426.7	0	52.1	0
1958 -59	8.20	0	2251.32	0
1959 -60	33.85	51.60	147.30	1890.03
1960 -61	8.00	95.10	386.49	1635.90
1961 -62	7.30	37.90	905.57	1201.73

Through this brief review of the history of black spot at Letaba Estates, it is evident that it took about eight years from the time the disease was first observed until it

became an epidemic.. One may also speculate that a sound knowledge of the epidemiology of the disease and the implementation of quarantine measures might have delayed the epidemic. This point is discussed later .

E. ECONOMIC INPORTANCE OF BLACK SPOT.

Black spot causes unsightly lesions which spoils the sales appeal of the fmuit. The effect of the disease on tree health must be regarded as negligible if anything at all. The relatively few lesions that occur on the leaves can not possibly effect the efficiency of the leaves on a tree as a whole.

Letaba Estates produce oranges primarily for the export markets. The tolerance for black spot at the packhouse is zero for export fruit. The infected fruit is sold on the internal markets or goes to the juice factory.

As far as the export markets are concerned, the attractiveness of the fruit surface is an important factor in determining the sales value of the product. A fruit with spots is less attractive than the unblemished one , ceteris paribus. Should it be possible to educate the consumers that the internal quality of the spotted fruit is as good or even better than the one without spots, black spot will loose considerable economic prestige. Such a campaign would be a formidable undertaking indeed.

An accurate assessment of the direct and indirect losses to the South African Citrus Industry on account of black spot, is a difficult task. Most workers try to estimate the losses. According to Loest (1958) "enormous financial losses are suffered" annually. Kiely (1948) estimated that the annual losses due to black spot vary betweer £A50,000 ani £A 150,000 in New South Wales, Australia.

For the purpose of this discussion, Letaba Estates will be taken as a unit for the determination of direct losses.

1. DIRECT EFFECT.

In order to investigate the direct economic effect of the black spot disease at Letaba Estates, the 1960 - 1961 season was taken as an example, mainly because complete records were kept since 1960 and market results were available.

With a total of 192,000 trees during 1960 - 1961, (all varieties and various age groups) Letaba Estates produced631,055 cases (± 72 lb per case) of oranges. To control black spot 7,199,875 gallons of spray mixture were

applied at a total cost of R54,388 (including operational costs, labour, cost of materials, etc.) In spite of the capital spent on control, 10.1% of the total crop failed export standards as a result of black spot. This represents 63,737 cases or 148,719 pockets which had to be marketed locally. The average nett realisation per pocket for the season was 21.83 cents, or R32,465 for all the diseased fruit. But the packing cost was 9.47 cents per pocket or R14,084 for all the diseased fruit. That leaves the grower R18,381 to pay for production, picking, etc.

If 100% disease control could have been achieved by spraying and the additional 63,737 cases did not influence the overseas market prices, then at 278.07 cents per case, R177,233 could have been realised. Packing cost of these fruits at 71.93 cents per case would have been R45.846 which left a gross realisation of R131,387.

The loss is therefore R131,387 - R18,381 = R113,006, plus the cost of spraying (R54,388) which brings the total figure to R167,394.

Summary of economic effect of black spot on the citrus crop at Letaba Estates 1960 - 1961 season :-

No. gallons spray mixture applied: 7,199,875
Spraying cost (operational, materials, etc.)..... R54,388 A
Total No. cases (72 lb) produced.... 631,055
No. cases culled due to black spot.. 63,737 (a)
i.e. 148,719 pockets (b)

Nett realisation per case F.O.R. Growers station (export) ..... 278.07 cents (c)

Loss non-exportable cases due to black spot (a x c) ..... R177,233 (d)

Nett realisation per pooket F.O.R. growers station (local market) ..... 21.83 cents (e)

Realisation from pockets (local) (b x e) ..... R32.465 (f)

Total loss (c - f) ..... R144,768 B

Packing cost if fruits were exported @ 71.93 cent per case ..... R45,846 (g)

Packing cost for local market @ 9.47 cent per pocket ..... R14,084 (h)

Difference : (g - h) ..... R31,762 C

Total expense (losses) caused by disease (A + B - C) R167,394

The overall losses vary from year to year and depend on the degree of control achieved by spraying and the local and overseas market results. The control programme will

remain fairly constant for the next number of years. Although the cost of control measures during the 1961 - 1962 season was almost the same as the previous year, only 0.8% of the fruit could not be exported on account of black spot. The final overseas market results are not yet available, but it is estimated that the total losses on account of black spot during the 1961 - 1962 season, were reduced to approximately R65,000 (including cost of control measures).

2. INDIRECT EFFECTS.

Apart from the direct and calculable losses, the occurrance of the disease has further repercussions.

At present copper fungicides are used to control black spot. There is no fungicide outside the copper group which is quite as effective, except Dithane Z78 which may be used under special circumstances. There is therefore, every chance that copper fungicides will be used for many more years. The danger of copper toxicity in our soils can not be overlooked. Although there is no cause for alarm yet, according to Mc Onie (1961), toxicity is an imminent problem.

Direct spray damage to fruit can show up in several ways. The most alarming fact is that a symptom which was described as "wind damage" in the past, occur by far more on fruit which had been sprayed with copper fungicides. This lesion is possibly caused by an insect or mite, but the damage is undoubtly accentuated by the application of copper fungicides. This kind of spray damage caused severe losses during the past three seasons in the Nelspruit area and appeared last season for the first time at Letaba.

It is customary in South Africa to spray calcium arsenate on the Valencia orange variety during October or November for early maturity (Basson, 1959). The writer observed that where calcium arsenate is sprayed as a combined spray with a copper fungicide, there is no effect on the sugar - acid ratio. Subsequently, Mr. Conradie* carried out experiments over two seasons and confirmed the above observation. The earlier Valencia oranges are picked the less are the chances of black spot to develop. It is also easier for the packhouse to continue packing, than to have breaks of several weeks per season with an idle labour force.

* Entomologist, Letaba Estates. Unpublished data.

Kiely (1950) and Wager (1952) claimed that applications of Bordeaux mixture cause an increase in the incidence of red scale (Aonidiella aurantii) and other insect pests. There is no irrefutable evidence that fixed copper fungicides have the same effect, but it is possible.

Three to four spray applications are normally carried out for the control of black spot. The spray programme requires a big labour force at a time when other cultural practices such as weeding, irrigation etc, need attention. With the native labour which becomes scarcer and wages going up, this may become a real problem in future.

During a packing season such as 1960 when a high percentage of the fruit is infected, the packhouse performance is seriously effected. When the packhouse output is slowed down, the season is extended and chances are better for lesion development, with the result that less fruit can be exported. This creates great inaccuracies in crop estimates and booking of shipping space which may result in payment of dead freight. The position was so bad during the 1960 packing season that a considerable amount of capital was spent to enlarge the packhouse. The erection of a juice factory at Letaba Estates at the cost of R1,000,000 was partly due to the losses caused by black spot.

Although perfectly lesion-free fruit may be packed, considerable disease development may take place in transit to the coast. This is especially a problem with Valencia orange fruits which are harvested from the end of August onwards. Repacking has to be done at the coast which is a costly procedure. Losses may be so great in transit over long distances that exports through Durban and Capetown ports have to be stopped completely. This actually happened in 1960 and 1961. Lourenco Marques can only handle a limited amount of fruit. Glut conditions develop inevitably on local markets during bumper crop seasons.

Finally, there is the question: How would the market prices be effected if black spot was non-existing and all the fruit was available for export? This is difficult to evaluate because the export market is extended every year. It is the writer's opinion that the depressing effect on the oversea's prices would not have been great. The effect on the local market, which is limited would be considerable. The less fruit available for local markets, the higher the price will be.

Exports to the United States of America would be welcomed by the South African Citrus Industry. One factor which hampers possible exports to the U.S.A. is black spot, mainly because of American import regulations which protect their own industry against the introduction of this disease.

II EPIPHYTOLOGY

A. SOURCES OF INOCULUM

1. INTRODUCTION

Under Australian conditions Kiely (1949) showed that the first infections were established in the early stages of fruit development, shortly after petal-fall until approximately 5 months later. These infections survive in a latent form in the outer tissues of the flavedo until the onset of warm weather the following spring when the fruits mature. Earlier Australian workers accepted that these latent infections were initiated by pycnidiospores, produced upon black spot lesions of affected ripe fruits. This view was based on considerable circumstantial evidence, since the time of lesion development on mature fruit coincided with the time of fruit setting in Australia. Furthermore, new lesions continued to develop on apparently healthy mature fruit, hanging on the trees during the subsequent 3 to 4 months after blossoming. This period of lesion development coincided with the period during which the following season's young fruit, which was also hanging on the trees, were susceptible to infection. Kiely (1948) proved, however, that the removal of diseased fruit, prior to blossoming, did not reduce the severity of the disease the following season.

Kiely examined dead wood and twigs on the trees with negative results but found numerous pycnidia and eventually perithecia of Guignardia citricarpa on dead leaves under the trees. It is difficult to judge to what extent Kiely was influenced by the work of Frey and Keitt (1925) and Keitt and Jones (1926) on Venturia inaequalis (CKe) Wint., but he was evidently aware of these publications and he saw the similarity between the life cycles of the two organisms. The fact is that Kiely considered ascospores on dead leaves as the most important source of inoculum.

Sueda (1941) and Schüepp (1961) believed that the fungus spread from infected stems into fruit tissues and that subsequent activity of the fungus lead to lesion development.

Enough information is now available to assume that infection of fruit may be caused by ascospores or pycnidiospores or by mycelium (systemic infection). It is accepted that ascospores are more important than pycnidiospores or mycelium under most circumstances, but it would be wrong to ignore other sources of inoculum in investigational work.

Wager (1952) inoculated smooth lemon trees with ascospores from dead leaves and another lot with pycnidiospores from ripe fruits. About 9 months later typical black spot lesions were observed on young leaves which appeared at the time of the inoculation. In a similar experiment Wager also produced fruit lesions. The infected material with which these trees were inoculated was placed in wire baskets over the trees. It is also known that dead leaves may harbour large numbers of pycnidia (Kiely,1948). The fact that Wager found ascospores on the dead leaves with which his inoculations were carried out did not rule out the possibility of the presence of pycnidiospores. There is therefore, a certain factor of doubt about these results. Ascospores were not yet found on fruit, and Wager therefore proved, like Kiely (1948) that pycnidiospores caused infection.

A survey of the sources of inoculum was undertaken at Letaba Estates during the course of these studies.

2. OCCURRENCE OF PYCNIDIA

Pycnidia, containing typical pycnidiospores were found in large numbers on dead leaves under the trees throughout the year but were more abundant during the summer months. These fruiting bodies were mostly found on leaves from one month to three months after the leaves dropped and developed in greater numbers on old leaves than young leaves when picked and exposed to natural conditions on the ground in orchards. Pycnidia also developed sooner and in greater numbers when leaves dropped during the period from October to March. These are the hottest and rainiest months of the year.

Pycnidia were observed in black spot lesions on out-of- season and in- season fruit throughout the year shortly after the lesions appeared. This aspect was already described by Kiely (1948), Wager (1952) and Calavan (1960).

On 17th December, 1959 a dead twig of about 3 to 5 mm. thick was picked from an old healthy Valencia tree on which pycnidia were found. These pycnidia were isolated with a sharp needle and examined microscopically after they were flattened between two glass slides. Five of these pycnidia contained spores, which were identical to pycnidiospores of P. citricarpa. Efforts to grow cultures from these spores on P.D.A.-medium failed.

Large numbers of twigs were examined after this but the pycnidia of Phoma citricarpa were not seen again on twigs on trees. It is possible, however, that pycnidia are more prevalent in certain years on dead twigs and fruit stalks on the trees than this record indicates.

On dead twigs on the orchard "floor" pycnidia with pycnidiospores were found twice. The first twig with pycnidia was in an advanced stage of decay and was covered by leaf litter under an old Valencia tree. These pycnidia were found in large numbers, grouped together in patches of about 5 cm. in diameter. Fruiting bodies of Diplodia natalensis were also prevalent, but did not occur among the pycnidia of Phoma citricarpa. This observation was made on 15th January, 1960.

The second twig with pycnidia was found on 19th March, 1960 in the same orchard. This twig was as thick as a pencil and was covered with grass and other weeds. The pycnidia were not grouped together but were scattered over the bark surface between pycnidia of Diplodia and fruiting bodies of other fungi.

Schüepp (1961) also mentioned that pycnidia of Phoma citricarpa were found on dead twigs.

3. OCCURRENCE OF PERITHECIA

a, Citrus

During the course of these studies, hundreds of twigs picked from old and young trees as well as twigs which were collected from the ground, were examined for perithecia. Results were negative except once.

A dead twig was collected under old Valencia trees on plot 786 at Letaba Estates on 2nd February, 1960, on which altogether 8 perithecia with ripe ascospores were found. More perithecia-like structures were found but they contained no spores. It was possible that spores had already been released.

The perithecia which contained spores were isolated with a sharp needle, crushed between two glass slides and compared with perithecia and spores of Guignardia citricarpa from dry leaves. No differences were observed.

An intensive investigation was carried out on dead twigs in this particular orchard and others, but results remained negative.

It is possible that pycnidia and perithecia of G. citricarpa occur more frequently on dead branches or twigs on trees or under trees than our records suggest, but they are difficult to detect. At Letaba, dead twigs are usually covered with fruiting bodies of Colletotrichum species, Diplodia and other fungus species. This makes an identification of perithecia and pycnidia of G. citricarpa most difficult.

b. Other Hosts

Kiely (1948) discovered the perithecial stage of Guignardia citricarpa in Australia and Wager (1952) was the first to observe perithecia in South Africa on dead citrus leaves. Another noteworthy observation by Kiely was that perithecia of G. citricarpa occurred not only on citrus leaves but also on leaves of many other plant species, such as Telopea speciossima, Smilax australis, Syncarpia laurifolia, Ceratopetalum gummiferum, Callistemon lanccolatus, Dendrobium speciosum, and Camellia japonica.

Latent infections were also found in the leaves of Magnolia, Illex, Rubus and Amygadalis species.

Wager (1952) reported that pycnidiospores were found on leaves of Eucalyptus species after they were subjected to the wilting treatment described by Kiely (1948). Wager also reported that ascospores were found on dead leaves of Smilax kraussiana.

Schüepp (1961) claimed that he found the fungus on dead leaves of Lagerstroemia indica, Royena lycioides and Combretum suluense. The author also found perithecia with ascospores on dead rose leaves. The morphology of the perithecia and spores were rather similar to those on Citrus leaves but no infection studies on citrus were carried out. The host range of G. citricarpa appears to be very wide, but studies may show that the fungus has a number of strains.

No one has so far been able to prove that inoculum from various host plants other than citrus can infect citrus. Mc Onie (unpublished report, 1962) reported that this aspect is being investigated.

4. INVESTIGATIONS

In order to get some idea of the importance of the different sources of inoculum, a trial was laid out on three year old Valencia trees in the field. Lack of suitable laboratory facilities and skilled assistance determined the course of the experiment. As the results are regarded as important, the experiment will be discussed in detail.

a. Methods and Materials

The different treatments were as follows:-

Treatment A. Dry twigs were picked from old Valencia trees where black spot was severe on the fruit the previous season. From the same orchard, dead twigs were collected under the trees. These twigs were placed on wire mesh frames (9' x 9') over the young trees on 10th October, 1959 (Plate 1). On 30th November, 1959 the old twigs on the wire frames were removed and replaced with fresh dry twigs, collected from the same locality as before. About 10 lb. of dry twigs were placed above 4 trees as shown in the plan.

Treatment B. Mature Valencia oranges, showing large numbers of black spot lesions with pycnidia of Phoma citricarpa were placed on wire mesh frames as in A. One hundred infected fruits were placed above each of four trees. The oranges were evenly spaced over the entire frame. On 10th October, 1959 the first batch of inoculum was placed over the trees.

The old fruits were removed on 25th October, 15th November, 1st December, 1959 and replaced with fresh, infected Valencia oranges on each occasion.

Treatment C. Green leaves, showing black spot lesions were picked from the same trees as were used for treatment A, and also from another block of old debilitated Valencia trees where black spot was severe the previous year. In many of these lesions pycnidia with pycnidiospores were observed About 500 of these leaves were placed above each of four trees on the same dates as in treatment B.

Treatment D. Dead leaves, were collected from the orchard "floor" under badly infected old Valencia orange trees. About 10 lb. of dry leaves were placed under each of four young Valencia trees about 75 feet away from the nearest tree of the previous treatment. A wire mesh, anchored in the ground, was placed over the leaves under each tree. The first lots of dead leaves were placed under the trees on 15th October, 1959. A large number of these leaves were examined for the presence of ascospores, but although pycnidiospores were prevalent, ripe perithecia were found on less than one per cent of the leaves.

On 15th November, 1959 a fresh lot of leaves was collected and used to replace the previous lots. Examination showed ripe perithecia on about four percent of these leaves. It was hoped that perithecia would develop further under young trees to provide enough inoculum.

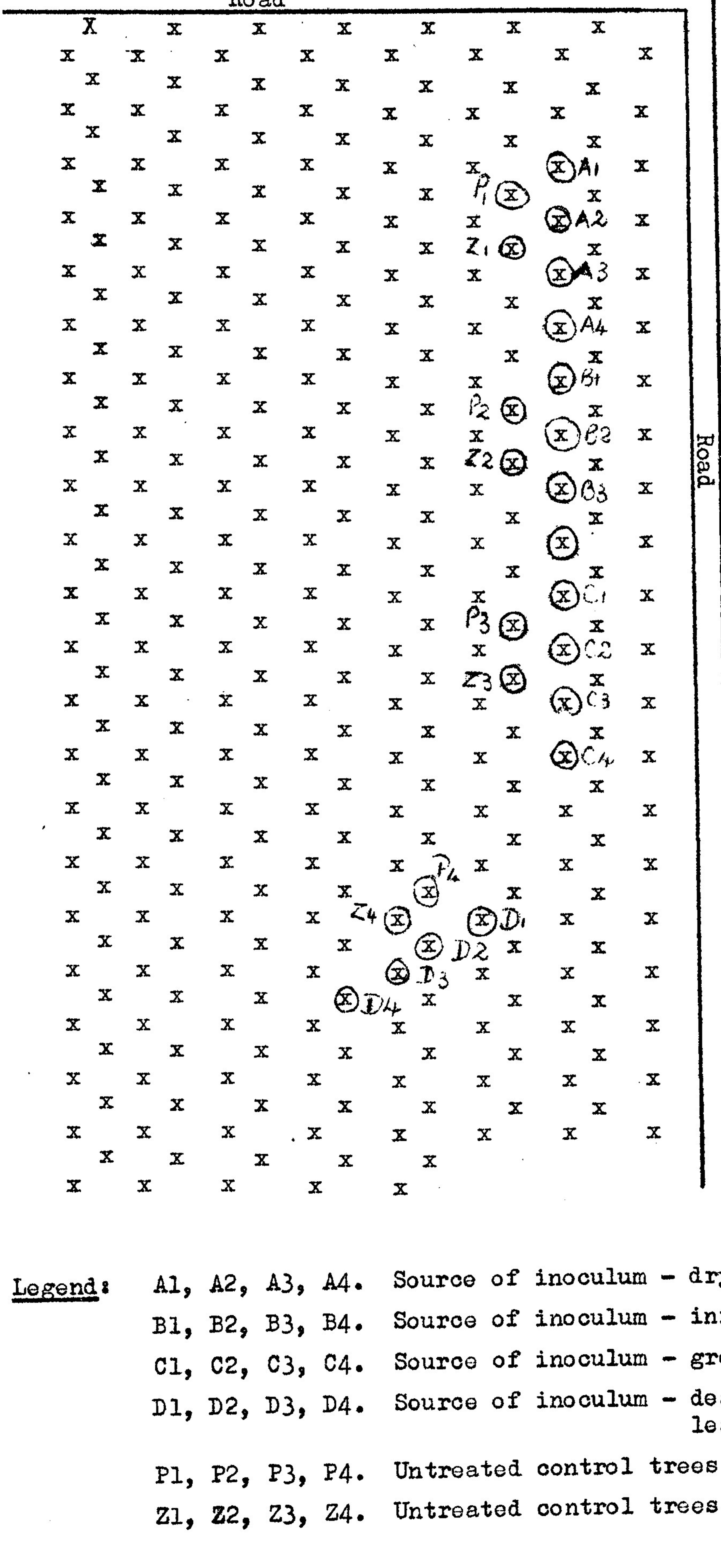
Before this experiment commenced all out-of-season fruit and as much dead wood as possible were removed from the experimental trees.

Plate 1

Wooden frame with wire-netting used in a trial to test the importance of various sources of inoculum.

On 15th February 1960 the wire frames and all sources of inoculum were removed from the experimental block.

PLAN OF EXPERIMENT:



Legend: A1, A2, A3, A4. Source of inoculum - dry twigs

B1, B2, B3, B4. Source of inoculum - infected oranges

C1, C2, C3, C4. Source of inoculum - green leaves

D1, D2, D3, D4. Source of inoculum - dead citrus leaves

P1, P2, P3, P4. Untreated control trees (first harvest)

Z1, Z2, Z3, Z4. Untreated control trees (2nd harvest)

b. Results:

On 8th August, 1960, one tree per treatment, viz. A1, B1, C1, and D1 and an untreated control tree (P1, P2, P3 and P4) for each treated tree were picked and examined for black spot and "melanose". (see plan)

TABLE 3

Percentage fruit infected with black spot and "melanose" on 8th August, 1960 after exposing the fruit to different sources of inoculum during the first four months after blossoming.

Treatment	Code	Number of fruits	Percentage fruit with black spot			Percentage fruit with "melanose"
			% <5 spots per fruit	% >5 spots per fruit	Total %	
Twigs	A1	259	13.9	15.4	29.3	4.6
Control	P1	268	6.3	10.9	17.2	1.1
Fruit	B1	322	12.4	26.1	38.5	33.9
Control	P2	541	9.6	11.3	20.9	2.4
Green leaves	C1	188	2.1	36.7	38.8	73.4
Control	P3	356	2.8	9.0	11.8	3.6
Dead leaves	D1	275	8.0	5.1	13.1	5.8
Control	P4	272	6.2	10.0	16.2	1.5

On 29th September the rest of the experimental trees were harvested and examined for black spot and "melanose". For every 3 trees per treatment, an untreated tree in the adjacent row was harvested for comparison (Z1, Z2, Z3 and Z4).

TABLE 4.

Results on the incidence of black spot and "melanose" on the fruit of young Valencia orange trees after the application of inoculum from different citrus plant materials.

Treatment	Code Number	Number of fruits harvested	Percentage fruit infected with black spot				Percentage fruit with "melanose"	
			<5 spots per fruit	>5 spots per fruit	Total % black spot	Mean of total	Total	Mean
Twigs	A2	218	38.5	26.6	65.1		10.1	
	A3	235	27.2	27.5	54.7		4.5	
	A4	546	54.2	17.5	71.7	63.8	5.5	6.7
Control	Z1	496	10.7	13.7	24.4		0.2	
Fruit	B2	333	27.2	39.2	66.4		55.6	
	B3	293	9.9	70.9	80.8		56.0	
	B4	164	3.6	6.8	10.4	52.5	57.3	56.3
Control	Z2	333	5.4	11.1	16.5		0.3	
Green leaves	C2	318	46.8	6.4	53.2		37.4	
	C3	342	44.7	15.6	60.3		57.6	
	C4	368	35.1	8.9	44.0	52.5	41.6	45.5
Control	Z3	330	16.6	12.8	29.4		0.6	
Dead leaves	D2	293	1.3	11.0	12.3		3.1	
	D3	325	11.7	15.7	27.4		0.0	
	D4	220	27.9	24.4	52.3	30.7	0.0	1.0
Control	Z4	221	9.9	10.9	20.8	22.8*	6.3	1.9

* mean percentage fruit with black spot for all controls.

\+ mean percentage fruit with "melanose" for all controls.

5. DISCUSSION

On both dates of harvesting inoculum from twigs, fruit and leaves above the trees caused an appreciably higher incidence of black spot than in the controls or where dead leaves were placed under the trees. Tree B4 had an unexpected low incidence of black spot, for which there may be many explanations. The tree did not look different from the others, except that it was slightly smaller. Where dead leaves were placed under the trees the incidence of black spot was not higher than the untreated controls, except D4 (Table 4). This tree, (D4) was as healthy as the others with no outward signs of debilitation.

It was stated before, that ripe perithecia were found on only a low percentage of the dead leaves which were used in treatment D. It is almost impossible to give a true reflection of the inoculum potential on such leaves. But, the leaves came from a badly infected old Valencia orchard. A large number of leaves on which ascospores were found were placed under each tree. All this suggests that ascospore inoculum was present. It is known that fruit on young trees is less prone to black spot than fruit on old trees. It is also possible that the ascospore density must be greater for young trees to cause infection and symptom expression. The spore density around D4 might have been higher than D1, D2, and D3. The inoculum potential could have varied considerably throughout this trial.

The results seem to discredit the idea that ascospores are important in the epidemiology of black spot, but such a conclusion is undoubtly premature. Environmental conditions in a young orchard are very different from those in an orchard of old trees. There may also be considerable physiological differences between fruits from young trees and fruits from old trees.

A large percentage of black spot lesions on the fruit of trees where infected fruits were used as an inoculum source, must have been due to infection by pycnidiospores. Ascospores were never found on fruit and there was no doubt about the presence of pycnidiospores. Like all the other trees a certain amount of "natural" infection took place as shown in the results of the untreated trees.

Where green leaves with black spot lesions were used as a source of inoculum pycnidiospores were likely to have been the cause of infection. When the leaves were removed they were examined and, although pycnidia developed in large numbers while the leaves were above the trees, not a single perithecium was found. Yet, the human eye is fallible and a possibility of ascospore infection from these leaves can not be excluded.

There is considerable doubt as to the cause of infection where twigs were used as a source of inoculum. Examination of the twigs revealed large numbers of fruiting bodies of Diplodia and Colletotrichum species but no pycnidia or perithecia of G. citricarpa were observed. Nevertheless, the possibility that one or both kinds of spores were present cannot be discarded. Neither can one ignore the possibility of infection by mycelium fragments which could have been washed down by rains from the twigs.

The incidence of "melanose" forms an interesting pattern. "Melanose" symptoms were rare in the untreated controls, the twig treatment and where dead leaves under the trees were used as a source of inoculum, but abundant where green leaves and infected fruit were used. No pycnidia or perithecia of Diaporthe citri were observed on any of the citrus plant materials used as inoculum sources. According to Fawcett and Lee (1925), fruiting bodies (pynidia) of D. citri are found on dead branches, but very rarely on fruit.

If one disregards treatment A, where considerable doubt exists as to the cause of black spot infection, "melanose" was severe on the fruit where there was a reasonable degree of certainty that pycnidiospores were the cause of infection (treatments B and C).

It is also possible that the so-called "melanose" symptoms are caused by pycnidiospores and/or ascospores of G. citricarpa when infection takes place at a certain stage of fruit development. There is more evidence in support of this theory which will be discussed later. It is perhaps unfair to make firm conclusions on the results of this experiment, which has many shortcomings. The results indicate strongly, however, that ascospores from dead leaves are not the only source of infection and that dead twigs, infected fruit and green leaves with lesions must not be overlooked entirely.

B. SEASONAL DEVELOPMENT OF PERITHECIA

The role of ascospores in the epidemiology of black spot was discussed by Kiely (1948) and Wager (1952).

Kiely, in particular discussed conditions which must be fulfilled for ascospore development. He demonstrated that spermatogonia, pycnidia and perithecia developed from latent infections present in green leaves. Alternative wetting and drying of green leaves favoured the development of these fructifications. Kiely also pointed out that leaves which dropped during wet weather were often overgrown by acervuli of Colletotrichum gloeosporioides or were broken down by bacteria without developing fructifications of C. citricarpa. He also found that leaves that fell during moderately hot weather and wilted initially, when evening dews followed hot weather, ~~leaves~~ were most liable to develop fruiting structures. Very hot weather during the first few days after leaf drop did not favour fructification.

It appears therefore that, as in the case of Venturia inaequalis (CKe) Wint. and related fungi, climatic conditions and time of leaf fall vitally influence perithecium formation and ascospore development. Kiely (1948) maintained that ascospores are present in the orchard atmosphere throughout the year, but our results revealed that spores are only trapped during periods of rain and that many more ascospores are caught during summer than the winter months.

1. INVESTIGATIONS

In order to investigate the effect of the time of leaf fall on perithecium development, leaves approximately one to two years old, were picked at different times of the year and exposed to orchard conditions. In all cases the leaves were picked from old severely infected Valencia orange trees. At each date as indicated in Tables 5, 6 and 7 below, 50 leaves were picked by hand. Wooden boxes filled with a sandy loam orchard soil were buried in an old orange orchard. The soil in the boxes was level with the soil in the orchard. The leaves were placed on the soil in the boxes and covered with wire mesh for protection. The leaves were examined for perithecium development at least once a week and sometimes more often.

TABLE 5.

Effect of time when leaves were picked on ascospore development when leaves were exposed to natural conditions, 1959 - 1960.

Date when leaves were picked	Number of days after picking		Date when first ripe ascospores were observed	Rain during exposure period	
	Spermatogonia observed	First ripe Ascospores observed		Amount (mm)	No. rainy days
10/8/59	20	88	6/11/59	46.2	10
1/9/59	15	70	10/11/59	52.3	10
30/9/59	16	55	24/11/59	64.0	15
25/10/59	-	54	18/12/59	199.9	19
25/11/59	11	49	13/ 1/60	282.0	17
3/ 1/60	10	51	23/ 2/60	185.9	22
14/ 2/60	14	56	9/ 4/60	102.6	17
Mean	14.3	60.4	-	133.3	15.7

TABLE 6

Effect of time when leaves were picked on ascospore development when leaves were exposed to natural conditions 1960 - 1961.

Date when leaves were picked	First ripe ascospores observed		Rain during exposure period	
	No. days after picking	Date	Amount (mm)	No. rainy days
25/ 5/60	-	20/ 9/60+	-	-
20/ 6/60	130	28/10/60≠	32.4	7
23/ 7/60	104	4/11/60	26.6	6
21/ 8/60	75	4/11/60	26.6	6
1/11/60	50	21/12/60	282.5	16
Mean	89.8	-	92.0	8.8

\+ Asci with immature spores. Decay too far advanced for further observations.

≠ Only fragments of leaves left.

TABLE 7

Effect of time ~~of~~ ~~time~~ when leaves were picked on ascospore development when leaves were exposed to natural conditions 1961 - 1962.

Date when leaves were picked	First ripe ascospores observed		Rain during exposure period	
	No. days after picking	Date	Amount (mm)	No. rainy days
6/ 6/61	145	29/10/61	87.7	10
12/ 7/61	119	8/11/61	101.7	8
8/ 8/61	92	8/11/61	83.2	7
1/ 9/61	-	8/11/61*	73.7	5
10/10/61	63	12/12/61	91.1	7
Mean	104.8	-	87.5	7.4

* Asci with immature ascospores were observed, but the date of the first mature ascospores was not recorded.

2. DISCUSSION

It is remarkable that in all three seasons during which these observations were made, ascospores matured rapidly towards the end of October to the beginning of November. In most cases immature asci were observed long before the first ripe ascospores were seen. Rain, dew, atmospheric humidity and temperature play important roles in the development of perithecia (Kiely, 1948). Observations during 1959 - 1960 seem to indicate that there was a correlation between the period of ascospore formation and the amount and frequency of rain, but results during the subsequent seasons did not support these observations. Rain or water is undoubtedly an important factor for perithecium development.

One point must be made clear: The fact that ripe ascospores were found on a certain date does not indicate that all the perithecia on such leaves were ripe at that time. In most cases ripe perithecia were found on the same leaves for long periods - up to six weeks. During the 1961-62 season for example, the first ascospores were found towards the end of October to beginning of November, but the same leaves were literally loaded with ripe ascospores towards the end of November. This observation is important.

Under Letaba conditions considerable leaf-drop occurs from the end of May to the end of July, largely due to damage caused by citrus red mite (Panonychus citri Mc Gregor). The spores which develop on these leaves may play an important role in the epidemiology of black spot.

Presumably most of these leaves produce ripe perithecia during November, which is a rainy month. During spells of rain these spores are discharged and may cause infection. If rains are delayed, until the end of November (which was the case in 1961) large numbers of spores are available to cause infection with the onset of the summer rains. The spore trap results confirmed this.

On the other hand, relatively small numbers of perithecia with ripe spores were found on leaf fragments during June, July, August and September. As these observations were made on dead leaves which occur naturally under orchard conditions, there is no record when they actually dropped. These leaves were usually very old and decayed and seldom harboured many perithecia. The determining factors for ascospore maturation are still obscure, and predictions of ascospore availability cannot be made by studying weather records, yet. By exposing leaves at different intervals every year and by careful examination of these leaves, as well as dead leaves which occur naturally in the orchards, one can gain valuable information whereby infection periods can be predicted. This method was used last season at Letaba Estates with extremely good results. Regular examinations of dead leaves are laborious, but worthwhile. If such data can be correlated with weather records, predictions should be comparatively easy and more reliable. Investigations on these lines are strongly recommended.

C. DISCHARGE OF ASCOSPORES

Kiely (1948) observed that the ascospores of G. citricarpa were liberated with explosive violence when a dead citrus leaf with mature asci was moistened with water. A perithecium was regarded as "ripe" when the asci protruded partially from the "ostiole" of the perithecium after wetting for about 30 minutes.

1. DISTANCE OF EJECTION OF SPORES

The nature of ascospore liberation was studied by basically the same method as used by Kiely (1948).

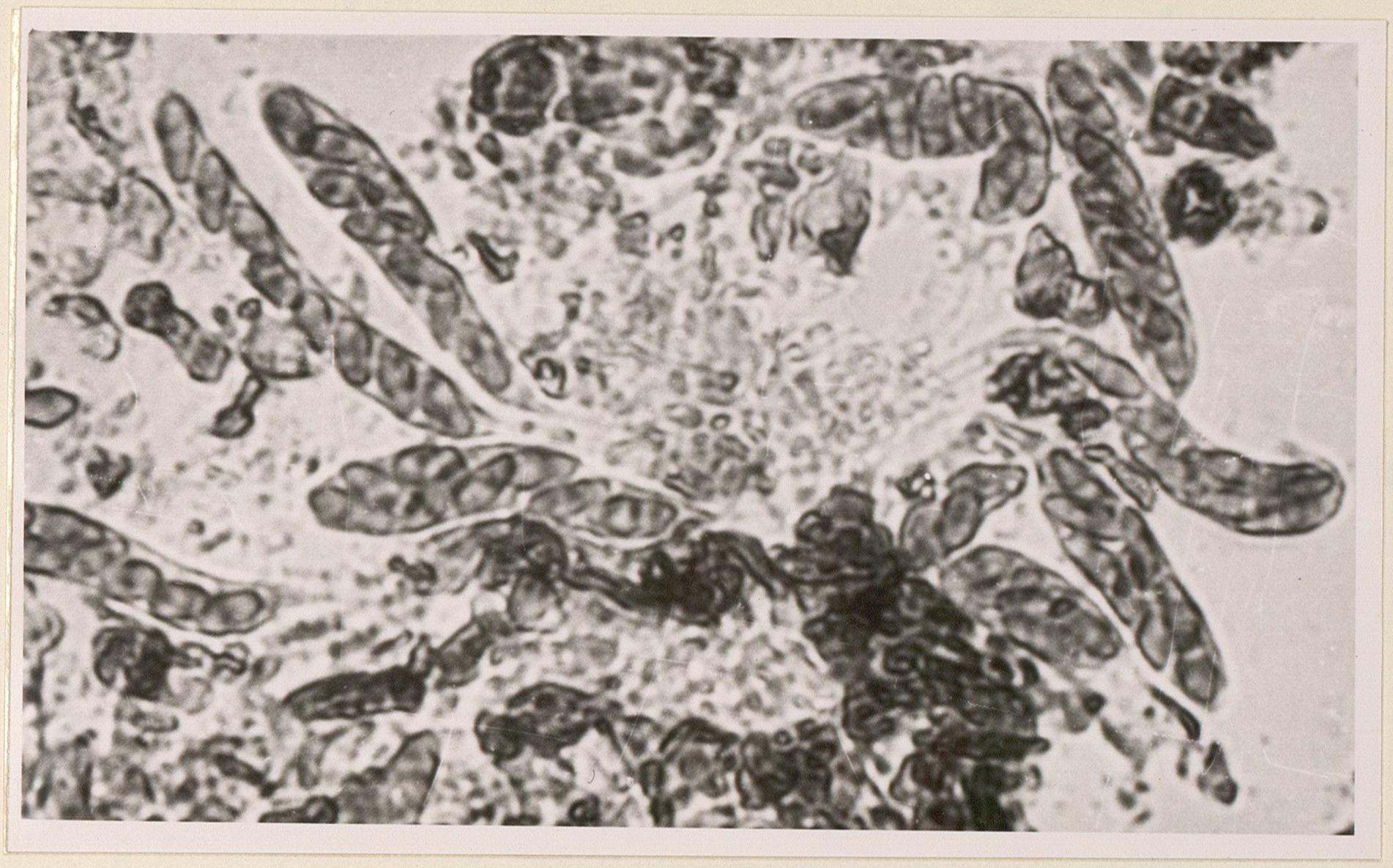


PLATE 2. Asci with ripe ascospores of G. citricarpa after pressing the perithecium between two glass-slides.

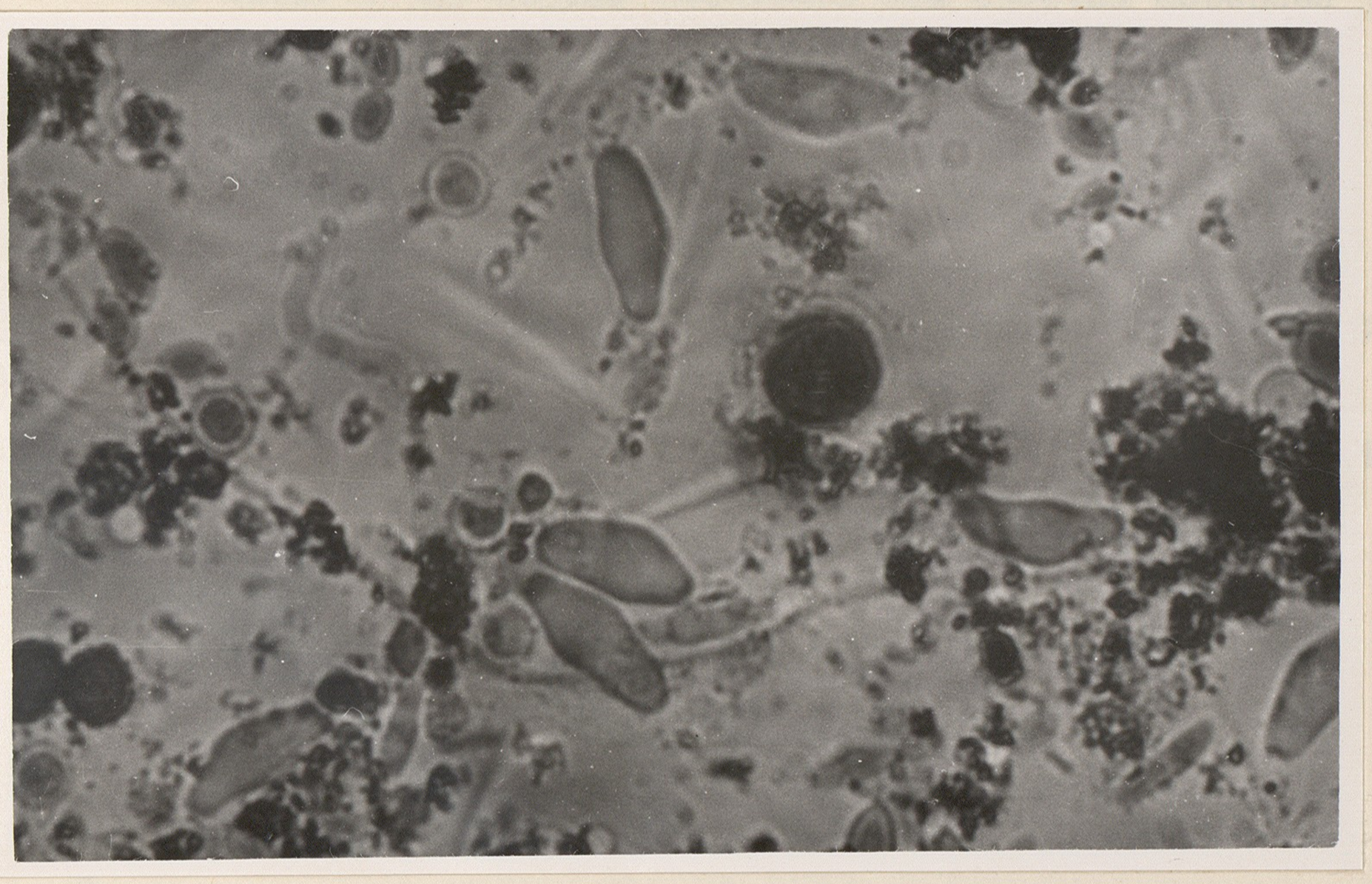


PLATE 3. Ascospores of G. citricarpa, caught with a Hirst spore trap on vaseline-coated glass-slides.

a. Method

One centimeter square pieces were cut from dead leaves bearing mature ascospores. These pieces were placed inside a Petri dish on two layers of filter paper in a centre position with perithecia uppermost. A microscope slide, treated with vaseline was fixed with sticky tape to the inside of the Petri dish cover. The filter paper and the pieces of leaf were wetted with tap water and the dish replaced. The distance between the leaf tissue and the vaseline slide was regulated by inserting thin slices of cork under the filter papers, for shorter distances, or by lifting the dish cover for longer distances. Distances between the leaf tisue and the slide were estimated with the aid of calipers in each case.

b Results

The slides were examined at hourly intervals. The shorter the distance between the slide and the leaf tissue, the more spores were found. At a distance of 1.2 cm, only an occasional spore was found. The number did not increase after 14 hours at 1.2 cm, 0.9 cm, and 0.7 cm but did increase when the distance was shorter. Kiely (1948) maintained that the distance to which a particular ascospore was ejected depended on the time after the first ejection commenced in a particular perithecium and that the vertical throw decreased as the time interval increased.

This type of experiment was repeated several times with minor modifications, but the general picture remained the same. These results are in agreement with Kiely's observations.

2. EFFECT OF TEMPERATURE ON ASCOSPORE LIBERATION

Using the same method as described above, 10 Petri dishes were prepared with slides and leaf pieces. Five dishes were placed in a refrigerator at 5°C and five at 25°C for 1 hour. The dishes were then taken out and the filter paper and leaf pieces were thoroughly moistened with tap water which was also kept at the respective temperatures. The Petri dishes were placed back in the refrigerator and incubator respectively, but in such a position that the spores could be shot down on to the vaseline slides. All the slides were examined microscopically after two hours.

Fresh vaseline coated slides were put in the same position as the previous ones and examined after 12 hours.

TABLE 8

Summary of number of ascospores liberated 2 and 12 hours after wetting at 5°C and 25°C.

Temperature	Time after wetting	No. spores ejected
5°C	2 hours	100
25°C	2 hours	170
5°C	12 hours	261
25°C	12 hours	312

This experiment shows that ascospores are liberated at cold temperatures.

3. EFFECT OF WATER ON EJECTION OF ASCOSPORES

Water plays an important role in the liberation processes of spores of most fungi. For the liberation of ascospores in the Ascomycetes, water plays an essential role.

Kiely (1948) maintained that ascospores were trapped "consistently throughout the spring, summer and autumn" under Australian conditions and the number of spores trapped, "did not appear to be correlated with periods of rainfall". He believed that dew was important for the liberation of ascospores in Australia.

With the aid of Hirst Spore trap (Hirst 1952) ascospores have been trapped on vaseline coated microscope slides since 1959. These slides were carefully examined under the microscope and detailed records were kept of the numbers of spores caught, as well as of weather conditions.

Spores were never found in the orchard atmosphere in the absence of rain. Neither were any spores caught during or after flood irrigations, not even when irrigation water was applied during the period November to February. A possible explanation for this is that irrigation water cools off the soil and, although spores are released, they do not reach the convection streams and therefore never reach the spore trap orifice. Preliminary temperature measurements indicated that this theory may be valid. Mc Onie (unpublished report, 1962), who has used the same technique of trapping ascospores since 1960, presented data which is in general agreement with the writer's results.

He maintained that irrigation water is given mainly during winter when mature ascospores are not abundant and that the water penetrates sandy soils too rapidly to cause thorough wetting of the dead leaves. This explanation is not accepted here. The writer trapped large numbers of ascospores in June 1961 during rain, but no spores were caught in the same orchard during flood irrigations. But under the right atmospheric conditions, (e.g. on a cold day) it is possible that ascospores will reach the fruit on the trees after an irrigation via air currents. One does not expect that such spores will infect since there is no free water on the fruit for germination and penetration. On the other hand, if such spores are able to remain viable for long periods on the fruit in the absence of water, the picture is changed. Results so far indicate however, that this aspect is not important in practice under Letaba conditions. It will be surprising if ascospores are not trapped during sprinkle irrigation operations. Letaba Estates introduced sprinkle irrigation on a limited scale lately and this aspect should be investigated.

Rain was found to be essential for ascospores to be liberated in nature. The amount of rain was seldom important. Also, spores were not caught with every rain. On 7th December, 1960 a heavy thunder shower fell from 2.00 p.m. to 2.30 p.m. The temperatures were 84°F at 2.00 p.m. and 82°F at 2.30 p.m. During the first hour after the rain commenced, 2491 spores were caught. Rain continued again from 4 p.m. onwards till late that night, but no further spores were caught. Altogether 47.5 mm rain was recorded for the whole period.

On 26th November, 1961 a very slight drizzle started at 5.30 p.m. and continued for 16 hours. During the first hour after the rain commenced 11 spores were caught. Thereafter, between 1 and 32 spores were trapped every hour for 12 hours. Altogether 1.5 mm rain was recorded.

The following experiment demonstrates how perithecia from the same leaf and treated the same way vary in behaviour.

On 21st March, 1961 a leaf with numerous mature perithecia was collected from the orchard "floor" in an old Valencia orange grove.

Five pieces (1 sq. cm) were cut from this leaf. To the inside of each of 5 Petri dish lids a piece of leaf was placed on four layers of filter paper. A piece of fine gauze wire was placed over each bit of leaf and filter paper and fixed with sticky tape. The leaf tissues and filter paper were thoroughly wetted with tap water. A microscope slide, coated with vaseline, was placed directly under each bit of leaf on a layer of wet filter paper in the Petri dish. These slides were removed at hourly intervals and replaced with other vaseline coated slides. All dishes were kept in an incubator at 30°C.

On the slides of the first Petri dish 560 ascospores were observed after the first hour. The number of spores liberated during the second hour were 185, the third hour 81, the fourth hour 12, and thereafter nothing.

From the piece of leaf in the second Petri dish no spores were ejected after the first three hours. After four hours 26 spores were counted, but 102 were liberated after five hours, 42 after six hours, 45 after seven hours 20 after eight hours, nine after nine hours and thereafter nothing.

Examination of the slides in the third dish showed no spores for nine days. On the 9th day it was placed in the sun for $\frac{1}{2}$ hour and moistened again. Spores were only ejected for three hours and never again.

In the fourth dish 20 spores were caught after the first hour. Between 6 and 41 spores were counted for the next ten hours. There was nothing for two hours and then a consistent, but low number of spores were observed for seven days. When this piece of leaf was dried in the sun for $\frac{1}{2}$ hour on the eighth day and moistened again only 5 spores were caught during the first hour, but none afterwards.

In the fifth dish the pattern was much the same as in the first, but spores were observed for seven hours after wetting.

An interesting observation was that in all cases large numbers of malformed ascospores were observed just before spore liberation stopped completely. These spores varied much in size and shape and are shown on Plate 4.

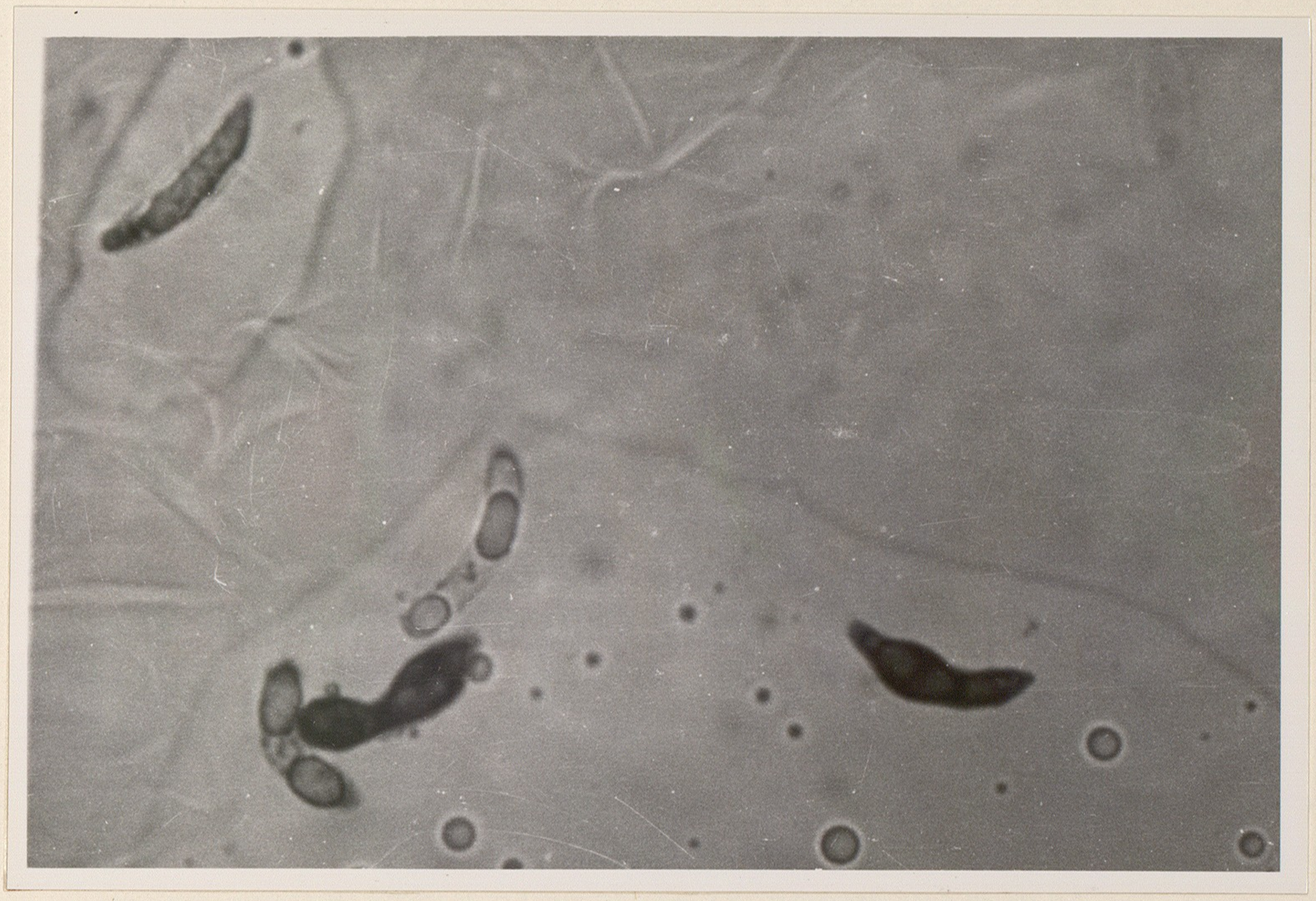


PLATE 4. Malformed ascospores of G. citricarpa.

These spores were given ample opportunity to germinate and although normal spores germinated freely, the malformed spores failed to germinate even after nearly 100 hours at 25°C on P.D.A. medium. These malformed spores were frequently observed on slides of the Hirst spore trap.

A possible explanation for the difference in spore liberation from the various pieces of the same leaf may be due to variations in perithecial age and maturity. It was often observed that perithecia may develop first on one particular site on a leaf and when the leaf was placed in another position new perithecia developed at another site. On the same leaf one may therefore find perithecia in various stages of development. From some bits of leaf all the spores were released during a short period, while in another case spores were released over several days.

4. ASCOSPORE DISCHARGE THROUGH THE YEAR

The consensus of opinion among all research workers who published after 1948 is that ascospores on dead leaves under the trees constitute the major source of inoculum for the development of black spot. The evidence for this assumption is circumstantial and it may still take many years before this theory is proved (or disproved).

An examination of dead leaves for the presence of ascospores provided useful information in the past as a guide of the inoculum potential. It must be borne in mind, however, that spores may be released, but fail to reach air currents for some reason. Under these circumstances the spores are of no importance. In order to determine the presence of ascospores in the orchard atmosphere during the infection period a Hirst volumetric spore trap was used (Hirst, 1952). This instrument was in operation since September 1959.

a. 1959 - 1960 season

The trap was placed in the centre of a plot of old Valencia trees on a stand, with the orifice 5 feet above the ground. Electric power was not available at that stage and the suction pump was operated by a J.A.P. petrol engine. This engine worked fairly well during the initial stages but gave considerable trouble later. This was unfortunate because a breakdown occurred sometimes during a period when spores were being caught. A native boy was on duty all the time to report break downs, but when a break down occurred during the night information was lost.

The trap was in operation from 2nd September 1959 and the first rain fell on 13th September (11.3mm). It rained again on 26th September (0.6 mm) and 19th October (3.5 mm) but no spores were recorded during that period as shown in Figure 1. The first spores were recorded on 20th October. Until the middle of November comparatively low numbers of spores were caught, but the numbers increased considerably from mid-November onwards. Although the results for this season are incomplete , the relatively low numbers of spores trapped during September and October were important information as later discussions will show.

b. <u>1960 - 1961 season</u>

The trap was placed in a 20-year old Valencia orange grove. Once again the orifice was 5 feet above the ground. The spore trap operated from 1st September 1960. The results are presented in Figure 2.

From mid-December 1960 to mid-March 1961, the spore trap was not in operation due to an overseas tour by the writer. The results are presented for the rest of the season. As in the previous season it rained twice during September but no spores were caught. During October rain was recorded on four days as in the previous year but spores were caught only on two occasions. A total of 50 spores were caught during October 1960 compared with 22 for October the previous year. On 9th November, 320 spores were caught and again 56 on 10th November but nothing was caught on the 11th, in spite of 48.1 mm rain for that day. This must be regarded as a possible infection period as 3 wet days followed the rather heavy spore discharge of 9th November.

Although a large number of spores were caught on 14th November, the wet period was probably too short to cause many infections.

After 14th November spores were trapped regularly during spells of rain. The number of spores trapped during the first half of December was higher than for any other month. It is of interest that considerable numbers of spores were caught in June 1961.

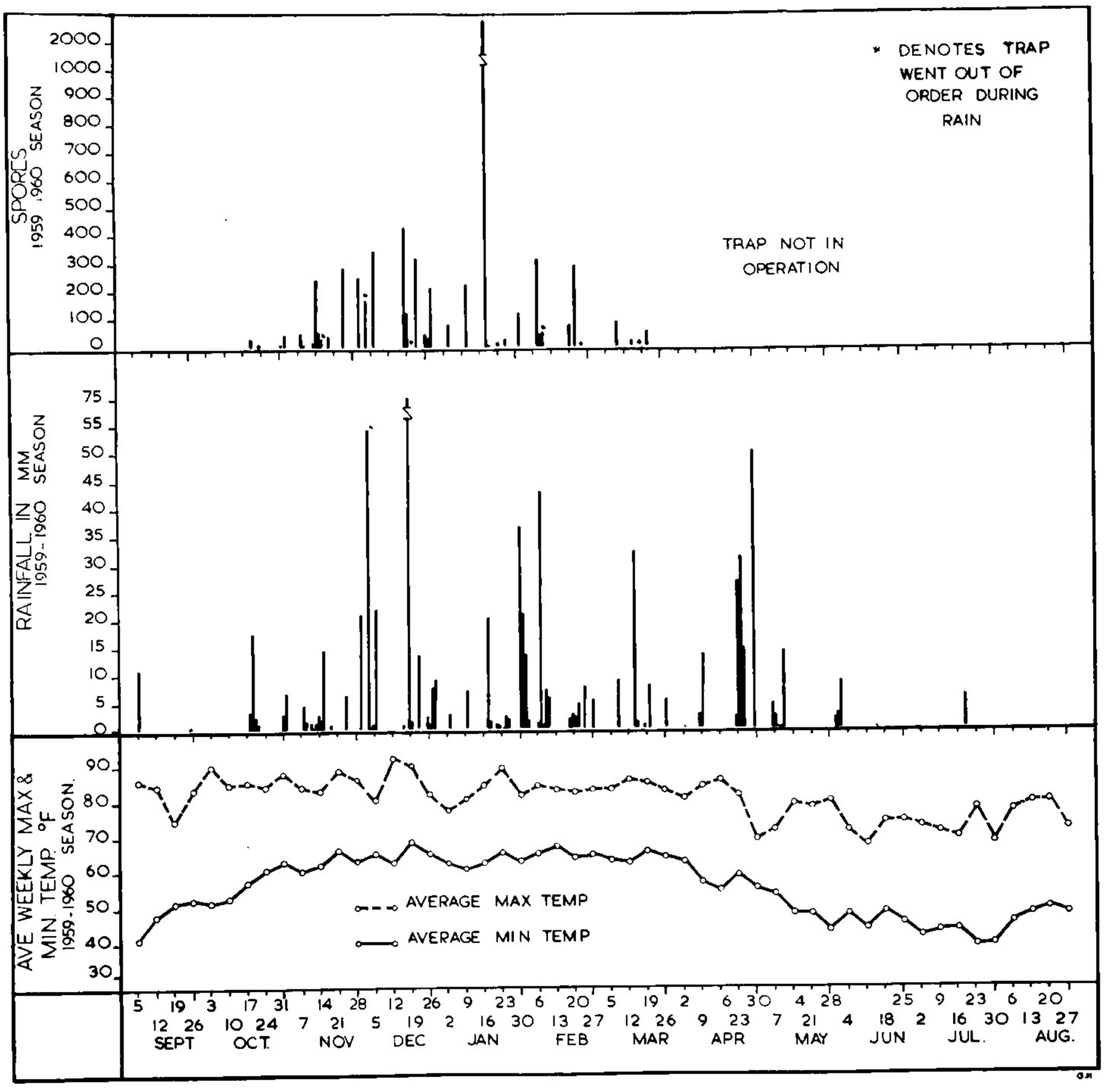


FIG. 1. Graphic representation of the ascospore discharges of G. citricarpa, rainfall and average weekly maximum and minimum temperatures during the 1959-1960 citrus season at Letaba Estates.

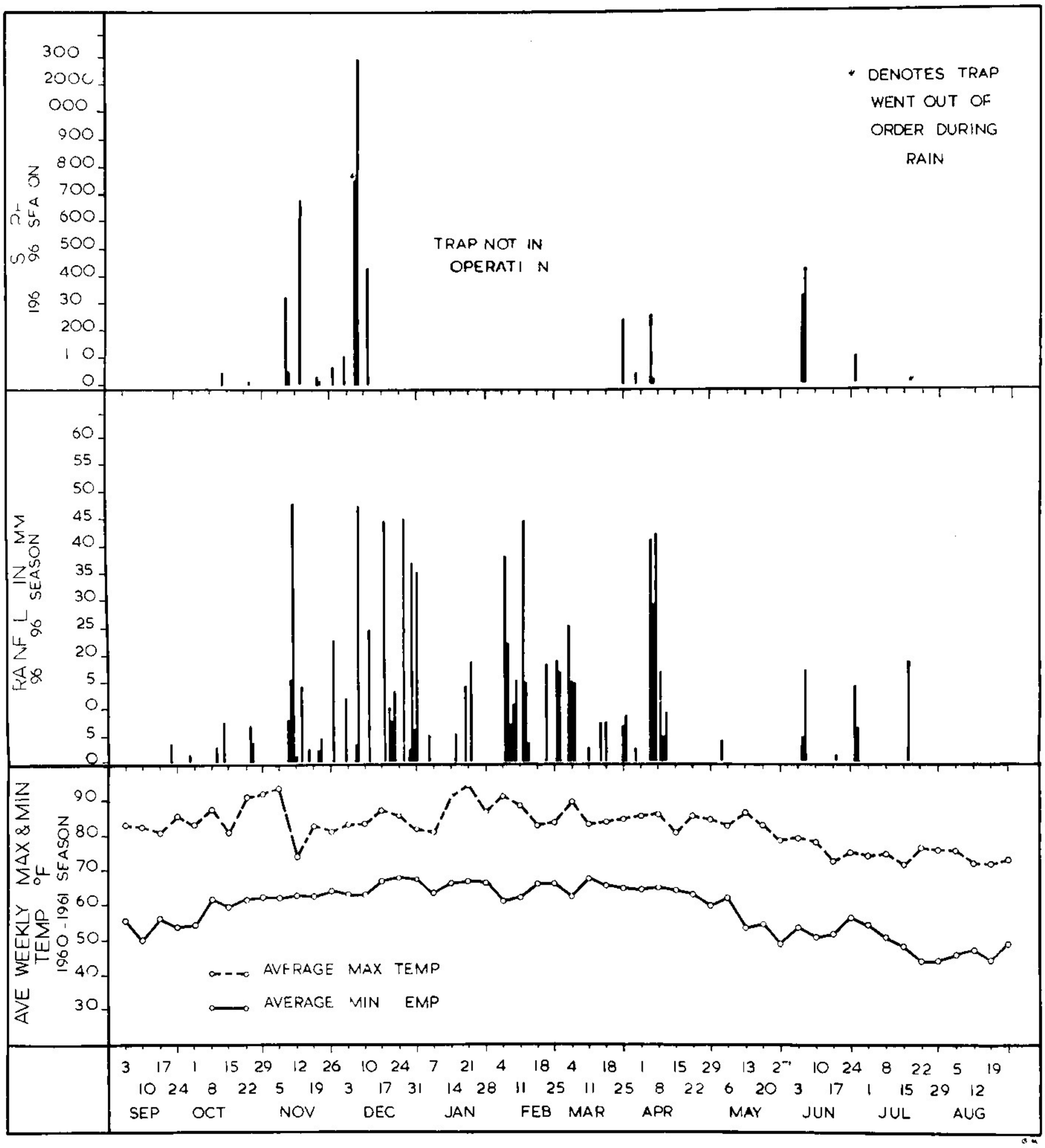


FIG. 2. Graphic representation of ascospore discharges of G. citricarpa, rainfall and average weekly maximum and minimum temperatures during the 1959-1960 citrus season at Letaba Estates.

c. 1961 - 1962 season

The trap was operated by an electric motor during the whole season. This was a great improvement on the petrol motor.

During the 1961 - 1962 season the trap operated in a 12-year old Valencia orange orchard, with the orifice 3 feet above ground. The results are presented as graphs in Figure 3.

Although this was the only season when ascospores were observed on spore trap slides during September, the numbers were comparatively low (6). The months October, November and December were drier than usual. Long periods of continual rain did not occur during November and December as in the previous seasons. The number of spores trapped during November, December, January and February nevertheless were relatively high as in the previous seasons.

5. DISCUSSION

It is of interest that from 8th to 15th November 1959 there was only one rainless day and 375 spores were trapped during that period. A spray experiment carried out at the same site where the spore trap operated showed that considerable infection took place during that period.

The second highest number of spores ever caught on one slide was recorded on 19th January 1960 when 20.3 mm rain was measured. Not nearly so many spores were caught on any other occasion in that year although it rained more than 20.3 mm on 9 different occasions while the spore trap was in operation. It often happened that no spores were caught during light rains.

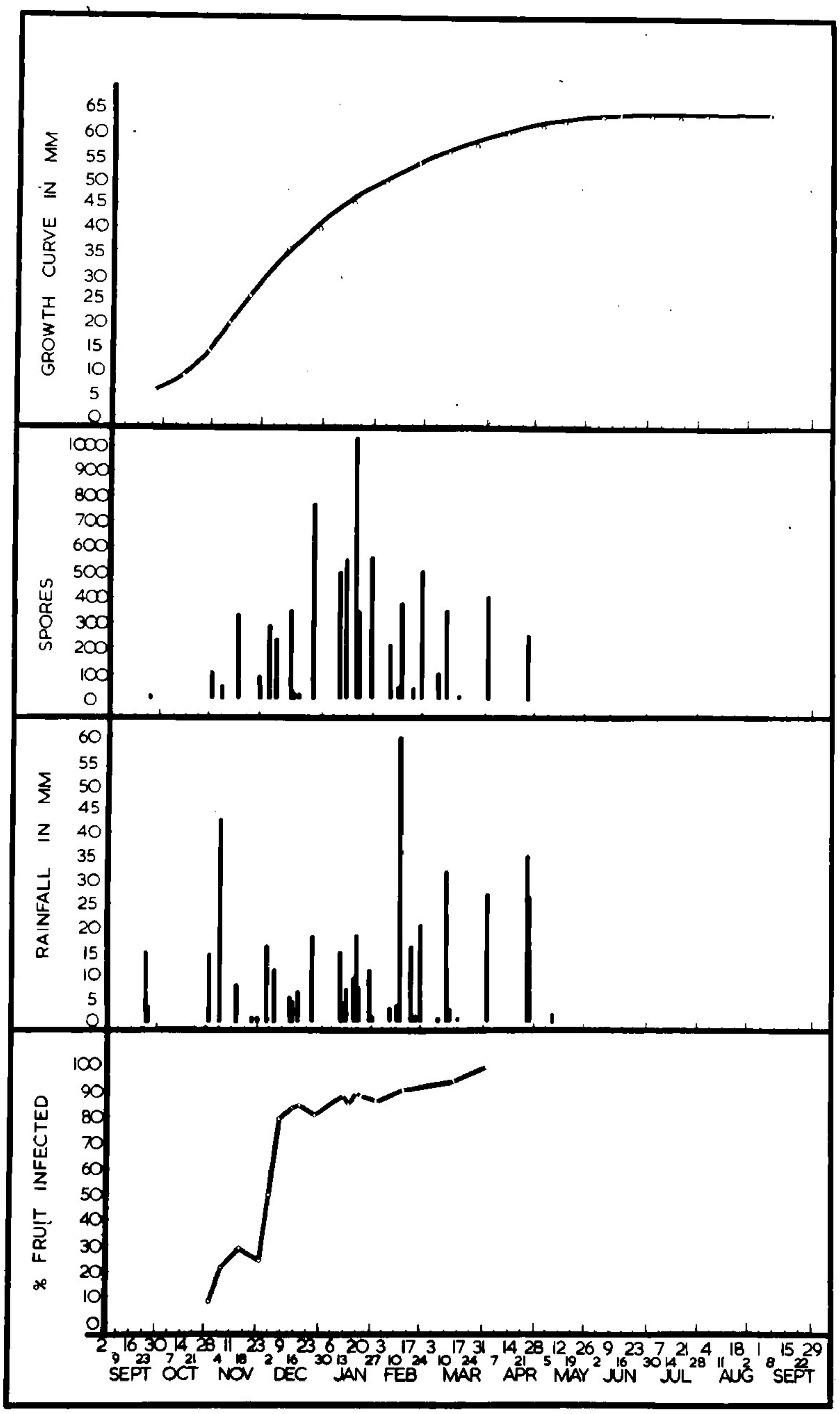


FIG. 3. Graphic representation of Valencia orange fruit growth, ascospores trapped, rainfall and progressive increase of disease incidence during the 1961-1962 citrus season at Letaba Estates.

D. GERMINATION OF ASCOSPORES

Germination studies were carried out with ejected ascospores which matured under natural conditions in an old Valencia orange orchard. Leaf pieces, with mature ascospores were soaked in distilled water and fixed to the inside of a Petri dish cover as described before. The spores were allowed to shoot out for 25 minutes onto a microscope slide, which was treated with a thick layer of potato-dextrose-agar with a pH of 5.9. The slides were removed carefully and placed in Petri dishes, lined with moist filter paper. Altogether nine slides were prepared as above. The number of spores per slide varied from 89 to 421. Three slides were placed at each of the following temperatures: 15°C, room temperature (21°- 23°C) and 30°C.

The results are given below:-

TABLE 9. Germination of ascospores at different temperatures on P.D.A. medium, (pH 5.9).

Temp.	Percentage spores germinated after 15 hours	Ø 22-23 hrs.	Ø 36-38 hrs.
15°C	0.0	0.0	9.2
21-23°C	3.2	29.3	51.1
29.5° C	15.7	41.1	68.0

Ø It was impossible to count all the slides within an hour.

It was originally decided to examine these spores at intervals until 100% germination was obtained. This was impossible because saprophytic fungi overgrew the ascospores and observations had to be abandoned.

Although observations on ascospore germination were carried out on several occasions, using surface sterilized citrus leaves, water suspensions and artificial agar medium, the percentage of spores which germinated was never more than about 75%.

Germinating spores did not absorb analine blue stain, although the germ tubes were stained. The jelly caps at the ends of germinating spores became clearly visible under the microscope. The germ tubes appeared at the centre of the spores and not at the ends as described by Wager (1952). More than one germ tube was never observed per spore, although the young hypha may branch immediately after leaving

the spore.

Appressoria are brown in colour, and thick walled. There is usually a hook at the end of the appressorium.

Appressorium formation was observed in one case, 18 hours after the spores were shot out. In other cases up to 75 hours passed before appressoria were formed, and occasionally no appressoria were formed at all.

E. INFECTION

1. SYSTEMIC INFECTION

Sueda (1941) claimed that infection of fruit and leaves occurs by the movement of mycelium from infected tissues. He established that the fungus spreads to new citrus plants through the grafts which are already infected, but that the growth of the fungus in the tissues of the host is slower than the host. Schüepp (1960), without giving much detail, claimed that the mycelium of G.citricarpa grew more than 10 inches through citrus plant tissue within 3 months.

a. Investigations

On 11th November 1959, ten buds from old, heavily infested Valencia orange trees were T-grafted on 3 young Valencia trees in pots which were obtained from Zebediela Estates. These young trees were kept on the verandah of the laboratory where they were protected from rain and received no more than two hours sunlight per day.

Nine of the buds grew. On 9th March 1960, nearly four months after grafting, pieces of bark, 3 mm. square and 2 mm. thick were cut from the new shoots. Three pieces were cut at $\frac{1}{2}$" distances from the base of the shoot and then every inch, till a distance of $5\frac{1}{2}$ inches. Altogether 162 pieces of bark were cut from the 9 shoots. These pieces were first washed for 1 minute in 90% alcohol and then for $\frac{1}{2}$ minute in 0.1% mercuric chloride. After five washings in sterilized water they were placed on P.D.A. slants. Colletotrichum gloeosporioides grew out of 10 isolates. P. citricarpa grew out of 2 isolations. These two cultures grew from two bits of bark, isolated from the same shoot, $\frac{1}{2}$ inch from the original bud.

Eight months after grafting, all the leaves from these shoots were picked and treated as prescribed by Kiely (1948) to allow fruiting bodies of G. citricarpa to develop. Control leaves of the same age were collected from an old Valencia orange tree. After submitting these leaves for $3\frac{1}{2}$ months to this wilt treatment none of the leaves from the grafts showed any signs of fructifications while those leaves

from the old Valencia orange trees (control) showed spermatogonia after 3 weeks, and large numbers of pycnidia developed later.

To investigate whether mycelium infects fruit systemically, ten young Valencia trees in pots were left outside from September 1959 to August 1960. From these 10 trees 24 fruits were harvested in August and all showed "melanose" and typical black spot lesions. Five of these trees were then placed under the verandah of the laboratory, while the other five were left outside. All the trees flowered normally and set fruit. On 28th August 1961, 13 fruits were harvested from the trees under the verandah and 18 from the trees outside. All the fruit from the trees which were left outside showed "melanose" symptoms and an average of 7.9 black spot lesions per fruit. No disease symptoms developed on the fruit of the trees which were kept out of the rain. After incubation of the fruit for 14 days at 28°C an average of 19.0 spots developed on those which remained outside, but no symptoms developed on the fruit which were kept inside.

Ten isolations per fruit were made at random from each of the "clean" fruits and the apparently uninfected areas of the infected fruit after the incubation period. Not a single isolation from the "clean" fruit produced any fungus growth while 24% of the isolations from the outside fruit produced Colletotrichum gloeosporioides and 15% Phoma citricarpa.

During the 1961-62 season those trees which were under the verandah were placed out in a 20 year old Valencia orange orchard and those trees which were outside were placed on the verandah. Only one fruit matured on the verandah, but no symptoms appeared, not even after incubation for three weeks. Seven fruits matured on the trees in the orchard and all were showing "melanose" and black spot symptoms.

b. Conclusion

The evidence of movement of mycelium into newly formed twigs of Valencia orange trees is rather negative except in one case where the mycelium moved approximately $\frac{1}{2}$ inch in 4 months. No evidence could be found that fruits become infected by the movements of mycelium. Where fruits were bagged at the time of blossoming or shortly afterwards to prevent infection by spores as described later, the fruit remained lesion free. (See Tables 12 and 13).

It may be argued that factors such as rain, sunlight and high temperatures are essential for the movement of the mycelium from infected tissues to newly formed twigs, leaves

and fruit. It is conspicuous however, that neither Sueda (1941) nor Schüepp (1960 and 1961) who were strong supporters of the "systemic infection theory"mentioned any of the above factors. In the light of the information so far, systemic infection seems to be of minor importance in the epidemiology of black spot on oranges. Schüepp's results were apparently obtained with lemon trees which were very susceptible to black spot.

2. INFECTION WITH PYCNIDIOSPORES

Kiely (1948) indicated that infections were caused by pycnidiospores produced in cultures on sterilized Valencia rind tissues. Wager (1952) also presented evidence of pycnidiospore infection.

It was decided to investigate the possibility of pycnidiospore infection.

a. Investigations

The methods used were similar to Kiely's but slight modifications were found necessary.

On 18th November 1959 several hundreds of unopened out-of-season blossoms on five old Valencia trees were enclosed in brown paper bags which were reinforced on the seams with paraffin wax. On 3rd January 1960 the bags were removed on a warm sunny day.Only 73 fruits set. All these fruits were carefully cleaned with distilled water and cottonwool to remove dust or any foreign spores that might have been present.

From 28 slant cultures of P. citricarpa, isolated from infected Valencia fruit rinds, a spore suspension was obtained by scraping the surface of the cultures gently with a blunt sterilized needle and washing the spores off with sterile water. A spore suspension containing approximately 1,500 spores per cc. was eventually obtained. Fifty of the fruits were then dipped repeatedly in the spore suspension. Thin strips of absorbent cottonwool were then dipped in the spore suspension and placed loosely over each fruit. After this, a small plastic bag, containing wet cottonwool was slipped over all the fruits and tied with string, with non-absorbent cottonwool between the twig and the plastic bag to prevent any spores that might be washed in by rain. All this was enclosed in a brown paper bag. After four days the plastic bags were removed, and the brown paper bags were replaced.

On 2nd February 1960, 25 fruits were treated in the same way.

The ages of the cultures used for the inoculations were

25 days and 31 days respectively. Tiny fragments of mycelium were also present in the spore suspension.

On 1st October 1960, when the fruit appeared to be mature, all were picked and examined. The fruits were stored for 14 days at 27°C and examined again. The results are given in Table 10.

TABLE:10 Table showing black spot lesion development on fruit after inoculation with pycnidiospores of Phoma citricarpa.

Treatment No.	Dates of inoculation		No.fruit surviving	Total No. Black spot lesions.		No.fruit showing Melanose symptoms
	1	2		At picking	14 days incubation	
A	3/1/60	26/2/60	11	5 (3fruits)	13 (5fruits)	2
B	3/1/60	-	7	2 (1fruit)	4 (2fruits)	0
C	control		9	0	0	0

The results in the above table indicate that pycnidiospores infected the young fruits..

The presence of "melanose" symptoms is another indication that P.citricarpa is is under suspicion as the cause of this particular kind of symptom.

Laboratory experiments showed that the fungus enters through stomata of leaves. Five young Valencia orange leaves (approximately one month old) were picked from young trees which were growing in pots under the laboratory verandah. These leaves were thoroughly cleaned with cottonwool and distilled water. One leaf was placed with its underside up in each of five Petri dishes which were lined with moist filter paper. From a three week old P.citricarpa culture on P.D.A. medium, blocks of 2 cm. square were cut and placed on the leaves. These blocks and the leaves were kept moist with sterile water. The Petri dishes were sealed with scotch tape and placed in an incubator at 27°C. After 72 hours the leaves were examined microscopically and numerous germinating pycnidiospores were observed as well as mycelium penetration through the stomata. By using the cellulose acetate film method, described by Petersen (1956) a photograph was taken of this phenomenon (Plate 5.)

In other experiments where pycnidiospores were used for infection studies on leaves as described above no success was achieved. The penetration of the stomata as shown on Plate 5. was presumably the result of mycelium infection and not by germinating pycnidiospores.

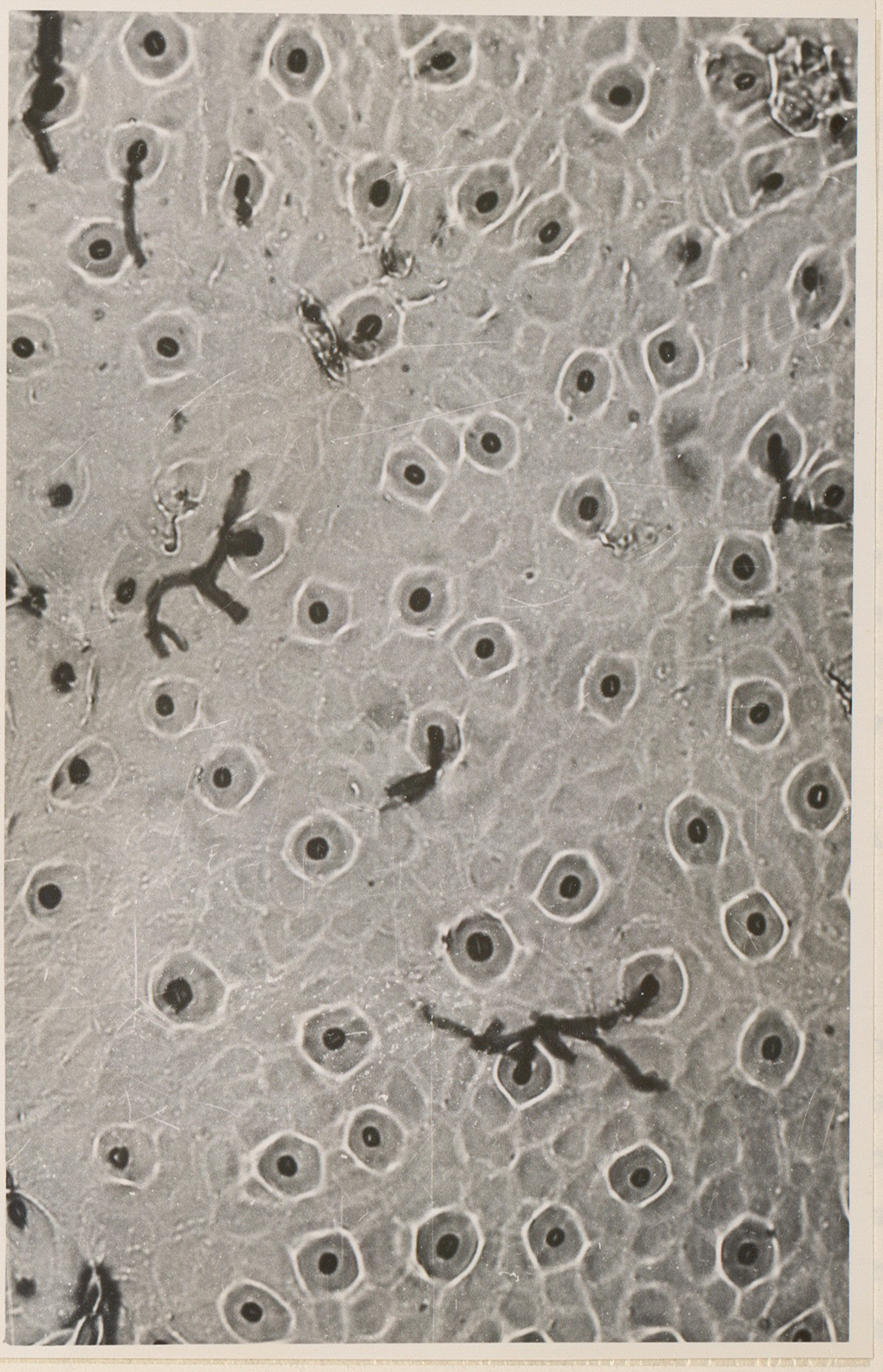


PLATE 5. Mycelium penetrating stomata of a citrus leaf.

b. Conclusion

Results presented by Kiely (1948) Wager (1952) and the results discussed here, show that pycnidiospores can cause infection. The relative importance of pycnidiospores in nature will depend on the availability of inoculum in the tree; on whether or not they are airborne; their viability and weather conditions.

During continuous spells of soft rain, pycnidiospores ooze out and into the water drops on the fruit, carrying concentrated spore suspensions. Tear stain marks are often observed on fruit which might have been caused by such drops containing heavy spore loads.

3. INFECTION WITH ASCOSPORES

The fact that ascospores did not develop freely on the artificial media used in these studies hampered investigations on infection. Dead Valencia orange leaves with ripe ascospores were used in these studies. These leaves were carefully examined for the presence of other fungi. Five leaves were eventually found with very many ripe perithecia and no fruiting bodies of other fungi. It is of course highly possible that saprophytic organisms were present in the leaves.

a. Investigations

This experiment was carried out on the fruit of an old Valencia tree. Large numbers of blossoms were covered with brown paper bags on 1st. September 1960. On 3rd December the paper bags were removed and the fruit thoroughly cleaned with distilled water and cottonwool. The old leaves with the ascospores were soaked in water for about 10 minutes. A leaf was placed on each of five fruits to allow the spores to shoot out onto the rind of the young fruits. As described before, a thin strip of moistened cottonwool was placed over each leaf and covered with a small polythene bag containing moist cottonwool. Paper bags were slipped over the plastic bags and were firmly fastened. Two days later, the bags were removed and the old leaves and cottonwool were moistened again. The plastic bags were removed five days after commencement of the experiment, but brown paper bags were replaced until the fruits were picked on the 15th September 1960.

Seventeen fruits which were left as controls received exactly the same treatments as the 5 inoculated fruits, except that the old leaves with the ascospore inoculum were left out.

When the fruits were picked none of the control fruit showed any disease lesions, but on one fruit two lesions

appeared on the "shoulders" after incubation of 14 days at 27°C. These two lesions might have been the result of spores that washed in along the stalk by rains. The possibility of systemic infection as described earlier cannot be excluded however. Of the five inoculated fruit, 3 showed typical black spot lesions (hard spot and freckle spot) but two exhibited "melanose" symptoms round the area where the leaves were situated on the fruit. (See Plate 6.) After incubation all five fruits developed hard and virulent spots. On the two fruits showing "melanose" hard spots developed within five days, just below the "melanose" ring but after 14 days about one third of the rind surfaces developed into one big virulent spot, showing myriads of pycnidia.

The leaves with which the fruits were infected, were thoroughly examined before the inoculations. It is fairly certain that no fruiting bodies of Diaporthe or Phomopsis were present on these leaves.

It is suggested that a heavy spore suspension of G.citricarpa which caused numerous infections close together, gave rise to these "melanose" symptoms.

b. Conclusion

Kiely (1948), Calavan (1960) and Schüepp (1960) considered ascospores as the most important source of infection in the epidemiology of black spot. Although much basic research is still necessary on infection, considerable information is available to substantiate the theories of the above workers.

4. INFECTION PERIOD

Earlier workers, both here and in Australia, showed that the best control is obtained by applying a number of copper fungicidal sprays during the first few months after blossoming. The major break-through was made by McCleery (1939) when he showed that "the main infection period extends from blossoming until approximately 20 weeks later". Kiely (1950) concluded ....."if orange crops were to be protected from black spot, preventure spraying programmes would have to start when the fruit was quite small, as infection commenced apparently shortly after blossoming....."

Wager evidently accepted that Kiely's results are valid under South African conditions. Wager (1950) recommended that the first spray should be applied at the two-third petal-drop stage followed by two more sprays at six weekly intervals. This recommendation was followed by the Citrus and

PLATE 6. A Valencia orange showing "Melanose" symptoms after the fruit was infected with ascospores on a dead citrus leaf.
Note that no symptoms developed under the leaf, but around the area occupied by the dead leaf.

Horticultural Research Station at Nelspruit. Wager (1952) reported that: "In different seasons.....both good and bad results have been obtained when either the first or 3rd sprays had been omitted, or when all three sprays were two or three weeks later than they should have been applied. It probably all depends entirely on how much rain or dew there is at that time of the year.".

Kotze, (1961) and unpublished reports of A.P.Fochessati ø and K.C. McOnie+ indicated that the onset of the infection period is much later than the end of September (petal-drop).

For the establishment of a fungus epidemic, the synchronization of three conditions are required, viz:

a. Availability of a susceptible host.
b. Availability of the pathogen.
c. Optimum weather conditions for infection.

Evidence that fruit susceptibility decreases with time will be discussed later. The pathogen may be abundant in the citrus orchards, but the availability of viable spores depends largely on climatic conditions. Weather conditions play a dominating role in the development of the spores, their liberation, distribution, germination and infection. An infection period during the stage of fruit susceptibility is therefore guided by weather conditions and not the calender. (Miller, 1959).

In Table II it can be seen that the average rainfall at Letaba Estates for September is only 17 mm. The blossoming period is from the end of August to the 3rd week of September.

ø African Explosives and Chemical Industries Limited - unpublished report 1961.

+ S.African Citrus Exchange, unpublished report 1962.

TABLE 11. Rainfall in mm. at Letaba Estates 1945-1962.

Months	Monthly mean 1945-1962	1959-1960	1960-1961	1961-1962
Sept.	17.0	11.9	5.1	21.7
Oct.	46.2	24.4	21.6	14.0
Nov.	84.6	67.1	118.4	49.3
Dec.	137.7	244.3	310.6	63.6
Jan.	168.1	40.6	42.9	74.5
Feb.	146.8	159.3	208.3	53.7
March.	117.3	59.4	89.4	35.7
April.	50.3	140.3	143.3	89.8
May	24.9	21.6	4.1	4.2
June	8.4	13.5	42.5	0.0
July	7.4	8.4	18.5	0.0
Aug.	5.3	0.0	9.5	2.0
Total	814.0	790.8	1014.2	408.5

The full petal-drop stage is usually reached towards the end of September. Small fruits are therefore available for infection from the beginning of October onwards. The average rainfall for October is 46.2 mm. which is higher than the recorded October rainfall for any of the three seasons during which this investigation was carried out. The number of ascospores trapped during October were comparatively low for the three seasons.

Preliminary investigations were carried out during the 1959-60 season on the infection period. This was done by

a. taking rain records,

b. trapping ascospores,

c. covering fruit at different stages and

d. spraying experiments.

The results a. and b. were given in Figure 1.

Brown paper bags were placed over approximately 50 fruits on the following dates: 15th October, 1959, 10th November, 1959, 19th November, 1959, 23rd December,1959, 20th January, 1960. On 15th October 1959 an additional 50 fruits were tagged, but were never covered. This experiment was carried out on five old Valencia trees which received no sprays for the control of black spot. All the bags were removed on 31st July 1960 to allow the fruits to colour and mature under natural conditions. The fruits were harvested on 19th. September 1960 and stored in the laboratory at room temperature for 15 days before examining. The results are

tabulated below:

TABLE:12. The effect of bagging of fruit at different intervals on the incidence of black spot during the 1959-1960 season.

Period of exposure to infection	No.of fruits surviving	Total number of lesions	Average number of lesions per fruit
Blossoming 15/10/59	10	8	0.8
" 10/11/59	21	28	1.3
" 19/11/59	18	108	6.0
" 23/12/59	25	1757	70.3
" 20/ 1/60	26	2080	80.0
" Never covered	34	3100	91.2

One can deduce from this experiment that little infection took place between blossoming and 10th November 1959. Infection that occurred from 10th to 19th November resulted in an increase from 1.3 to 6.0 spots per fruit. From 19th November to 23rd December there was a sharp increase in the number of spots, from 6.0 to 70.3 per fruit. It is possible that some infection took place even after January.

By following a staggered spray schedule the effect of spraying with Bordeaux mixture ($2\frac{1}{2}$: 2: 100) on the incidence of black spot was investigated, during the 1959-1960 season.

This was a randomized block experiment with 7 treatments, each with 10 replications of single tree plots. An orchard of old Valencia trees were used. Records were taken during the first week of September 1960.

TABLE 13. Table showing dates when Bordeaux mixture was applied and the results obtained in an experiment to determine the infection period of black spot at Letaba, during the 1959-1960 season.

Treatment No.	Dates of Spraying ø 11th Sept 1959	+ 30th Sept 1959	23rd Oct. 1959	14th Nov. 1959	4th Dec. 1959	31st Dec 1959	Mean % fruit infected	Inverse Arc sine transformation
1	x	x	x	x	x	x	15.32	21.8
2	0	x	x	x	x	x	18.07	23.3
3	0	0	x	x	x	x	22.78	27.2
4	0	0	0	x	x	x	20.68	26.3
5	0	0	0	0	x	x	64.69	53.7
6	0	0	0	0	0	x	75.33	60.8
7	0	0	0	0	0	0	67.59	56.0

(p=0.05)11.6
(p=0.01)17.2

ø Full blossom
\+ 90% petal drop
x sprayed
0 not sprayed

All the trees were harvested during the second week of September 1960 and all the fruit was examined.

The results showed that there are no significant differences between the treatments where spraying commenced on 11th September 1959 until 14th November 1959. Where spraying was delayed to 4th December no better results were achieved than where no sprays had been applied. The results in this experiment agree with those obtained in the bagging and follow closely the ascospore liberation pattern.

An identical experiment was done at Muden, Natal. The results of that experiment were similar to the one above, except that the critical time for spraying was in December, about 3 weeks later than Letaba.

Results on ascospore trapping and weather conditions for the 1961-1962 season are given in Figure 3.

In order to determine the period of fruit susceptibility and to get further information on the infection period another bagging experiment was carried out. Twenty uniform trees in a 12 year old Valencia orange orchard in which the spore trap operated, were selected for this purpose. There were 10 treatments, using two trees per treatment on which 100 fruits were tagged and covered with brown paper bags, except for the periods of exposure as set out below. All fruits were covered since the beginning of petal drop (20th September) except those in treatment 1 and 10. The fruits were harvested on the 15th August 1962 and stored in the laboratory at 22-26°C until 1st September when all fruits were examined.

TABLE 14 Table showing the incidence of black spot and "melanose" as a result of exposure of Valencia orange fruits for approximately monthly periods during the 1961-1962 season. Rainfall (in mm.) and the number of ascospores trapped during the respective periods are also given.

Period of exposure to natural infection	Total No. survived fruit	% Fruit with B.S. lesions.	Av. No. lesions per infected fruit	% Fruit with "Melanose" lesions	Av. No. "Melanose" spots per infected fruit	Rain (mm)	No. of ascospores trapped
1) Blossom-15th Oct.	18	0.0	0.0	0.0	0.0	21.7	6
2) 15th Oct. - 15th Nov.	12	16.7	17.0	16.6	5.8	60.3	386
3) 15th Nov. - 15th Dec.	18	88.9	8.1	22.2	2.0	41.0	991
4) 15th Dec. - 15th Jan.	8	100.0	14.5	37.5	9.0	52.3	1834
5) 15th Jan. - 15th Feb.	24	100.0	>105.	37.5	117.4	115.3	2592
6) 15th Feb. - 15th Mar.	26	30.8	8.6	3.8	15.0	75.2	1011
7) 15th Mar. - 15th Apr.	32	0.0	0.0	0.0	0.0	27.0	423
8) 15th Apr. - 15th May	23	0.0	0.0	0.0	0.0	66.7	257
9) Never exposed	25	0.0	0.0	0.0	0.0	-	-
10) Always exposed	44	100.0	>86.	43.2	112.0	459.5	7500

According to the results in this experiment, no infection occurred before 15th October. The rainfall was only 21.0 mm. till then and very few ascospores were trapped for the corresponding period. The first infection occurred between 15th October and 15th November. Thereafter a sharp increase in the disease incidence occurred as a result of infection until 15th February. Infection which occurred between mid-January and mid-February caused so many lesions that they coalesced and an accurate count was not possible. This also applies to the treatment where the fruits were exposed throughout the season. Only 30.8 of the fruit became infected from mid-February and to mid-March. Exposure after 15th March caused no disease lesions, despite considerable rains and the presence of ascospores. This seems to indicate resistance in the fruit of some nature. There is a striking and rather unexpected resemblance between the periods of black spot and "melanose" infection.

To confirm the experiment just described, another was carried out in the same block of trees. Approximately 150 fruits were covered on two trees each time after a spell of rain during the 1961-62 season. The object of this was to establish the amount of infection that took place during each period of rain and to get some idea on the period of wetness required for infection. In July 1962 all the paper bags were removed to allow natural colour development and ripening. All the experimental fruits were harvested on 15th August and stored in the laboratory at 22° - 25°C until 1st September. All the fruits were then examined for the incidence of black spot and "melanose" lesions. The results are summerized in Table 15.

TABLE:15. The effect of covering oranges after spells of rain and ascospore discharges at different stages during the infection period on the incidence of black spot and "melanose" during 1961-1962 season.

Treatment: Period of Exposure	Total No. Surviving fruit	% Fruit with Black spot lesions			% Fruit with "Melanose" lesions	Rain (mm) before covering	Approx number hours wet	No. Ascospores during rain
		<5 spots	>5 spots	Total				
Blossoming- 30/10/61	63	6.3	1.6	7.9	1.6	14.0	20	110
" - 6/11/61	86	19.8	1.2	21.0	3.5	38.0	45	43
" - 16/11/61	75	26.7	1.3	28.0	1.3	8.3	40	338
" - 27/11/61	96	21.9	2.1	24.0	3.1	1.5	10	92
" - 2/12/61	93	46.3	3.2	49.5	2.2	16.7	22	291
" - 8/12/61	106	45.3	34.0	79.3	8.5	11.1	38	238
" - 15/12/61	97	50.5	33.0	83.5	13.4	10.2	40	365
" - 19/12/61	89	51.7	32.6	84.3	9.0	7.1	15	12
" - 27/12/61	109	39.5	41.3	80.8	7.3	18.5	30	757
" - 12/ 1/62	96	41.7	46.9	88.6	20.8	19.7	29	506
" - 15/ 1/62	92	37.0	47.8	84.8	19.6	7.0	15	559
" - 19/ 1/62	96	20.8	68.8	89.6	15.6	28.0	40	1047
" - 22/ 1/62	78	26.9	61.5	88.4	19.2	7.5	26	348
" - 30/ 1/62	89	31.0	55.2	86.2	18.4	12.3	27	568
" - 15/ 2/62	65	15.4	75.4	90.8	24.6	64.0	24	422
" - 15/ 3/62	90	7.8	86.7	94.5	20.0	34.0	33	355
" - 2/ 4/62	89	7.9	92.1	100.0	13.5	14.0	26	411

$x^2 = 573.3$
DF = 16

Dead leaves were regularly examined after the beginning of September. Until the end of November ripe perithecia were found only occasionally on comparatively few leaves, but immature perithecia with asci with non-differentiated spores were prevalent. From the end of November onwards, ripe perithecia appeared on a high percentage of dead leaves. This information, incidentally was used with great success to indicate the best times to apply the commercial black spot spray programme at Letaba Estates.

Until the end of October 7.9% of the fruits became infected but only 1.6% showed more than 5 spots per fruit. Until 2nd December the percentage fruit with less than 5 spots increased but the percentage with more than 5 spots remained fairly constant. From then on fruit became severely infected.

Less fruit fell in the less than 5 spot category after 19th January and progressively more in the "severe" category. This seems to indicate that infection occurred during that period. The results of this experiment are given in a graph in Figure 3.

For further information on the infection period a randomized block experiment was laid out on 12-year old Valencia orange trees. There were 7 treatments as set out in Table 16 with 8 replications of single tree plots per treatment.

TABLE: 16. Table showing dates when different trees were sprayed with 2 lb Perenox per 100 gallons water and the effect of the different treatments on the incidence of black spot.

Treatment	SPRAY DATES					% Fruit showing black spot lesions	Inverse Arc sine transformation
No.	10/10/61	1/11/61	22/11/61	13/12/61	3/1/62		
1	X	X	X	X	X	0.22	2.15
2	X	X	X	X	0	0.73	4.40
3	0	X	X	X	0	0.93	5.48
4	0	X	X	0	0	1.70	7.33
5	X	0	X	0	X	0.42	3.65
6	0	X	X	0	X	0.25	2.80
7	0	0	0	0	0	32.04	-
X = Sprayed				without control		(p=0.05)	1.87
0 = Not sprayed						(p=0.01)	2.52

The fruit was harvested during the 1st week of September 1962. All treatments showed a low incidence of black spot. If picking could have been postponed to a later date, more lesions would have developed to show bigger differences between treatments, but that would have caused great inconvenience to the packhouse which finished operations early in September. In the statistical analysis, the arc sine transformation was applied but the results of the untreated control were omitted to detect differences between the other treatments.

Evidently, spraying on 10th October, 1st November and 13th December had little effect on the control of the disease. The sprays which were applied on 22nd November and 3rd January were apparently important.

To a certain extent these results confirm the data obtained with bagging.

5. DISCUSSION

Investigations over three seasons lead to the conclusion that the seasonal pattern of the discharge of ascospores is

greatly influenced by the occurrence of rain. Hardly any ascospores were trapped during September and comparatively few were recorded in October. Correspondingly few ripe perithecia were found on the dead leaves during that time. A close relationship was found between the onset of ascospore discharges, the onset of rains and the onset of the infection period.

The presence of spores in the air which settle on the susceptible plant tissues are of no importance unless the weather is suitable for infection. When a wet period lasted a few hours only, no infection resulted despite the presence of ascospores. Ascospores germinate and form thick-walled appressoria. Whether these appressoria can resist unfavourable weather conditions is a matter to be investigated. If they can resist hot and dry periods, they may cause infection when conditions become more favourable.

In February 1960 about three weeks after the petal-drop stage paper bags were placed over out-of-season fruits of young Valencia orange trees. The paper bags were removed on the day when the fruits were picked (15th September 1960). Eleven of the seventeen fruits showed black spot lesions. These symptoms could only have been the result of infections during the first three weeks after blossoming. Considerable rain and ascospores were recorded during this period. This observation, together with Kiely's (1948) data suggest that fruit is susceptible to infection immediately after blossoming. The fact that little or no infection occurred during September and October must therefore be due to the prevailing weather conditions and subsequently the absence of inoculum at that time.

It was pointed out that ascospores were scarce on dead leaves during September and October. A spell of rain during that period may have serious consequences even in the absence of ascospores, but where pycnidiospores are available. This may be an important factor in the control of black spot especially if pycnidiospores are airborne. This aspect should be investigated.

Results obtained with bagging suggest that fruit become resistant to infection after February. This explains why spraying with copper fungicides after January has little effect on the control of the disease.

Fochessati (1959) suggested that the poor control of black spot which growers sometimes experience despite a three or four-spray programme, is due to the increase in fruit-growth. He pointed out that the concentration of the

fungicide will decrease in proportion to the increase of the surface area of the fruit. According to his results, the concentration of copper per unit surface will be approximately 16 times lower at the time of the second application (i.e. when the first spray was applied at petal-drop and the second six weeks later). As the season advances the rate of fruit growth decreases and the influence of this factor on the residual deposit of fungicide becomes less important.

From the growth curve in Figure 3 the increase of surface area can be calculated. Consider a 3-spray programme, starting at petal drop with a time interval of 6 weeks between the sprays. Suppose that a fruit is sprayed at petal-drop stage; that no weathering occurs and no redistribution of the fungicide takes place. At petal drop (25th September) the surface area of the fruit is 79 sq.mm. and six weeks later the surface area is approximately 1257 sq.mm. Twelve weeks after petal-drop the surface area is approximately 4540 sq. mm. With no redistribution of the fungicide, the unprotected surface area six weeks after the first spray, is 1257-79 = 1178 sq. mm., but six weeks after the second spray application 4540-1257 = 3283 sq. mm. will be unprotected. The unprotected surface area between the first and second spray is therefore relatively smaller than the unprotected surface area between the second and the third spray.

Consider another and more practical possibility. In practice a copper fungicide is used and redistribution does occur. If we ignore the effect of weathering, the concentration of the fungicide per surface area of the fruit, will be approximately 16 times lower at the time when the second spray (6 weeks later) is due. If a spray is applied six weeks after petal drop, the fungicide concentration will be 3.6 times lower than the initial deposit, at the time when the third spray is due (12 weeks after petal drop).

It appears therefore that the interval between the first and second spray should be shorter than the interval between the second and the third application. This was exactly what Fochessati proposed. Our own results showed however, that little or no infection takes place from petal drop to the end of October under Letaba conditions. Under normal circumstances, protection during the first month after petal drop is not required. Our studies on the infection period suggest that a three-spray programme which commences towards the end of October should provide better protection throughout the season than a 3-spray programme which starts

at petal drop. A programme which commences later in the season will be less affected by the "dilution" of the fungicide due to fruit growth.

F. INCIDENCE OF DISEASE.

Black spot lesions occur primarily on fruit but symptoms also appear on leaves and twigs (Calavan 1960).

1. THE OCCURRENCE OF SYMPTOMS ON LEAVES.

Descriptions of leaf symptoms were given by Kiely (1948), Wager (1952), Calavan (1960) and Schuepp (1961).

Kiely (1948) stated that leaf symptoms in the Gosford district of New South Wales are rare on orange leaves but more common on lemon leaves. Kiely claimed that senile leaves are more likely to show black spot lesions and that pycnidia only develop occasionally on leaves.

At Letaba Estates leaf symptoms are now scarce. It is the writer's impression that leaf symptoms were more common a few years ago. In 1959 and 1960 thousands of leaves with lesions were collected from old Valencia trees in plots 616, 785 and 786 for experimental purposes. During 1961 and 1962 leaf symptoms were extremely scarce. In June 1962 it took two Native boys nearly 1 hour to collect 200 leaves with lesions from plot 786.

The apparent decrease in the number of affected leaves may be due to an intensive pruning programme during the last three seasons and general improvement in tree condition. An improved spray programme might also have contributed to the lower incidence of leaf symptoms.

In order to obtain some information on the prevalence of leaf symptoms, observations were made at three localities at Letaba Estates in September, 1959. Plots 118, 179 and 181 were selected for these observations. These plots were divided into two equal areas with 40-year old trees on one area and young 7 year old trees on the other. From each plot 100 leaves were sampled at random from two old trees and 100 leaves from two young trees. Care was taken that all data trees were apparently healthy with no signs of decline.

TABLE 17.

Table showing the incidence of black spot leaf symptoms on young and old Valencia trees at Letaba Estates, September 1959.

Plot No.	Tree No.	% leaves with Black spot lesions.		Average number of lesions per affected leaf.	
		Young trees	Old trees	Young trees	Old trees
118	1	0	2	-	2.0
	2	0	0	-	-
179	3	0	7	-	12.0
	4	1	4	1.0	1.0
181	5	1	2	1.0	2.5
	6	0	0	-	-
Total		2	15	-	-
Average		0.33	2.5	-	-

The trees from which data were taken were planted on the same soil type and environmental conditions for the old, and young trees could not have varied greatly. The results in Table 17 indicate that old trees have more leaves with black spot lesions than young trees.

2. THE OCCURRENCE OF FRUIT SYMPTOMS

If disease symptoms occurred only on leaves, black spot would have been of no economic importance. The fruit is severely affected however.

Kiely (1948), Wager (1952), Calavan (1960) and Schüepp (1961) described the disease symptoms on fruit in detail. Kiely (1948) found it convenient to classify the fruit symptoms into 3 categories, viz. "hard-spot" or "shot-hole spot", "freckle spot" and "virulent" or "spreading spot". There are various intergradations but Kiely's classification is accepted and will be followed in this report.

a. Hard spot: The type of lesion that develops, depends mainly on the prevailing temperature and the maturity of the fruit. Hard spots appear from the beginning of March onwards on the in-season Navel fruits at Letaba and about one month to six weeks later on Valencia oranges. Fruit of old debilitated trees show symptoms sooner than fruit on normal trees. The fruit rind may still be green when the first symptoms are observed, but in such cases a yellow halo surrounds each lesion.

Hard spot is primarily a pre-harvest symptom but also develops as a post-harvest symptom, especially when fruits are harvested early in the season. Like the other types of black spot symptoms hard spot develop more on that side of the fruit which was exposed to the sun prior to picking.

b. Freckle Spot: Freckle spots usually occur on Navel oranges from May onwards and was seldom noticed on Valencia oranges before June. This type of lesion appears after considerable yellow colour has developed in the rind and is nearly always found on that side of the fruit which is exposed to the sun.

Freckle spot was so named by Kiely because numerous small light-brown to red spots appear simultaneously on the fruit. More than one wave of freckle spots may occur on the same fruit (Calavan 1961). The older spots are darker in colour. Individual spots may be so close together that they coalesce to form one big lesion which is rather similar to a tear stain "melanose" lesion. The coalesced freckle-spot lesions often develop into a virulent black spot lesion.

Calavan (1960) believed that freckle spots originate from numerous individual infections. It is possible that freckle spots are caused by dense populations of pycnidiospores or ascospores in water drops during rainy periods.

c. Virulent Spot: From the beginning of August onwards virulent spots appear. The appearance of this type of symptom seems to coincide with peak maturity of fruit and the onset of warmer weather. This type of spot usually causes heavy losses to post-harvest fruit in transit to the coast. In most cases virulent spot arises from apparently healthy but latently infected rinds.

d. "Melanose" Symptoms: According to Stevens (1912), melanose is caused by Phomopsis citri. It is not the intention to challenge any of the previous workers' results on melanose.

At Letaba Estates symptoms popularly called "melanose" appear on fruit of all citrus varieties. During the course of the investigational work at Letaba Estates, strong circumstantial evidence was obtained which indicates that these "melanose" symptoms (or at least some of the symptoms) are caused by the same organism which is responsible for black spot, viz. Guignardia (Phoma) citricarpa.

"Melanose" symptoms are usually observed towards the beginning of February on Navel oranges at Letaba. On Valencia

oranges these symptoms appear about one month later. The most common symptoms are small raised brown dots up to two millimeters in diameter. These spots start on green fruits as yellow dots, which become darker and eventually brown to black. While the fruit is still green, a yellow halo of several millimeters may surround the actual spot. The yellow halo disappears completely as the fruit matures, but a tinge of green may remain round the spot; some of these spots may develop into typical black spot lesions, or the spot itself may be raised and may look like a scale insect at a glance. The necrotic corky tissue may be scratched off. When these lesions occur close together this may form a continuous brown crust.

Other symptoms look like a small brown fungus colony growing on the fruit surface. There is no hard necrotic crust in the centre. These lesions may be up to five millimeters in diameter. The centre part usually collapses with time and a typical "hard spot" lesion arises. This type of symptom is less common than the previous one.

"Tear streak" symptoms are also commonly found which are probably the results of heavy spore suspensions in rain drops.

3. ISOLATIONS FROM DIFFERENT FRUIT SYMPTOMS.

Large numbers of isolations were made from the various symptoms from 1959 to 1962.

a. Methods and Materials: Infected fruits were obtained from Letaba Estates or from farms in the Letaba district. Isolations were made at various depths of the fruit rind, by cutting small pieces with a sharp razorblade. These pieces of rind tissue were first washed in 90% alcohol for 1 minute and then rinsed 3 times in sterile water. Mercuric chloride at 0.1% was used for the next sterilization which lasted $\frac{1}{2}$ minute. The pieces of tissue were then rinsed five times in sterile water, before the pieces were placed on P.D.A. medium. This method was standardized and used throughout for this type of work.

b. Results: The results of isolations carried out during the course of this investigation are given in table18 and 19.

TABLE 18

Isolations from different types of black spot and "melanose" lesions and fungi isolated at various dates from different orange fruits 1959 - 1961.

Date	Fruit	Type of Lesion	Depth of isolation	No. of isolation.	Fungi isolated		
					Phoma	Colletotrichum	Others
8/8/59	Navel	Hard	Surface	25	0	11	1
"	"	Virulent	"	25	4	13	1
10/10/59	Valencia	Hard	1 mm deep	25	1	4	0
"	"	Freckle	Surface	25	6	10	1
"	"	Virulent	Surface	25	8	11	1
"	"	Tear Streak "melanose"	Surface	25	15	4	0
8/6/60	Navel	Hard	Surface	50	1	7	1
"	"	Freckle	Surface	50	20	15	2
"	"	Melanose	Surface	50	27	17	2
"	"	Melanose crust	1 mm deep	50	31	4	0
7/7/60	Letaba early	Hard	Surface	20	0	4	2
"	"	Freckle	Surface	20	5	12	0
"	"	Virulent	Surface	20	6	10	1
"	"	Melanose	Surface	25	19	3	0
30/5/61	Navel	Scabby Melanose	Surface	20	12	10	0
"	"	Scabby Melanose	1 mm deep	20	15	0	0
"	"	Melanose green tinge	Surface	20	13	6	0
"	"	Melanose no green tinge	Surface	20	11	7	0
"	"	Hard spot	Border	20	0	4	0
"	"	Hard spot	2 mm deep	20	0	0	0
"	"	Freckle	Surface	20	5	12	0
"	"	Freckle	1 mm deep	20	3	0	0
"	"	Healthy tissue no symptoms	Surface	50	3	4	1

TABLE 19

Results of isolations from "melanose" lesions on Valencia fruits, shortly after the first lesions appeared until 1st August, 1962.

Date	No. of Isolations	Fungi Isolated		
		Phoma	Colletotrichum	Other
21/2/62	100	52	38	8
9/3/62	100	59	22	12
26/3/62	100	70	14	11
12/4 '62	100	70	16	1
2/5/62	100	71	25	3
18/5/62	100	72	17	4
1/6/62	100	70	19	1
2/7/62	100	67	15	2
1/8/62	100	68	9	6

TABLE 20

Results of isolations made from symptom-free, unsprayed, in-season Valencia fruit at various intervals during the fruit growing season 1961 - 1962.

Date	No. of isolations	Fungi Isolated		
		Phoma	Colletotrichum	Other
2/10/61	100	0	0	0
7/10/61	100	0	0	0
30/10/61	100	0	0	0
15/11/61	100	0	15	0
3/12/61	100	1	32	0
17/12/61	100	2	30	1
4/ 1/62	82	1	32	0
16/ 1/62	100	2	40	4
2/ 2/62	100	2	73	1
21/ 2/62	100	2	53	7
9/ 3/62	100	3	30	6
26/ 3/62	100	5	18	4
12/ 4/62	100	6	18	3
2/ 5/62	100	3	26	5
18/ 5/62	100	3	17	4
1/ 6/62	100	3	18	4
2/ 7/62	100	4	12	4
1/ 8/62	100	2	9	6

c. Conclusions: Colletotrichum was regularly isolated from all types of fruit lesions, even from apparently healthy tissues. Wager (1952) found that Colletotrichum gloeosporioides was present universally in citrus leaf and fruit tissues, but attached no special importance to that result.

Calavan (1960) believed that Phoma citricarpa as well as C. gloeosporioides are important in lesion development. He concluded that ...."it is not apparent whether or not either fungus, or both together are able to develop pre-harvest lesions from latent infections without the involvements of an outside factor to weaken some cells in the outer peel".

Inoculations with cultures of P. citricarpa and C. gloeosporioides by the writer showed that both these fungi cause lesions on mature Navel fruits. The rate of lesion development was slower with P. citricarpa than with C. gloeosporioides.

Kiely (1948) maintained that in the course of hard spot development a limited amount of fungal growth takes place in response to a favourable combination of environmental factors, but some physiological factors, associated with the immaturity of the rind cells slow up the rate of growth of the fungus, finally killing it. In Table 18 it can be seen that P. citricarpa was only occasionally isolated from hard spot lesions. Although Calavan (1960) was more successful in isolating Phoma from hard spot lesions, his isolations were carried out in April, which is very early in the season and the lesions must have been relatively young.

Isolations from freckle and virulent spots yielded Phoma cultures consistently.

It was easier however, to isolate Phoma citricarpa from "melanose" lesions than from any of the recognised black spot lesions. In table 19 results are given of isolations made from in-season Valencia fruits from February to August. Phoma citricarpa grew out of 52% to 72% of the pieces of fruit lesions. Phomopsis citri was never isolated from these lesions. Isolations from "melanose" symptoms on fruit from a farm in the Letaba district, where typical black spot lesions have never been observed in the past, yielded 85% Phoma.

It may be argued that the potato-dextrose- agar is not a suitable medium for Phomopsis. This is doubtful. Fawcett and Lee (1926) maintained that Phomopsis citri grows well on "standard nutrient glucose agar" and it is almost certain that Wager (1953) used P.D.A. medium in his studies on "melanose".

If Phoma was present throughout the peel (systemic), isolations from apparently healthy tissue (Table 20) should have yielded more Phoma. This was not the case. The Phoma isolated from healthy fruits is probably due to spore infections earlier on.

4. SEASONAL DEVELOPMENT OF THE DISEASE

It was already mentioned that "melanose" symptoms usually appear towards the beginning of February and about a month later on Valencia oranges. Hard spot symptoms made their appearance from the beginning of March, followed by freckle spot and virulent spot.

a. Method: At Letaba Estates fruit is picked and transported to the packhouse in trailers which normally contain the equivalent of 100 field boxes of approximately 2 tons of fruit. From each trailer a sample of 50 fruits was taken at random at the packhouse and examined for black spot and "melanose", throughout the season.

In order to follow the development of black spot and "melanose" on a seasonal basis, 100 unsprayed Valencia fruits on 12 year old trees were tagged and the number of lesions which developed during the season was counted fortnightly.

b. Results: In Figure 4 it can be seen that the percentage fruit with black spot lesions (hard spot, freckle spot and virulent spot) increased sharply from the beginning of August onwards during the 1960 picking season. At that stage Valencia oranges were completely coloured and the fruits were reaching peak maturity. The period also coincided with a marked increase in temperature. Towards the end of August 1960, when more than 20% of the fruit became diseased, the badly infected fruit was culled in the orchard to ease matters at the packhouse.

It will be seen in Figure 4 that at the end of the Valencia orange packing season close to 90% of the fruit harvested at that stage, showed black spot lesions. The importance of black spot is demonstrated in no uncertain manner, especially if one bears in mind that four copper fungicidal sprays were applied earlier that season.

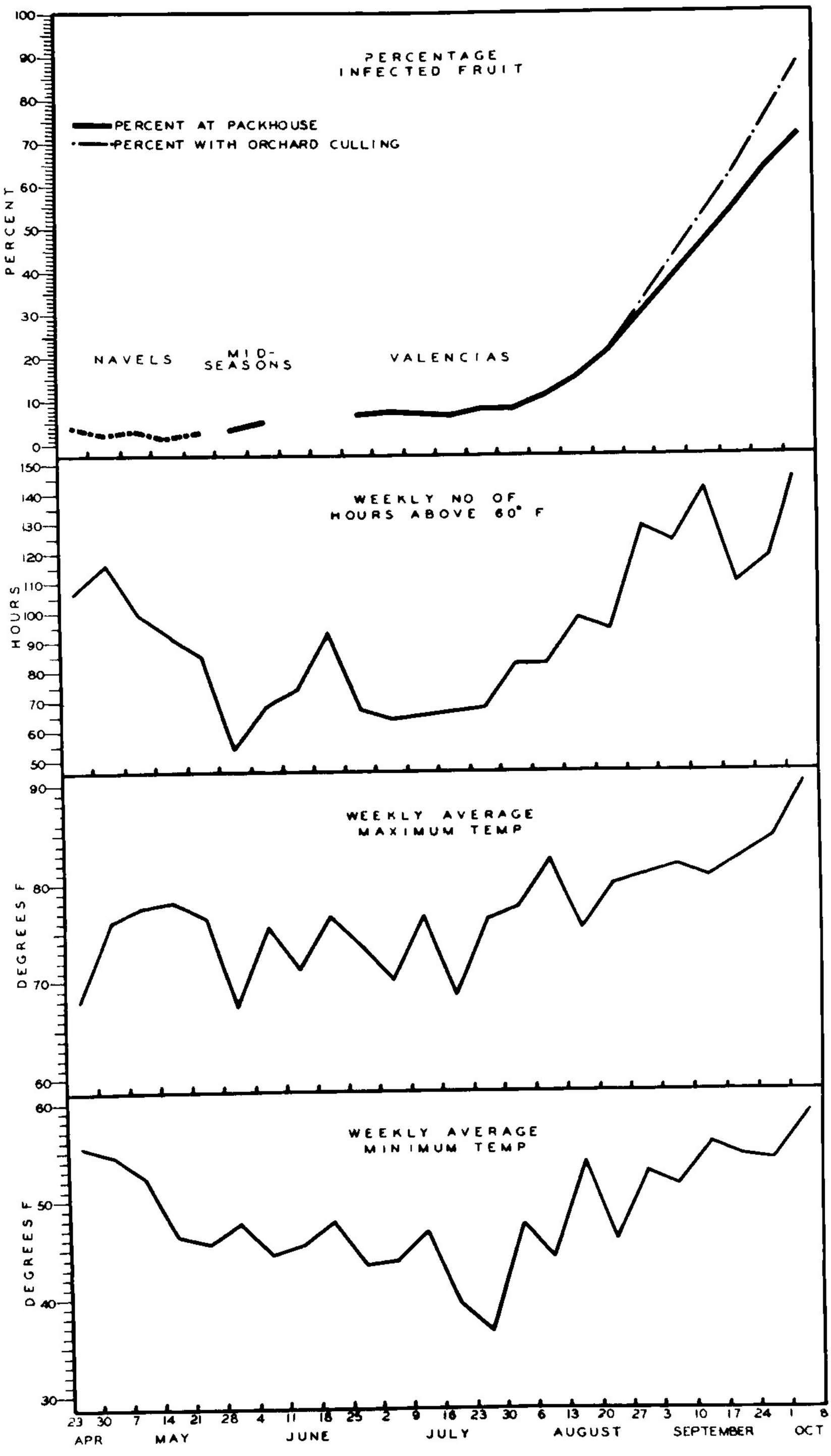


FIG. 4. Graphic representation of the increase of black spot during the 1959-1960 citrus picking season at Letaba Estates. The weekly no. of hours above 60°f as well as the average weekly maximum and minimum temperatures are given.

The packhouse report for the 1960 packing season indicated that the percentage fruit with "melanose" decreased as the season progressed. Although regular checks were made on the standards used for culling at the packhouse, it was thought that the decrease was due to the human factor, seeing that black spot was so severe. Subsequent results showed that some of the "melanose" symptoms developed into black spot symptoms and that at least part of the decrease of "melanose" might have been due to this fact.

In Figure 5 the development of "melanose" and black spot during 1962 can be seen in the centre and bottom graphs. On the fruit of these young unsprayed Valencia orange trees the first "melanose" symptoms were observed towards the middle of February. Counts were made since the beginning of April. The number of "melanose" symptoms increased rapidly until the beginning of May. A slight increase occurred until mid-June and from then on the number of "melanose" spots decreased. The black spot lesions (hard and freckle spot) increased steadily from the beginning of June until early September when the fruit had to be harvested. The graphs show clearly an increase in both the percentage of fruit infected with black spot and the number of spots. Some "melanose" spots developed into black spot lesions.

When the percentage infected fruit is determined as in this experiment, the curve is inclined to flatten when "near-saturation" point is reached. This fact makes evaluation of differences between treatments difficult. It will be observed that by counting the number of spots, an ever-rising curve was obtained. The disadvantage is that when the incidence of the disease is severe, an accurate count of spots becomes most difficult, as the lesions coalesce. For the purpose of the black spot curve in the centre graph (Figure 5), the number of countable spots were recorded, but to show that more "uncountable" spots were present an arrow was used to indicate this on the curve. If all the spots could have been counted the end point of the black spot curve (for number of spots) would have been much higher.

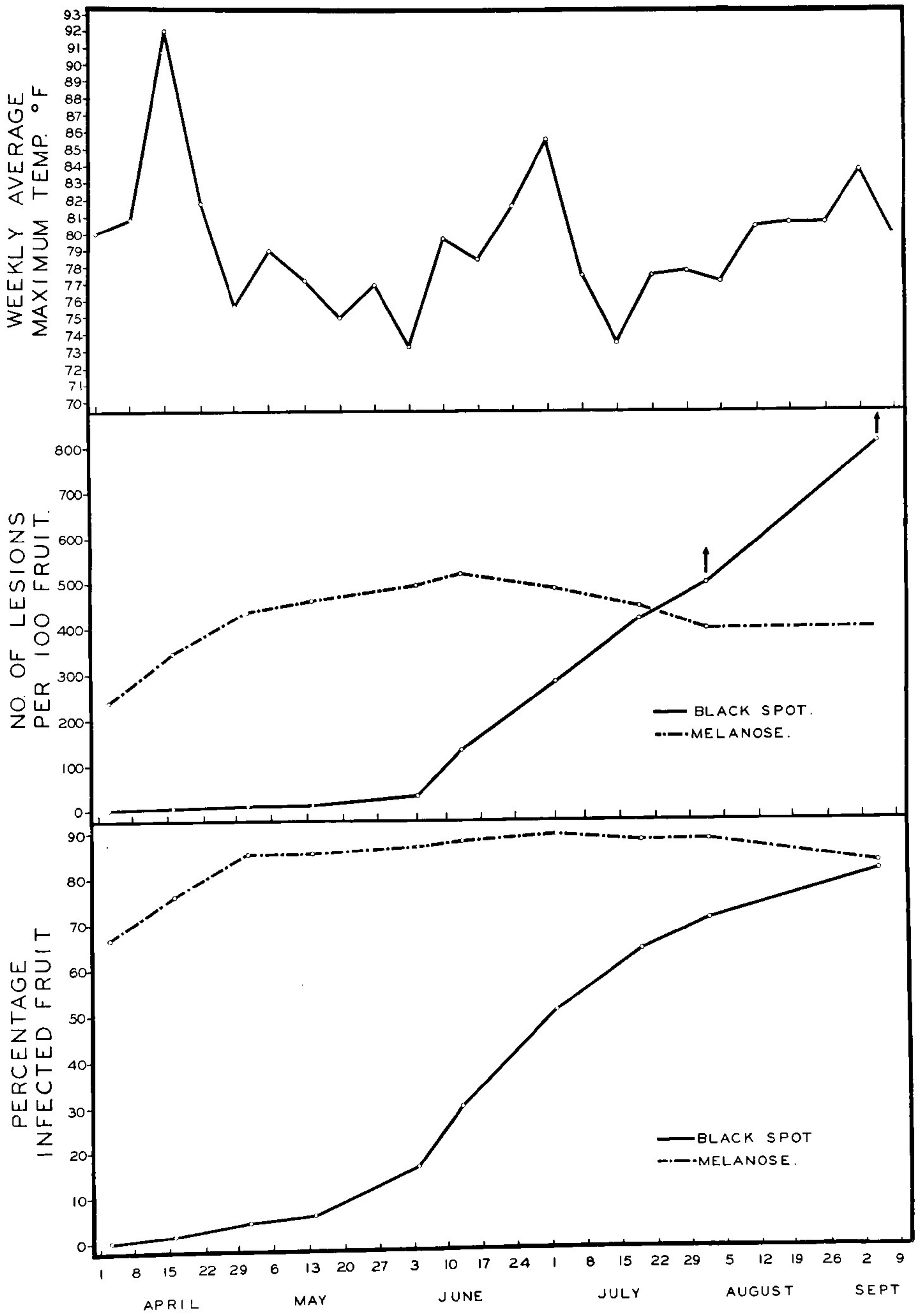


FIG. 5. Graphic representation of the weekly average maximum temperatures, the numbers of black spot and "melanose" lesions which developed during the season and the percentages of fruit with black spot and "melanose" lesions during the 1961-1962 citrus season at Letaba Estates.

These results indicate that excellent use can be made in black spot research by adopting the table of multiple infection transformation of percentages to infections as described by Gregory (1948). According to the results presented here, the error will be an under estimation of the actual number of spots, as the number of lesions in the case of black spot appear to be much higher than Gregory's table indicates.

5. DISTRIBUTION OF DISEASE IN TREES

Kiely (1957) observed that black spot develop more on the fruit on the Northern half of trees and recommended that those fruits should be picked as soon as possible in the season. Calavan (1960) found at Nelspruit that more black spot lesions developed on the North-Western side of trees than on the South-Eastern side. He found that after 14 days storage between 75°F and 92°F there was no difference between the two sides. This indicated that the number of latent infections on fruit of the N.W. and S.E. sides was the same. Calavan also found a higher incidence of black spot in the lower half of the tree than the top half. Kiely (1948) could find no differences between the upper and the lower portions of mature Valencia trees.

a. Methods: During the second week of September 1960 three trees in each of three unsprayed Valencia orange plots (9 trees) were divided in 4 quarters viz. North-East, North-West, South-East and South-West. The fruit from each quarter was stripped and examined for black spot.

On 28th June 1962 seven unsprayed trees in an old Valencia orange orchard were divided in 4 quarters viz. North, South, East and West. The fruit was stripped from each quarter and examined for black spot.

To determine the incidence of black spot on the fruit borne inside of the tree and those on the periphery, three unsprayed Valencia orange trees on each of three different plots were taken for record purposes.

Observations on the incidence of black spot in the upper and lower halves of trees were made in several experiments.

b. Results

TABLE 21

Summary of the incidence of black spot on the NE, NW, SE and SW quarters of trees at Letaba Estates 1960.

Quarter of tree	Mean % Fruit with black spot lesion*	Inverse arc sine transformation.
North-East	44.9	42.0
North-West	44.5	41.8
South-East	26.6	30.5
South-West	16.8	24.2
		p=0.05 10.7 -

* mean of 9 replications of single tree plots

TABLE 22

Summary of the incidence of black spot on the North, South, East and Western quarters on old Valencia orange trees at Letaba Estates in 1962.

Quarter of tree	Mean percentage fruit with black spot lesions*
North	50.0
South	34.9
East	33.5
West	50.0
	(p=0.05) 13.8

* mean of 7 replications of single tree plots.

TABLE 23

A comparison of the incidence of black spot inside and outside trees on the different plots of Valencia orange trees during 1959/60.

Pair Number	Percentage fruit with black spot lesions		Difference in percentage infected fruit.	Deviation from mean	D^2
	Inside	Outside			
1	31.5	45.0	13.5	-.2	.04
2	37.8	52.2	14.4	+.7	.49
3	34.3	47.6	13.2	-.4	.16
M = 13.7			ΣD^2 = 0.69		

$t = 13.4\sqrt{\frac{6}{.69}} = 39.52$

From Fisher's tables (1946)
$t \begin{pmatrix} N=3 \\ n=2 \end{pmatrix} = 4.30$

The difference of the incidence of black spot between the inside and outside fruit of the trees is therefore significant.

In another experiment a comparison was made between the upper and lower halves of trees, which received no sprays, one, two, three, four, five and six copper fungicide sprays.

TABLE 24

A comparison of the incidence of black spot in upper and lower halves of trees after receiving none to several sprays of Bordeaux mixture ($2\frac{1}{2}$:2:100) during the 1959 - 1960 season.

Block	Mean % Fruit infected *		Inverse arc sine transformation	
	Upper half	Lower half	Upper half	Lower half
No spray	83.54	51.65	66.0	46.0
1 spray	86.46	64.19	68.5	53.2
2 sprays	75.02	54.36	60.0	47.5
3 sprays	30.79	10.56	33.7	19.0
4 sprays	37.42	8.14	37.7	16.6
5 sprays	31.41	4.72	34.0	12.5
6 sprays	25.19	5.45	30.0	13.5
Mean	52.83	28.44	47.12	29.75

* Mean of 5 replications

L.S.D. for upper and lower leaves of trees (p=0.05) 6.21
(p=0.01) 9.19

There was significantly more diseased fruit in the upper halves of the trees than the lower halves.

c. Conclusions: From a control point of view it is important to know where the incidence of black spot is highest on the tree.

The results in Tables 21 and 22 indicate that the fruit on the Northern and North-Western aspects of the trees are more prone to black spot development under orchard conditions. These results agree with the report of Kiely (1948) and results of Calavan (1960).

Results in Table 23 confirm that the incidence of black spot is higher on the fruit on the outside of the tree, than on the inside, under orchard conditions.

According to Table 24 and results of other experiments which will be discussed later, black spot is more severe in the upper half than the lower. Kiely (1948) reported that in Australia no differences were found in the incidence of black spot between samples of fruit from the upper and lower portions of old Valencia trees. Calavan (1960) worked with relatively small numbers of fruit at Nelspruit, but indicated that more black spot occurred on the lower halves of trees than the upper halves. This is completely contrary to the observations at Letaba. This phenomenon is probably due to differences in climatic conditions in the upper and lower portions of the trees, during the period of disease development. The tops of trees receive more sunlight than the lower portions. It is unlikely that more infection takes place in the tree tops.

6. EFFECT OF DROUGHT ON BLACK SPOT DEVELOPMENT

Based on general observations Kiely (1950) stated that, hot, dry winds at the time of fruit ripening predispose citrus fruits to black spot development. No experimental evidence was presented. It was decided to investigate the effect of wilting of trees at various stages on the incidence of black spot.

a. Methods: In an irrigation experiment at Alkmaar, carried out by Mr. S.V. Hefer of the Citrus and Sub-tropical Horticultural Research Station, Nelspruit, observations were made which formed the basis of further experimentation at Letaba Estates.

Mr. Hefer's experiment consisted of 10 treatments which represented a wilting period at different stages of the year. Each treatment was assessed by three selected assessors for the incidence of black spot on 4th September 1959. The assessment indicated that wilting of trees during June, July, August and September prior to picking increased the black spot incidence.

b. Experimental layout: In May 1960 an experiment was commenced to evaluate the effect of drought periods at different times of the year on the incidence of black spot. An 18 year old Valencia orange orchard, uniform in size and without any signs of root disease, was selected for this experiment. The trees received four copper fungicidal sprays earlier in the season. Each treatment consisted of four trees. The locality of each treatment was randomized. To prevent interference by rain, the soil surface was covered with corrugated iron sheets which rested on bricks 1 foot above ground, to allow free movement of air. When no rain was expected the iron sheets were removed.

When the fruit was harvested in October 1960 three wilt treatments had been completed. After harvesting the experiment was continued. For the 1960-61 season the trees received a Parathion spray for the control of scale insects and one Perenox spray during the 3rd week of November, 1960.

c. Results

TABLE 25

Summary of the incidence of black spot after trees were wilted at different periods, prior to harvesting during 1960.

Treatment Number	Wilting period	% diseased fruit
1.	Beginning August to 2nd Sept. '60	31.5
2.	Mid-August to 19th September, 1960	24.2
3.	Beginning Sept. to 6th Oct. 1960	17.7
4.	Never wilted	17.4
	Least significant difference (p=0.05)	11.0

TABLE 26

Summary of the effect of wilting of trees during 1960 - 1961 season on the incidence of black spot.

Treatment number	Wilting period	% fruit with black spot
1.*	Beginning August to 2nd Sept. 1960	39.6
2.*	Mid-August to 19th Sept. 1960	37.6
3.*	Beginning Sept. to 6th Oct. 1960	32.3
4.	Beginning Nov. to 6th Dec. 1960	27.4
5.	End April to end May 1961	54.1
6.	End June to 24th July 1961	52.8
7.	End July to 24th August 1961	49.8
8.	Never wilted	31.0
	L.S.D. (p=0.05)	9.67
	(p=0.01)	13.16

* These trees were wilted before, or during blossoming.

d. Conclusions: By wilting trees during July and August, blossoming was delayed by several weeks, but the effect of drought on the incidence of black spot during that period was negligible. A period of drought from April onwards (prior to harvesting) increased the disease incidence significantly. Normally, little rain can be expected at Letaba from April to September. It appears therefore that if irrigations are regularly applied during the winter months to prevent wilting, the development of black spot can be reduced.

III. CONTROL OF THE DISEASE

A. QUARANTINE

When Doidge (1929) reported the occurrence of black spot near Pietermaritzburg, it was the first record of the presence of this disease in South Africa. No attempt was made at that time to establish the presence of latent infection in other areas. The disease caused no concern as it was thought at that time that climatic conditions in most of the citrus growing areas were unsuitable for a black spot epidemic. Wager (1952) showed that the disease was present in visible form or latently throughout the more important citrus growing areas except Citrusdal and Clanwilliam.

One may therefore speculate that with a sound knowledge of this disease and strict quarantine measures, soon after the discovery of black spot, the spread of the disease might have been retarded.

Wager (1952) stated that "there seems little doubt .... that nursery trees from the infected Pietermaritzburg area have been carrying either visible or latent infection to all parts of South Africa". The suggestion was then made that nursery trees should be stripped of all leaves before despatch to other areas. Sueda (1941) and Shüepp (1961) showed that the mycelium of the black spot fungus is present in young shoots and it seems unlikely that the disease spread can be eliminated, simply by stripping the leaves.

Where new citrus farms are established far away from infected citrus orchards it may pay dividends to practise quarantine methods by: (a) Planting trees from nurseries where black spot was never observed in any form (e.g. nursery trees from Citrusdal).

(b) Strict prohibition on the introduction of any plant material which may be a potential source of inoculum.

B. ORCHARD SANITATION

The term "orchard sanitation" refers to the removal of possible sources of inoculum from an orchard.

1. PRUNING

Kiely (1948) and Wager (1952) pointed out that the incidence of black spot is high on the fruit of old debilitated or "sick" trees. Darnell-Smith (1916) indicated that the removal of dead wood from trees contributed towards the control of the disease.

Dr. Loest[*] of the Citrus and Sub-tropical Horticultural Research Station at Nelspruit, observed that black spot was less severe on the fruit of lemon trees which were pruned than on fruit of unpruned trees. Dr. Loest demonstrated the effect of pruning on the incidence of black spot further on Valencia orange trees.

At Letaba Estates pruning of Valencia orange trees was done annually on commercial scale to rejuvenate old trees. Four systems of pruning were carried out:

(a) 'Dehorning', where all the branches are cut off about 4 to 5 feet above ground level. This is a rather drastic method of pruning and is only carried out on a small scale.

(b) 'Skeletonizing', where the trees are pruned, so that only the skeleton-like framework remains. In this system, all the small branches and dead wood are cut away, leaving only the main branches. (See Batchelor and Webber, 1948, p. 435).

(c) 'Haircut', where two to three feet of the canopy, all over the tree is cut off, but the tree is not skeletonized any further.

(d) 'Row pruning', where part of the canopy is removed between rows. This method is also called "hedge pruning".

Skeletonizing and row pruning are preferred to the other systems at Letaba Estates.

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[*] Private communication

Pruning was carried out for horticultural reasons, as it stimulates new growth and helps to restore tree vigour. Pruning also helps to increase fruit size. Observations indicated that black spot control was improved on pruned trees. This improved control can be due to the following factors or combination of factors.

(a) Better spraying and coverage of the fruit with the fungicide are more easily obtained when the trees are "open".

(b) Restoration of tree vigour is associated with disease resistance. Any factor which improves tree health and restores vigour should help to lower the incidence of black spot. Judicious pruning can therefore assist in obtaining better results with fungicidal sprays.

(c) Dead twigs may harbour spores, as shown earlier and a lower incidence of disease as a result of pruning may be due to the removal of inoculum sources.

2. ERADICATION OF THE ASCIGEROUS STAGE

a. Introduction

Keitt (1939) reported success in reducing the discharges of ascospores of *Venturia inaequalis* (Cke) Wint, the cause of apple scab, by spraying the over-wintering leaves with eradicant fungicides. Louw (1946) proved experimentally that orchard "floor" spraying with eradicant fungicides reduced the incidence of apple scab considerably. Hutton (1958) achieved great success on apple scab control in Australia when phenyl mercuric chloride was sprayed commercially. Phenyl mercuric chloride is used at times on a fairly large scale in the apple growing districts of South Africa to eradicate the ascigerous stage of *Venturia inaequalis*.

Since ascospore inoculum of *G. citricarpa* is regarded as the most important source of infection, experiments were carried out to find out if fungicides can effectively eliminate the ascigerous stage.

b. Preliminary Investigation.

Before laborious and expensive field experiments could be carried out it was necessary to find out which fungicides were the most effective against the perithecial stage on dead leaves.

(i) Methods and Materials

Green, but old leaves were picked from old Valencia orange trees where black spot was severe on the fruit. These leaves were divided into lots of 50 and dipped in the various chemicals as set out below:

A. Copper sulphate 0.5% dipped for 1 minute.
B. Phenyl mercuric chloride 0.1%, dipped for 1 min.
C. Phenyl mercuric chloride 0.5%, dipped for 1 min.
D. Phenyl mercuric chloride 1.0%, dipped for 1 min.
E. Phenyl mercuric chloride 0.5%, dipped for 5 min.
F. Cyprex (N-Dodecyl quanidine acetate) 0.1% dipped for 5 minutes.
G. Cyprex (N-Dodecyl quanidine acetate) 0.5% dipped for 5 minutes.
H. Sodium dinitro-O-cresylate, 0.5%, dipped for 1 min.
I. Sodium dinitro-O-cresylate, 0.5%, dipped for 5 min.
J. Ammonium sulphate 1.0%, dipped for 5 minutes.
K. Lime Sulphur 2.0%, dipped for 1 minute.
L. Untreated control.

These leaves were dipped and then placed in cement boxes filled with soil. To prevent rapid drying out, a thin layer of grass was put over the leaves to provide shade. During the first three weeks the leaves were moistened with tap water, but afterwards they were left entirely to natural climatic conditions.

(ii) Results:

After three weeks a sample of 5 leaves from each treatment were examined every 3 to 4 days for the presence of ripe perithecia. The first ripe asci were observed on several of the leaves 40 days after the experiment commenced. All the leaves were examined on the 40th day and thereafter. The results are summarized below.

TABLE 27

Summary of the effect of different chemicals on the development of perithecia on dead citrus leaves.

Treatment No.	Treatment	40 days after treatment			62 days after treatment		
		% leaves with Perithecia	Assessment of incidence of perithecia	Ripe ascospores observed	% leaves with perithecia	Assessment of incidence of perithecia	Ripe ascospores observed
A	$CuSO_4$ 0.5% 1 min.	100	+++++	Yes	100	+++++	Yes
B	PMC 0.1% 1 min.	72	+++	Yes	90	++++	Yes
C	PMC 0.5% 1 min.	54	++	No	60	++	Yes
D	PMC 1.0% 1 min.	4	+	No	8	+	No
E	PMC 0.5% 5 min.	4	+	No	6	+	No
F	Cyprex0.1% 5 min.	100	+++++	Yes	100	+++++	Yes
G	Cyprex0.5% 5 min.	100	+++++	Yes	100	+++++	Yes
H	DNOC 0.5% 1 min.	6	+	No	8	+	Yes
I	DNOC 0.5% 5 min.	6	+	No	6	+	No
J	NH_4SO_4 1.0% 5 min.	100	+++++	Yes	100	+++++	Yes
K	L. Sulphur 2.0% 1 min.	100	+++++	Yes	100	+++++	Yes
L	Control	100	+++++	Yes	100	+++++	Yes

\+ - very few perithecia present and scattered
++ - few perithecia present, scattered or in groups
+++ - perithecia present, but clearly less than control
++++ - abundant perithecia, but less than control
+++++ - abundant perithecia.

(iii) Conclusions

All the Phenyl mercuric chloride and sodium dinitro-O-cresylate treatments reduced the number of perithecia. The leaves in treatment B,C,D,E, H and I did not decay normally as the other leaves but remained paper-brown and intact. They disintergrated with time as a result of handling.

None of the treatments prevented perithecium formation completely, but although perithecia were observed in treatments D,E,H and I the asci appeared to be disorganised and ascospores were never observed, except in treatment H. The spores in the perithecia in treatment H varied in size and shape and appeared to be slightly bigger than normal spores. The asci were not erect but malformed and irregular in shape.

It appeared therefore that phenyl mercuric chloride and Sodium dinitro-O-cresylate were the most promising materials for a field experiment.

c. Field Trial

(i) Methods and Materials

A block of approximately 450 old Valencia orange trees with a bad black spot record was selected for this trial. The trees in this block were fairly uniform in size and healthy except for a few odd trees which were not used for record purposes. Before the trial commenced, all the fruit was stripped from the trees and removed. Pycnidiospore inoculum was therefore reduced as far as possible. The 450 trees were divided into 5 blocks, more or less equal in size. The treatments were as follows:

Treatment A

The dead leaves and orchard soil surface were sprayed on 27th August 1959, 2nd October 1959 and 15th November 1959 with 0.3% Sodium dinitro-O-cresylate. About 4 gallons were sprayed on the soil surface area occupied per tree.

The trees received 3 sprays of Bordeaux mixture ($2\frac{1}{2}$:2:100) which were applied on 18th September, 28th October 1959 and 3rd January 1960.

Treatment B

The dead leaves and orchard soil surface were sprayed on the same dates as A, with 0.3% phenyl mercuric chloride solution. About 4 gallons of this solution was sprayed on the soil surface area covered per tree.

The trees were not sprayed.

Treatment C

All the dead leaves and twigs under the trees were collected by hand by a team of Native women on 25th August, 29th and 30th September and again on 13th and 14th November, 1959. These leaves were burned outside the orchard. The orchard soil surface was then sprayed as in B. The trees were not sprayed.

Treatment C(a)

Ten trees only in C received 3 Bordeaux mixture sprays as A.

Treatment D

The orchard soil surface and the dead leaves were sprayed as B. The trees received 3 Bordeaux mixture sprays as A.

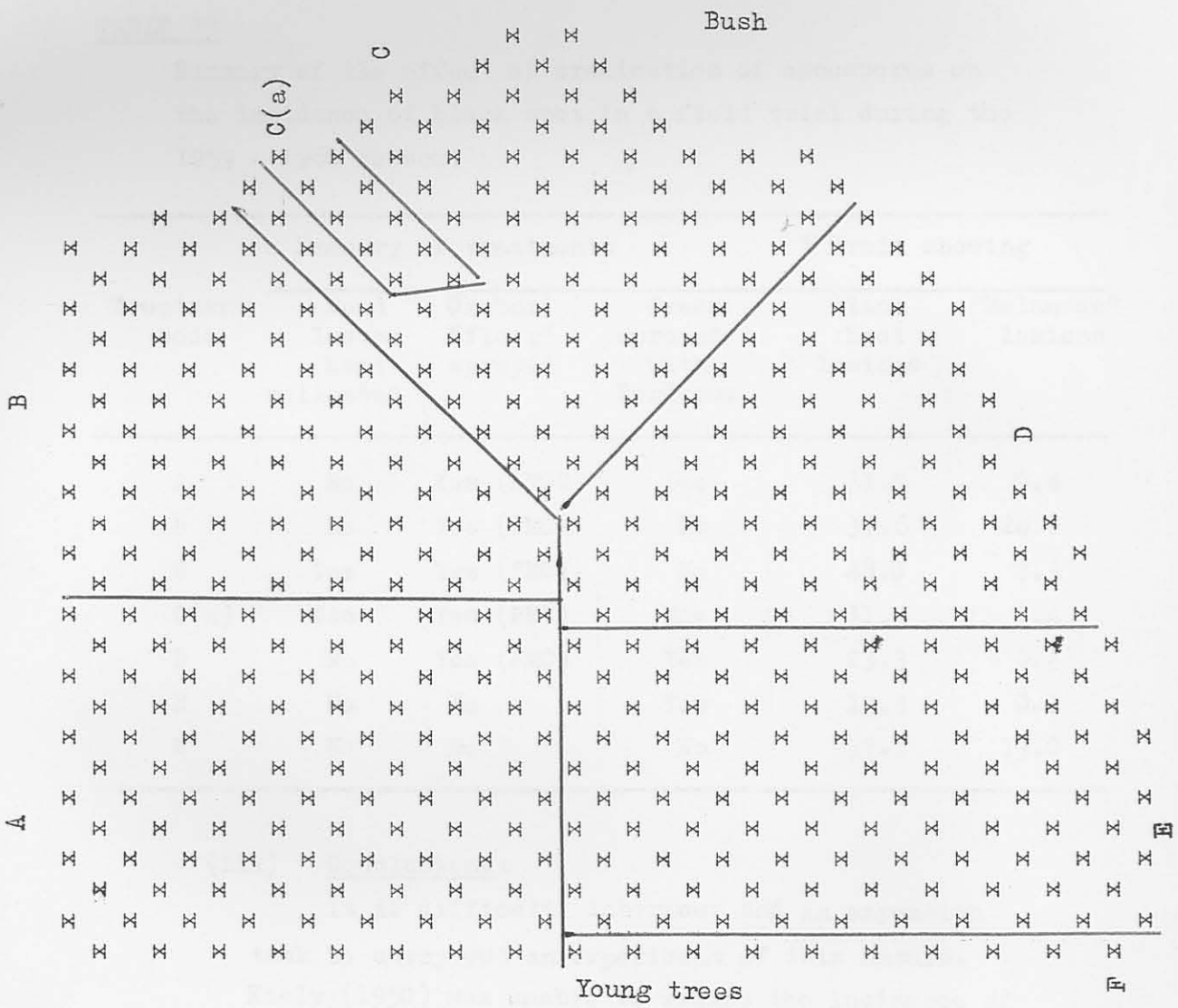
Treatment E

The trees received 3 Bordeaux mixture sprays on the same dates as A. The soil surface was not sprayed.

Treatment F

Unsprayed control.

Plan:



Legend:

Treatment A. DNOC on ground, (dead leaves not removed). Bordeaux on trees.

" B. PMC on ground only, (dead leaves not removed). No sprays on trees.

" C. Dead leaves collected by hand. Ground sprayed PMC. No sprays on trees.

" C(a) Same as C, but trees also sprayed with Bordeaux.

" D. PMC on ground, (dead leaves not removed). Bordeaux on trees.

" E. No floor treatment. Bordeaux on trees.

" F. Untreated control.

(ii) Results

From each treatment, ten trees were harvested during the first week of October 1960 in the centre of the experimental blocks. All the fruit was examined for black spot.

TABLE 28

Summary of the effect of eradication of ascospores on the incidence of black spot in a field trial during the 1959 - 1960 season.

Treatment code	Summary of treatments			% Fruit showing	
	Dead leaves hand collected	Orchard "floor" sprayed	Trees sprayed with Bordeaux	Black Spot lesions	"Melanose" lesions
A	No	Yes (DNOC)	Yes	31.9	0.4
B	No	Yes (PMC)	No	37.6	24.8
C	Yes	Yes (PMC)	No	48.0	7.3
C(a)	Yes	Yes (PMC)	Yes	31.7	0.4
D	No	Yes (PMC)	Yes	23.3	0.3
E	No	No	Yes	17.3	0.1
F	No	No	No	37.1	19.0

(iii) Conclusions:

It is difficult, laborious and an expensive task to carry out an experiment of this nature. Kiely (1950) was unable to reduce the incidence of black spot in an experiment where dead leaves were sprayed with copper sulphate plus calcium arsenite and ammonium sulphate.

He claimed that "the asci, both mature and immature, within the perithecia of G. citricarpa had been killed". but no explanation was given on what grounds that statement was made. Kiely blamed ascospores from other host plants for the poor result. The results in Table 27 show that copper sulphate and ammonium sulphate were ineffectivein controlling perithecium development.

The results of the trial at Letaba Estates are equally disappointing. Not even where all the dead leaves were removed by hand and the soil surface was sprayed was any improved control observed.

The nature of this trial makes a statistical analysis difficult and it is doubtful whether a statistical analysis will produce anything that cannot be seen in the results as presented.

Possible explanations for the negative results are:-

(a) "Floor" treatment should have been continued until February. Spores could have developed on the leaves which dropped after the 15th November and could have caused infection in January. In Table 14 results are presented which indicate that infection can take place later than January.

(b) Ascospores of G. citricarpa are airborne and could have been blown into the experimental trees from other orchards. If Kiely's theory is valid that ascospores which are produced on other host plants can cause infection on citrus, then considerable inoculum could have come from the adjacent natural bush.

(c) The citrus tree harbours inoculum on dead twigs, fruits and spotted green leaves and infection could have been caused from such sources. Infected fruit had been removed however, before the trial commenced and can therefore not be considered here.

The possibility of systemic infection seems remote, judging by the experimental evidence presented earlier. Large scale experimentation on the eradication of ascospores on an area basis, or on isolated citrus farms should be investigated. But it is doubtful whether this method of control will become a practical proposition.

C. EVALUATION OF PROTECTIVE FUNGICIDES (HV)

1. INTRODUCTION

Mc Cleery (1939) indicated that the main infection period extends from blossoming until approximately 20 weeks later. This marked a major break-through on the black spot problem. Since then, control measures were aimed at protection of fruit against spore infection during the first 4 to 5 months after blossoming. Copper containing fungicides were superior to other fungicides (Kiely 1950; Wager 1952; Kotze 1961). Kiely (1950) found that "weak" home-made Bordeaux mixture ($1\frac{1}{2}$ lb commercial copper sulphate plus $1\frac{1}{2}$ lb hydrated lime in 80 gallons water) gave satisfactory results under Australian conditions in a 3-spray programme. Stronger concentrations of Bordeaux mixture were also used, but both Kiely (1950) and Wager (1952) claimed undesirable effects on fruit quality. They also reported serious outbreaks of red scale (Aonidiella aurantii Maskell) after strong Bordeaux mixture sprays.

At the time when black spot research commenced at Letaba Estates, the official recommendation for black spot control by the Citrus and Sub-tropical Horticultural Research Station at Nelspruit, was 3 Bordeaux mixture ($2\frac{1}{2}$: $1\frac{1}{4}$: 100) applications, sprayed at 6 weekly intervals, and starting at petal-drop stage.

2. METHODS AND MATERIALS

a. Sites

Old Valencia orange trees were selected as experimental sites during the first year of investigation. Old trees may vary considerably in condition and as tree health may influence the incidence of disease, old trees were not favoured in later investigations. Young trees are easier to spray and harvest, and the disease incidence was high enough to show up treatment differences.

b. Experimental layout

The randomized block method was almost exclusively used. Different treatments were indicated by different colours, painted on metal plates 3 x 5 inches in size. The colour plates were fixed on the tree stems with nails, so that the experimental trees could be easily recognised from the direction of the spray pump.

Single to 4 tree plots were used, depending on the condition of the trees. The number of replications depended on the nature of the experiment, condition of the trees, and availability of experimental materials.

c. Spraying

All the spraying was carried out with high pressure spray machines, commonly used on citrus farms in South Africa. The pressure at the spray gun usually was between 400 and 450 lb per square inch. Trees were sprayed until all parts appeared to be properly wet, but excessive run-off was avoided. A very large tree received about 15 gallons of spray mixture.

d. Recording of results

Trees were usually harvested when the unsprayed control trees showed a high incidence of black spot, but before the fruit dropped as a result of black spot.

Fruit from experimental trees were usually stripped and the whole crop was examined by a team of specially trained Africans. The results were written down on special forms. In all cases the number (or percentage) of fruit with less than 5 spots, more than 5 spots and "melanose" was recorded. In this report, the total percentage fruit infected with black spot will be presented, unless the "light" and "heavy" categories bring out additional information. Results on "melanose" will only be presented when it is considered important.

e. Statistical Analysis of Results

The statistical methods as set out by Saunders and Rayner (1951) were followed for the analysis of most experiments. The incidence of disease was first expressed as a percentage of the total population of fruit. In data where some percentages fell below 5% or above 95% an inverse arc-sine transformation was applied and the percentages were than expressed in degrees. An analysis was then applied in one of the following ways:-

(a) A simple analysis was applied where the data only admitted of two factors (i.e. treatments and blocks)

(b) A complete analysis was done where three or more factors indicated possible significance (i.e. treatments, blocks, picking dates, etc.)

In both (a) and (b) above, an analysis of variance was obtained and by the "F-test" significance was ascertained . Where the significance was obtained the least significant differences (L.S.D.) was applied on the appropriate means.

f. Fungicides

(i) Preparation of Bordeaux mixture

Basically, the same method was followed for the preparation of Bordeaux mixture as recommended by Doidge (1910). "Standard Bordeaux" at Letaba consisted of $2\frac{1}{2}$ lb commercial copper sulphate (snow) and $1\frac{1}{2}$ - 2 lb high grade hydrated lime per 100 gallons of water. The copper sulphate was first dissolved in a small drum in enough water to bring the salt into solution. The copper sulphate solution was then poured into the big spray tank which was half filled with water. The lime was first mixed with water and while the spray tank was being filled after adding the copper sulphate, the lime suspension was slowly added. During the process the tank agitators were in operation to secure thorough mixing.

It was found that it is important to use fresh lime. It took about 2 lb of old lime to precipitate $2\frac{1}{2}$ lb of copper sulphate, while 1 lb was sufficient when fresh lime was used.

The concentration of Bordeaux mixture will be given in an abbreviated form (e.g. Bordeaux $2\frac{1}{2}$:$1\frac{1}{2}$: 100 will mean $2\frac{1}{2}$ lb copper sulphate and $1\frac{1}{2}$ lb lime, mixed in 100 gallons water).

(ii) List of fungicides:

Aerial Perenox, a wettable powder containing 50% metallic copper in the form of cuprous oxide. A special formulation for aerial application.

Agral 90, a liquid wetting agent containing 92% alkylated phenol-ethylene oxide condensate.

Alboleum, an emulsified light hydrocarbon oil, unsulphonated residue 94%.

Brestan, a wettable powder, containing 20% of an experimental organic tin compound (possibly Triphenyl tin acetate).

Brockman's Copper oxychloride, a wettable powder containing 50% metalic copper in the form of copper oxychloride.

Captan, a wettable powder containing 50% N-trichloromethyl mercapto-4-cyclohexene-1, 2-dicarboximide.

Ciba's Copper oxychloride, a wettable powder, containing 50% Cu in the form of copper oxychloride.

Commercial copper sulphate (Snow) containing ± 24% Cu.

Cop-o-Zinc, a wettable powder, containing 42% copper as basic copper sulphate and 11% zinc.

Coprantol, a wettable powder containing 50% metallic copper in the form of copper oxychloride.

Crag, a liquid containing 30% 2-heptadecyl glyoxalidine acetate in isopropanol solution.

Cuprosyl, a wettable powder containing 37.5% metallic copper in the form of copper oxychloride and 22.0% Zineb (Zinc ethylene bisdithiocarbamate).

Cyprex, a wettable powder, containing 65% N-Dodecylguanidine acetate.

Dithane Z78, a wettable powder containing 65% Zinc ethylene bis-dithiocarbamate. (Zineb)

Dithane M22, a wettable powder containing 80% Manganese ethylene bis-dithiocarbonate. (Maneb)

High grade hydrated spray lime

Hyamine 3500, an aqueous solution containing 50% of alkyl dimethyl bensyl ammonium chloride.

Nirit, a wettable powder containing 45% Dinitrophenyl thiocyanate plus 5.05% trace elements.

O-3818-B, an experimental fungicide containing "one part of nickel chloride and 2.85 parts of Zineb".

Omazine, a wettable powder. An experimental copper fungicide, containing copper dihydrazinium sulphate.

Oxychlor, a wettable powder containing 50% metallic copper in the form of copper oxychloride.

Perenox, a wettable powder containing 50% metallic copper in the form of cuprous oxide.

Phaltan, a wettable powder containing 50% N-trichloromethyl thiophthalimide.

Phygon XL, a wettable powder containing 50% 2, 3-dichloro-1, 4-naphthoquinone.

PMC, a water soluble powder containing 40% phenyl mercuric chloride.

Pomersol, a wettable powder containing 65% Tetramethylthiuram disulphide (thiram).

Sankyo Mercuric Bordeaux, a wettable powder containing 18% Basic copper sulphate and 0.71% phenyl mercuric chloride.

3. FIELD EXPERIMENTS

a. Copper fungicides

The object of this experiment was to evaluate different copper fungicides and to ascertain the effect of adjuvants to some treatments.

(i) Methods and Materials

This randomized block experiment was carried out on old Valencia orange trees. Four-tree plots were used with four replications per treatment. The fungicidal treatments are given in Table 29.

The sprays were applied with a conventional high volume spray-machine at a pressure of 400 lb per square inch. All the experimental trees, except the untreated control, were sprayed on the following dates:

Application No. 1 - 25th and 26th September, 1959.
Application No. 2 - 29th October and 1st November 1959.
Application No. 3 - 18th and 19th December, 1959.

(ii) Treatments and Results

The fruit was harvested during the 3rd week of September, 1960 and examined for black spot.

TABLE 29

Summary of different treatments and results of an experiment to evaluate various copper fungicides against black spot of citrus, during 1959 - 1960 season.

Treatment	Treatment Material per 100 gallons water	Mean % fruit with black spot	Inverse arc-sine transformation
A	Bordeaux (3:3:100) + Alboleum ½ gallon.	41.86	40.2
B	Bordeaux (1½:3:100)+ Alboleum ½ gallon.	55.05	47.9
C	Bordeaux (1½:1:100)+ Alboleum ½ gallon.	49.63	45.0
D	Bordeaux (2½:1½:100)+Alboleum ½ gallon.	44.13	41.5
E	Perenox 2 lb	11.00	18.9
F	Perenox 2 lb + lime ½ lb + Alboleum ½ gal	15.85	23.1
G	Perenox 2 lb + lime ½ lb + Agral ½ fl. oz.	15.26	22.1
H	Brockman's Copper Oxychloride 2 lb + Alboleum ½ gallon.	19.29	26.1
I	Oxychlor 2 lb + Alboleum ½ gallon	16.69	23.7
J	Ciba's Copper oxychloride 2 lb + Alboleum ½ gallon	29.83	32.6
K	Cuprosyl 2 lb	33.60	35.6
L	Omazine 2 lb	84.56	62.6
M	Unsprayed Control	89.74	72.9
	L.S.D. (p = 0.05)		12.8°

(iii) Conclusions

Bordeaux mixture appeared to be less efficient as the concentration of lime increased. This tendency was statistically non-significant, but it was also observed where lime was added to Perenox. All the Perenox treatments were significantly superior to any Bordeaux treatment. Although Perenox without additives gave the lowest incidence of black spot, it was not statistically better than Oxychlor and Brockman's copper oxychloride.

Although treatments E, F, G, H and I all gave better results than standard Bordeaux treatment, such a comparison is not fair, because the copper content in the bound copper fungicides is considerably higher than the copper in standard Bordeaux. De Villiers and Bester (unpublished data) and Fochessati (unpublished data) showed, however, that Perenox was better than Bordeaux mixture, at the same level of copper.

b. Organic fungicides

This experiment was carried out to evaluate some organic fungicides which were used with success against other plant diseases.

(i) Methods and Materials

This randomised block experiment was conducted on old Valencia orange trees. Four-tree plots were used, replicated 4 times. The sprays were applied with a conventional high volume spray machine at a pressure of 400 - 450 lb per square inch. All the experimental trees except the untreated control trees were sprayed on the following dates: 29th September, 23rd October, 20th November and 20th December 1959.

(ii) Treatments and Results

Harvesting and examination of fruit was carried out during September 1960. The results are summarized in Table 30.

TABLE 30

Results of an experiment to evaluate various organic fungicides against black spot of citrus during the 1959/60 season.

Treatment code	Treatments Material per 100 gallons water	Mean Actual % infected fruit	Inverse Arc sine transformation.
A	Bordeaux 2½:2:100 + Alboleum ½ gal.	13.35	21.18
B	Captan 2 lb	59.04	50.23
C	Captan 2 lb + Urea 5 lb	66.54	54.72
D	Captan 2 lb + PMC ¼ lb	41.35	39.62
E	Pomersol (TMTD) 2 lb	61.52	51.78
F	Dithane Z78 2 lb	35.36	35.95
G	Dithane M22 2 lb	24.93	29.83
H	Nirit 2 lb	86.96	69.10
I	Brestan 2 lb	74.16	60.40
J	Cyprex 1½ lb	59.85	50.78
K	Phygon XL ¾ lb	68.73	56.12
L	Unsprayed Control	72.48	58.48
	Least significant difference (p=0.05)		8.83
	(p=0.01)		11.85

(iii) Conclusions

Bordeaux mixture gave significantly better results than any of the other treatments. Dithane Z78 Dithane M22 and Captan plus phenyl mercuric chloride gave significantly better results than the unsprayed control treatment. The control achieved by the Captan plus PMC treatment must be attributed to PMC as Captan alone had no effect on the incidence of black spot. It is known that the organic fungicides lose their fungicidal value quicker than Copper fungicides. The organic fungicides may give better results when they are applied at shorter intervals.

Nirit and Brestan were extremely phytotoxic. Fruits in these treatments were severely russeted and leaf chlorosis was particularly severe in the Brestan treatment. It will be noticed that the incidence of black spot was significantly higher in the Nirit treatment than the unsprayed control. This phenomenon might have been due to the phytotoxic nature of Nirit which affected tree health and black spot symptom development could have been encouraged.

c. Organic fungicides (Second series)

This experiment was carried out to evaluate some more organic fungicides. In the light of personal experience with Captan plus phenyl mercuric chloride against apple scab (Venturia inaequalis) it was considered important to evaluate this combination again for the control of black spot.

(i) Methods and Materials

This randomized block experiment was carried out on old Valencia orange trees, using 4-tree plots, replicated 4 times per treatment. The fungicides and application rates are given in Table 31.

The dates of spraying were: 15th October, 6th November, 25th November and 30th December, 1959. Records were taken in September 1960.

(ii) Treatments and results

TABLE 31

Treatments and results of an experiment in which various organic fungicides were evaluated for the control of black spot during the 1959/60 season.

Treatment code	Material	Quantity per 100 gallons water	Actual % infected fruit	Arc sine transformation
A	O-3818-B	2 lb	59.1	50.8
B	Phaltan 50% WP	2 lb	68.6	56.0
C	Captan 50% WP plus PMC	1 lb ¼ lb	35.5	35.9
D	Crag	1½ pints	75.5	63.4
E	Hyamine	2 pints	51.9	46.6
F	Unsprayed control	-	75.4	61.8
	Least significant difference		(p=0.05)	15.11

(iii) Conclusions

The Captan plus PMC treatment yielded significantly cleaner fruit than the unsprayed control, which confirm the results of the previous experiment. All the other treatments failed to control the disease except Hyamine which was a border-line case.

Phenyl mercuric chloride which was included with Captan caused slight blemishes on the fruit, but despite this disadvantage further investigation on PMC was considered necessary.

d. Copper fungicides plus oil and PMC

Previous experiments indicated that phenyl mercuric chloride at a rate of 0.025% with Captan reduced black spot. Captan had no effect on the control of the disease. On the other hand, certain copper fungicides provided excellent protection against infection. When a good protective fungicide such as Perenox is combined with an eradicative fungicide such as phenyl mercuric chloride, the overall effect should be an improvement, other things being equal. The experiment was further expanded to include a mercuric Bordeaux mixture and a copper-zinc-mercury combination as well as other fungicides.

(i) Treatments and Results

The experiment was carried out on 20-year old Valencia trees, adopting a randomized block layout with 2 tree plots replicated 5 times.

The dates of spraying were: 4th October, 14th November and 30th December 1960.

The fruit was harvested during the second week of September 1961. The results are given below.

TABLE 32

Table showing the treatments and results in an experiment to evaluate different inorganic fungicides against citrus black spot.

Treatment code	Material	Rate per 100 gallons water	% Fruit with black spot.
A	Perenox plus Alboleum	1 lb 14 oz. ½ gallon	34.0
B	Perenox plus Alboleum	1½ lb ½ gallon	28.4
C	Perenox	1½ lb	39.3
D	Aerial Perenox plus Alboleum	1½ lb ½ gallon	42.4
E	Coprantol	1½ lb	41.3
F	Cop-O-Zinc plus PMC	1¾ lb 1 oz	47.6
G	Perenox plus PMC	1½ lb 1 oz.	57.8
H	Mercuric Bordeaux	2½ lb	55.9
I	Untreated control	-	65.7
	Least significant difference (p=0.05)		17.4

(ii) Conclusions

Although treatments A, B, C, D, E and F were significantly better than the unsprayed control treatment, the results were most disappointing. There were indications in other experiments that considerable infection took place after December, during this particular season and results might have been more striking if the first spray had been omitted and a spray applied in January instead.

In this experiment the inclusion of mercury with copper fungicide failed to give better control of black spot.

e. Evaluation of Dithane Z 78

(i) Methods and Materials

As shown earlier, Dithane Z-78 (Zineb) was one of the organic fungicides which showed promise for black spot control. It was known that considerable infection may take place during January and even later. To provide protection against infection during that period and to limit the number of sprays it was decided to use Dithane Z-78 during the early part of the season and to apply Perenox in January for further protection. Perenox was used throughout at 1 lb 14 oz. plus $\frac{1}{4}$ gallon Alboleum. Dithane Z-78 was used at 2 lb per 100 gallons water.

A simple randomized block method was employed, using 2-tree plots, replicated 5 times per treatment. The experiment was carried out on 20 year old Valencia trees.

TABLE 33

Summary of spray dates and materials applied, in an experiment to evaluate Dithane Z-78 and Perenox against citrus black spot during 1960 - 1961 season.

Treatment No	Spraying dates and materials			
	7/10/60	3/11/60	6/12/60	6/1/61
A	Perenox	Dithane	Dithane	Perenox
B	-	Dithane	Dithane	Perenox
C	Perenox	Perenox	Perenox	Perenox
D	Unsprayed control	-	-	-

One tree from each plot per treatment was harvested on 19th July 1961 and the rest of the experiment was harvested on 22nd August 1961.

(ii) Results

TABLE 34

Summary of results at two picking dates on the control of citrus black spot after applying Dithane Z-78 and Perenox spray schedules.

	First picking 19/7/61		Second picking 22/9/61		Arc-sine transformation. Whole treatment mean.
	Mean % fruit diseased	Arc-sine trans.	Mean % fruit diseased	Arc-sine trans.	
A	2.25	8.3	49.2	44.6	26.5
B	1.46	6.6	41.8	40.3	23.5
C	0.56	3.3	24.4	29.6	16.4
D	26.05	30.7	98.2	82.2	56.5
		(p=0.05) 4.22 (p=0.01) 5.92			(p=0.05) 6.77 (p=0.01) 9.56

TABLE 35

Summary of results on the control of "melanose" on 19th July, after applying Dithane Z-78 and Perenox spray schedules.

Treatment code	Mean % Fruit infected	Inverse arc-sine transformation
A	1.13	5.9
B	0.29	2.4
C	0.88	4.8
D	23.82	29.0
		(p=0.05) 3.26 (p=0.01) 4.57

(iii) Conclusions

For some inexplicable reason, treatment A where two Perenox and two Dithane Z-78 sprays were applied, was inferior to a straight Perenox treatment (treatment C) at the 5% level in July. At that time no significant difference existed between treatments B and C. Results on "melanose" showed that all fungicidal treatments gave effective control.

An earlier experiment showed that where Dithane Z-78 had been used in a straight programme, it was inferior to Bordeaux mixture. This experiment indicated that Dithane Z-78 could replace some of the copper fungicide sprays, provided the fruit was picked early in the season.

It will be shown later that Dithane Z-78 can be used for a special purpose where calcium arsenate is used for early harvesting. There is therefore no particular interest in the September result except for the indication that a relatively poor control will be obtained if picking of fruit is postponed until later in the season.

f. Evaluation of Dithane Z-78 (second series)

In view of the possibility of copper toxicity in the soils as a result of the annual applications of copper fungicides and other disadvantages of copper which will be discussed later, it was decided to evaluate Dithane Z-78 further.

(i) Methods and Materials

The experiment was laid out on 12 year old Valencia orange trees with 5 replications of single tree plots per treatment.

The dates of spraying were: 1st November, 5th December 1961 and 2nd January 1962.

(ii) Treatments and Results

TABLE 36

Summary of treatments and results on the control of black spot of citrus in an experiment to evaluate Dithane Z-78 during the 1961 - 1962 season.

Treatment code	Treatments: materials per 100 galls. water	% fruit with black spot	Inverse arcsine transformation.
A	Perenox 2 lb + Alboleum ½ gallon	0.70	4.18
B	Perenox 1 lb + Dithane 1 lb	0.24	2.66
C	Dithane 2 lb	2.52	9.08
D	Dithane 2 lb (first 2 sprays) then Perenox 2 lb	0.54	3.78
E	Dithane 2 lb + Alboleum ½ gallon	0.82	4.88
F	Untreated control	29.22	32.56
	Least significant difference	(p=0.05)	2.99
		(p=0.01)	4.00

(iii) Conclusions

The incidence of black spot was not very high at the time when this experiment was harvested, but it was not possible to postpone picking.

All the spray treatments were significantly better than the untreated control. Dithane Z-78 at 1 lb plus Perenox 1 lb, Dithane Z-78 plus Alboleum and Dithane Z-78 followed by Perenox were all as effective as the standard treatment (Perenox plus Alboleum).

The treatment where Dithane Z-78 was used throughout without Alboleum significantly inferior to all the other treatments except the unsprayed control.

4. DISCUSSION

Results of experiments by research workers over the last three decades, proved that copper-containing fungicides are superior to all other fungicides in the control of black spot. Until two years ago, "home-made" Bordeaux mixture at a strength of $2\frac{1}{2}:1\frac{1}{4}:100$ was preferred to other forms of copper and organic fungicides. It is true that users of Bordeaux mixture obtained reasonable results, but in some years up to 40% of the Valencia crop was lost on account of black spot.

Some of the results presented here and results of other experiments which were omitted to save space showed that several bound copper fungicides, obtainable commercially, are superior to Bordeaux mixture.

"Home-made" Bordeaux mixture has the advantage of being relatively cheap. In practice it has disadvantages. The copper sulphate is sometimes slow to dissolve and it frequently happened that spraying commenced while undissolved cristals were lying at the bottom of the spray tank. When the lime is old it may happen that all the copper sulphate is not precipitated and "copper burn" may result. Spraying and mixing are usually carried out by unskilled native labourers who are inclined to become careless, especially when working under pressure. Bordeaux mixture is also not compatible with Parathion preparations, with the result that two separate spray operations have to be carried out.

The copper fungicides give good results even when sprayed at comparatively long intervals, but there are also disadvantages,

which make it desirable to find another type of fungicide to use against black spot.

The main disadvantage of copper fungicides are:

(i) The build-up of copper in the soil may reach toxic levels which may have serious repercussions once that stage had been reached.

(2) Calcium arsenate which is sprayed for early maturity of Valencia fruit has very little or no effect when used with copper fungicides.

(3) Direct copper spray damage to fruit was an important culling factor during the last two years.

Mc Onie (unpublished report, 1962) maintained that no immediate danger of copper toxicity exists, but it stands to reason that we can not keep on spraying copper at rates of 40 to 60 lb metallic copper per acre annually without overstepping the danger mark.

To Letaba Estates and many other citrus growers, it is important for commercial reasons, to commence picking of Valencia oranges as early as possible. In order to get early maturity of the fruit, a spray of calcium arsenate is usually applied during October or November when the fruit is quite small. It is convenient and more economical to apply calcium arsenate with one of the black spot sprays. It was observed by the writer that where calcium arsenate had been applied as a combined spray with the copper fungicide, the effect of calcium arsenate on the sugar-acid ratio was slight or negligible. In an experiment during the 1959/60 season where calcium arsenate was sprayed in November with Bordeaux mixture the sugar-acid ratios in July were 6.2:1, 6.9:1 and 5.9:1 in the samples of 50 fruits, picked at random. Corresponding samples picked from trees which were sprayed in November with calcium arsenate alone, tested 8.6:1, 9.0:1 and 8.9:1.

Subsequent experiments by Mr. J.M. Conradie* confirmed the above observation. It was shown already that under certain circumstances, Dithane Z-78 can replace copper fungicide sprays. The question arises whether calcium arsenate, used with Dithane Z-78 will give the desired effect on early maturity.

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* Unpublished results of Mr. J.M. Conradie, Entomologist, Letaba Estates.

This aspect was investigated on large scale by spraying 4 plots (± 1000 trees) at Letaba Estates with 2 or 3 sprays of Dithane Z-78 followed by Perenox for the final application. Calcium arsenate was included in the first Dithane Z-78 spray of November. Excellent control of black spot was achieved with these large scale trials and calcium arsenate affected the sugar-acid ratio to such an extent that it was possible to pick these fruits early in July 1962. At that stage the incidence of black spot was less than one percent in these plots.

Mr. J.M. Conradie found also experimentally that where calcium arsenate was used with Dithane Z-78 the effect on early maturity was as good as where calcium arsenate was used alone.

Copper fungicides tend to cause unsightly marks on the fruit, particularly when sprayed during long spells of cloudy weather and under high humidity conditions. Fruit sprayed with Dithane Z-78 showed no more marks than the unsprayed control treatment.

It is therefore suggested that Dithane Z-78 can replace one, two or three copper fungicidal sprays in the black spot control programme, particularly in areas where black spot is not very severe or on young trees. It is regarded important that the final spray should be a copper fungicide. If calcium arsenate must be included it should be applied with a Dithane spray.

The cost of Dithane Z-78 is higher than the commonly used copper fungicides but the advantages of the former product may outweigh the initial cost difference.

D. TIMING OF PROTECTIVE SPRAYS

1. INTRODUCTION

Black spot is very severe on the fruit of old Valencia orange trees at Letaba. When these investigations commenced it was standard practice to apply a 3-spray programme of Bordeaux mixture ($2\frac{1}{2}$:$1\frac{1}{2}$:100) at 6-weekly intervals. The first spray was applied at petal-drop. Subsequent experimental results showed that the critical period for spraying usually starts towards the end of October, but this was not known when the first experiments were conducted. The theory evolved that the intervals between the sprays were too long and that more frequent sprays were necessary.

It was already indicated that very little infection occurred between blossoming and the beginning of November. The view was expressed by Kiely (1957) that each spray in the spray programme is of equal importance.

2. INVESTIGATIONS

a. Spray Intervals

This experiment was carried out to evaluate Bordeaux mixture, with and without oil at different intervals. It was further decided to evaluate Captan also at short intervals as well as a mixture of Captan plus Dithane Z-78.

(i) Method and Materials

All the trees were sprayed before the onset of the experiment with Parathion for the control of insect pests. The experiment was conducted in an old Valencia orange orchard. A randomized block design was adopted. Four-tree plots were used with four replications per treatment.

(ii) Treatments and Results

The different treatments and actual dates of spraying are given in Table 37. Records were taken in September 1960. The results are summarized in Table 37.

TABLE 37

Summary of treatments, spray intervals and results of an experiment to evaluate Bordeaux Mixture with and without oil, Captan and Captan plus Dithane Z 78 at approximately 15,30 and 45 day intervals.

Code	Treatments:	Actual Dates of Spraying							Mean % fruit with Blackspot	Inverse arc-sine transformation
A	Bordeaux Mixture 2½:2:100	16/9/59	1/10/59	16/10/59	31/10/59	16/11/59	4/12/59	31/12/59	10.8	19.1
B	- do -	16/9/59	-	16/10/59	-	16/11/59	-	31/12/59	19.5	26.2
C	- do -	16/9/59	-	-	31/10/59	-	-	31/12/59	14.1	21.9
D	Bordeaux Mixture Alboleum ½ gallon	16/9/59	1/10/59	16/10/59	31/10/59	16/11/59	4/12/59	31/12/59	4.9	12.7
E	- do -	16/9/59	-	16/10/59	-	16/11/59	-	31/12/59	22.3	28.1
F	- do -	16/9/59	-	-	31/10/59	-	-	31/12/59	14.3	21.8
G	Captan 2 lb	16/9/59	1/10/59	16/10/59	31/10/59	16/11/59	4/12/59	31/12/59	58.6	50.1
H	- do -	16/9/59	-	16/10/59	-	16/11/59	-	31/12/59	65.1	54.1
I	Captan 1 lb + Dithane Z 78 1 lb	16/9/59	-	16/10/59	-	16/11/59	-	31/12/59	61.2	51.8
J	Unsprayed Control	-	-	-	-	-	-	-	66.9	55.4

(P = 0.05) 10.97
(P = 0.01) 14.82

(iii) Conclusions

Due to climatic conditions the time intervals between spray dates could not be strictly adhered to as planned. Although there were slight differences in favour of Bordeaux plus oil, these differences were insignificant at all the spray intervals.

It is noteworthy that treatments A and D yielded 10.8% and 4.9% infected fruit respectively after receiving 7 Bordeaux sprays over a period of 3½ months after petal drop. Since these protective sprays were applied at only 15 day intervals the accumulative protective covering should have been at a maximum. It would therefore be reasonable to assume that the infection period continued long after December. Kiely (1950) indicated that in Australia on old trees the danger period for infection went up to 5 months after blossoming.

At a glance it appears that spraying at 45 day intervals gave better results than the 30 day intervals. It has already been indicated that large numbers of spores were caught during the beginning of November and that conditions were suitable for infection. Treatments C and F (45 day) were sprayed immediately prior to this period, while treatments B and E (30 day) were not due for sprays. It may therefore be concluded that better results can be obtained with 3 sprays which are well timed, than with 4 sprays which are just applied regardless of prevailing conditions. This was also proved in the commercial spray programme. During the 1960-1961 and 1961-1962 seasons, two well-timed copper sprays gave equally good results as four applications which were applied regardless of the fungus development.

An outstanding feature of this experiment is the complete failure of Captan and Captan plus Dithane Z-78. These fungicides loose their effectiveness much sooner than Bordeaux mixture or other fixed copper fungicides and the results might have been better if spraying continued after December for two more months.

Previous experiments showed that Dithane Z-78 when used at 0.2% gives reasonable control. In this experiment Dithane was used at 0.1% in combination with Captan.

b. Timing of Copper sprays and Dusting.

During the 1959 - 1960 season, experiments indicated that spraying during November was most important for the control of black spot. It was endeavoured in the following experiment to evaluate spraying during November only.

If mycelium (systemic) infection is negligible and infection will only take place during a rainy period, spraying immediately after rains should be evaluated. The effect of one spray (but double concentration) during November was considered worth trying.

It was noticed during dusting operations by aircraft that the dust was well distributed over the plant surface. After successful trial runs with the aircraft it was decided to evaluate dusting from the ground.

(i) Methods and Materials

A randomized block design was adopted, using three-tree plots, replicated 5 times per treatment. The experiment was carried out on 20 year old Valencia orange trees. The experimental trees which received the dust treatment, were surrounded by guard trees to prevent drift of the dust.

All sprays were applied at 400-450 lb per square inch and approximately 10 gallons of spray mixture were applied per tree. The dusting was carried out with a machine driven Knapsack applicator which delivered about 8 oz. of the dust per tree per application. The dust was made up by mixing Perenox and Vine sulphur so that the final mixture contained 25% Copper (metallic) and 48.5% sulphur.

(ii) Treatments and Results

The treatments are summarized in Table 38. The fruit was harvested in September 1961.

TABLE 38

Summary of treatments and results of an experiment to evaluate Perenox plus Alboleum and dusting for the control of black spot.

Treatment code	Material per 100 gallons water	Date of application	Mean % fruit infected	Arc-sine transformation
A	Perenox 1 lb 14 oz + Alboleum ½ gall.	16/11/60 after 29/11/60 long 23/12/60 rains 5/ 1/61	0.5	4.0
B	Same as A	29/10/60 5/12/60 24/ 1/61	1.5	6.7
C	Same as A	29/10/60 16/11/60 29/11/60	8.1	16.2
D	Perenox 4 lb + Alboleum ½ gall.	3/11/60	10.0	18.3
E	Perenox/Sulphur dust	11/10/60 29/10/60 24/11/60 23/12/60 15/ 1/61 31/ 1/61	3.8	11.1
F	Untreated control	-	21.4	27.5

(p=0.05) 3.75
(p=0.01) 5.12

(iii) Conclusions

In the control of endoparasitic fungi on plants, spraying is usually more effective than dusting. The idea behind this experiment was not to evaluate dusting versus spraying on a basis of equal number of applications, but merely to try out the method.

During the course of the experiment 12 ozs metallic copper and nearly 1½ lb sulphur were applied per tree in the dust treatment, compared with the normal orchard spraying (treatment B) where 4½ oz metallic copper and 1⅕ pint of Alboleum were used. The standard treatment was significantly better than dusting (p=0.05).

Treatment A where spraying was carried out only after three dull days with intermittent or continuous rain, the best control was achieved, but it was not significantly better than treatment B where normal orchard spraying was applied. Both treatments A and B were superior to treatment C where the November period only was covered. Three sprays in November with "normal" concentration of Perenox plus Alboleum gave no better control than one concentrated spray (treatment D).

All treatments were superior to untreated control.

No phytotoxic reactions were experienced in any treatment, but treatment E, which was dusted, was heavily infested with red scale.

c. Spraying of young Navel trees

The object of this experiment was to find out the effect of one, two and three sprays on the incidence of black spot on young Navel orange trees. It was also hoped that information would be obtained on the relative importance of each spray application. Information of this nature was regarded as important for working out spray programmes.

(i) Methods and Materials

The experiment was laid out on 8 year old Navel orange trees, using 3-tree plots, replicated 5 times per treatment. Perenox was used throughout at 1 lb 14 oz per 10^0 gallons water.

TABLE 39

Table showing treatments and spray dates in an experiment on young Navel orange trees to see the effect of one, two and three sprays on the incidence of black spot.

Treatment code	Material	Rate of applications	No. of sprays	Dates of spraying
A	Perenox	1 lb 14 oz	3	15th October 1960 15th November 1960 15th December 1960
B	Perenox	1 lb 14 oz	2	15th October 1960 15th November 1960
C	Perenox	1 lb 14 oz	1	15th October 1960
D	Unsprayed control		-	-

(ii) Results

One tree per plot from each of the 5 replications was harvested on three different occasions. This one was done to see how the incidence of the disease increased with time.

TABLE 40

Results of the effect of one, two and three sprays on the incidence of black spot on fruit of young Navel trees.

Treatment	Dates of picking.	Arc sine transformation of % fruit showing black spot lesions								Actual mean % fruit infested.
		Blocks					Total	Mean	Whole treatment mean	
		1	2	3	4	5				
A 3 sprays	4/5/61	0.0	3.4	4.0	5.9	3.6	16.9	3.4		0.6
	22/5/61	8.9	5.1	4.6	3.4	6.4	28.4	5.7		1.2
	12/6/61	0.0	0.0	0.0	2.8	0.0	2.8	0.5		0.1
	Total	8.9	8.5	8.6	12.1	10.0	48.1	9.6	3.2	
B 2 sprays	4/5/61	9.2	6.2	10.6	14.6	20.1	60.7	12.1		5.1
	22/5/61	10.0	14.4	14.6	21.8	16.2	77.0	15.4		7.4
	12/6/61	10.3	11.1	11.1	19.3	17.5	69.3	13.9		6.1
	Total	29.5	31.7	36.3	55.7	53.8	207.0	41.4	13.8	
C 1 spray	4/5/61	17.3	17.1	13.6	22.8	31.8	102.6	20.5		13.2
	22/5/61	18.1	19.6	18.6	23.0	24.5	103.8	20.7		12.7
	12/6/61	26.1	29.4	29.5	29.5	30.6	145.1	29.0		23.6
	Total	61.5	66.1	61.7	75.3	86.9	351.5	70.2	23.4	
D Control	4/5/61	26.3	26.1	26.2	26.6	39.9	145.1	29.0		23.9
	22/5/61	27.6	25.7	33.9	36.5	33.4	157.1	31.4		27.4
	12/6/61	31.4	33.4	34.1	35.1	39.1	173.1	34.6		32.3
	Total	85.3	85.2	94.2	98.2	112.4	475.3	95.0	31.7	

(p = 0.05) 3.58
(p = 0.01) 5.01

Analysis of Variance:

Component	D.F.	Sum of squares	Mean squares	Variation
Treatments	3	6,799.87	2,266.62	184.13 **
Blocks	4	386.48	96.62	7.85 **
Error (a)	12	147.66	12.31	-
Picking	2	108,03	54.02	6.25 **
Interaction	6	297.49	49.58	5.73 **
Error (b)	32	276.62	8.64	-

(iii) Conclusions

It is obvious from the results that three sprays gave good commercial control of black spot. There were significant differences between all the treatments and one may deduce further that the different spray applications were equally important in this experiment.

An interesting observation is the fact that significant block differences were found. The experimental orchard was situated next to an old Valencia orange orchard. The incidence of black spot increased progressively as the distance of the blocks from the old orchard decreased. A logical conclusion is that the spore density in the air close to the old orchard is higher than further away. This seems to confirm field observations. Young trees which were interplanted in old orchards always show a higher incidence of black spot than trees in a young orchard. One reason for the fact that fruit from young trees usually show less black spot than fruit from old trees, appears to be due to a lower spore dosage.

Although there was a considerable drop in temperature from May to June and July, there was a significant over-all increase in black spot at the later picking dates.

d. Spraying of Young Valencia trees

(i) Treatments

This experiment was similar to the preceding one except that young 8 year old Valencia trees were used, and harvesting took place later. For treatments, see Table 39.

(ii) Results

Like the previous experiment, records of the results were taken at three different dates.

TABLE 41

Results (inverse arc-sine transformation of percentages) of the effect of one, two and three sprays of Perenox on the incidence of black spot on fruit of young Valencia orange trees.

Treatment	Dates of picking	Arc sine transformation of % fruit showing black spot lesions								Actual mean % fruit infested.
		Blocks					Total	Mean	Whole treatment mean	
		1	2	3	4	5				
A 3 sprays	13/6/61	0	1.7	2.3	3.4	2.3	9.7	1.9		0.2
	4/8/61	0	3.2	3.4	0	4.8	11.4	2.3		0.3
	20/9/61	21.4	22.3	17.3	14.1	23.1	98.2	19.6		11.3
	Total	21.4	27.2	23.0	17.5	30.2	119.3	23.8	7.9	
B 2 sprays	13/6/61	0	6.2	0	0	5.7	11.9	2.4		0.4
	4/8/61	6.9	3.6	5.3	3.6	6.8	26.2	5.2		0.9
	30/9/61	23.4	24.8	26.9	31.2	41.3	147.6	29.5		24.2
	Total	30.3	34.6	32.2	34.8	53.8	185.7	37.1	12.4	
C 1 spray	13/6/61	17.2	11.2	13.7	26.8	24.7	93.6	18.7		11.3
	4/8/61	18.8	15.2	21.1	26.1	18.4	99.6	19.9		11.9
	30/9/61	57.0	49.2	51.6	67.9	56.7	282.4	56.5		69.6
	Total	93.0	75.6	86.4	120.8	99.8	475.6	95.1	31.7	
D Unsprayed	13/6/61	24.0	16.8	13.7	18.4	15.6	88.5	17.7		9.6
	4/8/61	30.1	23.7	24.4	26.2	26.2	130.6	26.1		19.5
	30/9/61	61.0	48.2	66.4	66.4	75.9	304.6	60.9		76.3
	Total	115.1	88.7	104.5	97.7	117.7	523.7	104.7	34.9	

Analysis of Variance

Component	Degrees of Freedom	Sum of squares	Mean squares	Variation	Significance
Treatment	3	8,258.29	2,752.76	92.219	***
Picking	2	6,324.48	3,162.24	158.588	***
Interaction	6	6,780.27	1,130.04	56.672	***

Analysis of Variance (contd)

Component	Degrees of freedom	Sum of squares	Mean squares	Variation	Significance
Blocks (Replications)	4	369.90	92.47	3.098	
Error (a)	12	358.23	29.85	-	
Error (b)	32	638.15	19.94		

Least significant differences:

Treatment means for all pickings: (i.e. 7.9 12.4, 31.7 and 34.9)	(p=0.05)	4.35
	(p=0.01)	6.09
Means of all treatments for each picking: (i.e. 10.2, 13.4 and 41.6)	(p=0.05)	2.88
	(p=0.01)	3.87

(iii) Conclusions

The results revealed that the second application (15/11/60) was, more important than the first and the last applications. It appears however, that when harvesting takes place later in the season, 3 sprays are essential. The last spray application did not appear to have much value where harvesting was carried out in June and August.

3. DISCUSSION

Kiely (1957) stated that each spray in the black spot control programme is of equal importance. Our results are in conflict with Kiely's statement. During the infection period, long dry spells may be experienced which make long intervals between sprays less risky. During very rainy ssasons with abundant inoculum and when the fungicide is washed off, more frequent sprays will be required. In Australia, Kiely (1949) recommended that "where definite intervals are specified between subsequent sprays, these should be carefully observed, as departure from them will result in less satisfactory disease control". These specified intervals are usually recommended long before the infection period starts and although such recommendations are most useful to the ordinary grower, we can not entirely agree with that statement. Conditions may vary greatly from season to season. During the 1961-62 season, the writer deviated considerably from the specified programme because of the peculiar season and excellent results were achieved.

Kiely (1957) found that fruit of old trees remain susceptible to infection longer than fruit of young trees. He recommended up to 4 Bordeaux sprays for old trees and two to three sprays for young trees. It was found at Letaba that 2 sprays (well-timed) gave good commercial control on young trees, but more sprays were required for old trees.

E. LOW VOLUME SPRAYING

1. INTRODUCTION

The basic principle of plant disease control with protective fungicides is to cover all the susceptible parts of the plant completely with the fungicide. This is an idealistic view, because in practice 100% coverage is seldom achieved.

All spraying of citrus trees in South Africa for pest and disease control is done with high volume machinery and trees are sprayed until dripping wet. This practice is mainly due to the fact that red scale (Aonidiella aurantii Maskell) is a serious pest in South Africa and thorough spraying is essential to achieve control. It became a habit to spray up to 2,000 gallons of spray mixture per acre for the control of black spot. This is a time consuming and laborious task and a great handicap on farms where labour problems exist. It has already been shown that black spot can be controlled by dusting.

Investigations on low volume spraying were done in steps:

(1) To establish whether the method has any merits for this particular problem.

(2) To evaluate low volume spraying versus high volume spraying on approximately the same level of material per unit basis.

(3) To evaluate different materials.

2. COVERAGE

When citrus leaves are examined after spraying with a high volume applicator, most of the surface area will be covered with a thin film of spray material, with bigger blobs where spray mixture accumulated before running off. In many cases one side of the leaf or fruit is well covered, while the other side has no spray material on it. With low volume spraying the results are similar, except that no film of spray material is formed, but tiny droplets of a highly concentrated material settle on the plant, leaving areas between the individual droplets uncovered. But, it is known that copper fungicides are active on fungal spores outside the area which is seen to be occupied by the drop (Horsfall, 1945; Morgan 1952).

Furthermore, infection takes place during rains or periods of wetness, but rain will also redistribute the copper fungicide, so that most of the open spaces will be covered.

To determine the penetration, coverage and distribution of droplets throughout the trees, glossy paper strips or glass slides were clipped onto leaves or fruit at all possible angles, and positions on the trees during spray operations. The method of Blodgett and Mader (1934) was also used extensively to determine the distribution of dried deposits. The coverage on vegetative organs on the outside of the trees was excellent but variable or poor in the centre of trees. Fortunately the incidence of black spot is usually much lower on the "inside" fruit.

3. DOSAGE PER UNIT

At Letaba the number of lesions that develop on leaves are so few that it seems reasonable to assume that the effect of black spot on tree health is negligible. The real damage is done to the fruit. Furthermore, disease symptoms develop more on the fruit on the "outside" of the tree than on the "inside" fruit. More symptoms develop on the fruit on the Northern half than the Southern half; more in the top part of the tree than the lower half and more symptoms develop on the exposed side of the fruit than the shady side.

In view of this distribution pattern of the disease, the question arises whether spraying of 15 and more gallons of spray mixture per tree is really neccessary. If the spraying is directed on the outside fruit, it seems fair to expect reasonable control. Preliminary tests with a power driven low volume applicator showed that coverage is far better on the outside leaves and fruit and that penetration to the interior of the trees is less effective.

a. Treatments

To put the above theory to test it was decided to try out high volume spraying with Standard Bordeaux mixture at 5, 10 and 15 gallons per tree, where normally 15 gallons would have been applied for disease control. At the same time a treatment was included in this experiment where a "Holder" Knapsack-type applicator was used. These trees were about 15 feet high and the low volume applicator was not able to cover the trees to that height successfully.

It was therefore decided to spray only as far as the machine could reach (± 10 feet) and to take ad hoc records only from the lower part of all trees.

TABLE 42

Table shows materials used, concentration and quantity of spray mixture per tree.

Treatment No.	Material	Quantity spray mixture applied per tree	Metallic Cu per tree per application.	Oil per tree per application
A	Bordeaux mixture 2½:2:100 plus ½% Alboleum	15 galls.	1½ oz	1.2 pint
B	Bordeaux mixture 2½:2:100 plus ½% Alboleum	10 galls.	1 oz	.8 pint
C	Bordeaux mixture 2½:2:100 plus ½% Alboleum	5 galls.	½ oz	.4 pint
D	Copper-in-oil	1 pint	1 oz	1.0 pint
E	Untreated control	-	-	-

Dates of application: 5/10/59; 4/11/59; 15/12/59.

Treatments A, B and C were applied with a conventional spray machine equipped with "Hardie" spray guns and No. 7 discs. The rates of application were determined with the aid of a stopwatch, after establishing the output per minute of each spray-gun, immediately before each spray application was carried out.

The "copper-in-oil" used in this experiment was mixed to contain ½ lb of metallic copper (in the form of copper oxychloride) per gallon.

b. Results

During the third week of September 1960 all the fruit was picked to a height of about 8 feet. This was done in order to see how effective the low volume application treatment was. Thereafter the whole trees were picked and examined for black spot.

TABLE 43

Effect of high volume spraying at different rates of application and low volume spraying on the control of black spot during 1959 - 1960 season.

Treatments	Lower half of trees only		Whole trees	
	% Fruit with black spot	Inverse arc sine transformation	% Fruit infected	Inverse arc sine transformation
A. Bordeaux + Alboleum 15 galls. per tree	5.4	13.2	20.5	26.9
B. Bordeaux + Alboleum 10 galls. per tree	18.9	25.1	30.3	33.3
C. Bordeaux + Alboleum 5 galls. per tree	27.3	31.0	38.2	38.2
D. Copper-in-oil 1 pint per tree.	9.2	16.2	39.3	38.7
E. Untreated Control	37.4	37.6	62.5	52.4
	(p=0.05)	11.9	(p=0.05)	7.15
	(p=0.01)	16.7	(p=0.01)	10.03

c. Conclusions

Results on the lower halves of the trees showed that only high volume spraying at 15 gallons per tree and low volume spraying are significantly better than the unsprayed control treatment at 1% level. There were no significant differences between these two treatments. Treatment B is only significantly different from the unsprayed control at 5% level.

Results taken over the entire tree showed no significant differences between treatments B, C and D but all treatments are significantly better than the unsprayed control. Treatment A was superior to C, D and E but not significantly better than B. A comparison of the low volume application on the basis of results taken over the entire tree is not valid, for reasons already given.

It is once again indicated that the incidence of black spot was higher in the upper portions of trees than the lower portions, although no effort was made here to prove this point statistically.

In this experiment a full cover spray (15 gallons per tree) was superior to applications of 10 and 5 gallons per tree. These data should not discourage further experimentation. It is possible that different spray nozzles or smaller discs which provide a mist of small droplets may give more encouraging results. At the time when this experiment was carried out No. 7 discs were used in the "Hardie" spray guns. The orifice of a No. 7 disc was subsequently found to be too large and gave a coarse droplet output. Since 1960, No. 5 discs have been used at Letaba Estates. The low volume treatment must be regarded as successful and further experiments were desirable.

4. EVALUATION OF FUNGICIDES

a. Treatments

In view of the encouraging results obtained in the previous experiment, it was decided to evaluate a few fungicides with low volume application. For this purpose a block of 11 year old Valencia orange trees were selected which were small enough to spray all parts with a low volume applicator. The different treatments are summarised in Table 44. The dates of spraying were 29th October, 22nd November and 29th December, 1960.
The Bordeaux plus Alboleum treatment (A) was applied with a conventional high volume machine. All the other treatments were applied with a Knapsack low-volume applicator.

b. Results

Records were taken on 28th, 29th and 30th August, 1961. The results are summarised in Table 44.

TABLE 44

Table showing different materials, concentrations, application rates per tree and control of black spot in a "low volume" experiment during the 1960 - 1961 season.

Treatment Code	Material and Concentration.	Material applied per tree per application			Mean % fruit with black spot	Arc sine transformation.
		Spray Mixture (pints)	Metallic Copper (lbs)	Oil (pints)		
A	Bordeaux mixt. 2½:2:100 + Alboleum ½ gallon	64.0	0.049	0.32	5.88	13.93
B	Copper-in-oil ½ lb metallic copper per gallon	1.2	0.075	1.08	2.40	8.67
C	Colloidox 2½ lb + Alboleum 2 gall. + water 18 galls.	3.0	0.009	0.30	12.05	20.12
D	Perenox 1 lb + Alboleum gall. + water 18 galls.	3.0	0.009	0.30	15.50	22.82
E	Perenox 4 lb + Alboleum 2 gall. + water 18 galls.	2.5	0.031	0.25	6.95	15.03
F	Cyprex 2 lb + Alboleum 2 gall. + water 18 galls.	2.8	-	0.28	24.50	29.67
G	Dithane M22 (Special) 3 lb + Alboleum 2 gal. + water 18 gallons	2.8	-	0.28	15.08	22.82
H	Untreated Control	-	-	-	31.45	34.08
					(p=0.05)	3.01
					(p=0.01)	4.03

c. Conclusion

Treatment B (copper-in-oil) was significantly better than any other treatment, while there was no difference in control of black spot between the standard high volume treatment (Bordeaux mixture) and treatment E (Perenox plus Alboleum, high concentration, low volume). All treatments were superior to unsprayed control.

5. DISCUSSION

It is possible to apply low volume (high concentration) spraying with success for the control of black spot. It would be advisable, however, to carry out further research on materials and different applicators. Preliminary trials carried out with a "Kinkelder" low volume machine showed that some modifications are necessary to this type of applicator before it can be used successfully in citrus.

Entomologists are rather sceptical about the control of red scale (Aonidiella aurantii) with low volume spraying. Few of the smaller growers can afford to have a low volume machine for black spot control only, but this is no problem to the bigger growers.

F. AERIAL SPRAYING

1. INTRODUCTION

In a study of the control of black spot, an interest in aerial spraying is a natural development for the following reasons.

(a) There appears to be a definite infection period where windborne spores play a dominating role.

(b) There are distinct possibilities of predicting an infection period by studying the prevalence of ascospores and their maturity on dead leaves. Aerial spraying is a quick operation and spraying can therefore be delayed until shortly before an infection period.

(c) If an infection occurred and a spray could be applied immediately afterwards with an eradicant fungicide it might be possible to achieve control. This, however, would have to be done quickly as a delay of a few days may be fatal.

(d) Conventional spraying is a slow, time consuming process for which a large labour force is necessary. It takes approximately 3 weeks to complete a spray round at Letaba Estates. During that period close to 2,000,000 gallons of spray material have to be pumped on the trees. For this task 200 Native labourers and about 20 Europeans are employed and 17 spray carts are used. If trees are big it may take 1,500 gallons to spray one acre of citrus trees and this has to be done 4 times per year.

(e) The occurrence of the disease seems to favour aerial spraying eg. the incidence of the disease is higher in the upper half of the tree than the lower half. The "outside" fruits are more prone to lesion development, than the fruit inside the tree, etc.

(f) Experimental results on low volume spraying were encouraging.

(g) Letaba is a relatively wind-free area and if the temperature allows it, spraying can be done almost all day.

(h) The topography at Letaba is suitable for aerial spraying.

2. PRELIMINARY TRIALS

Although dusting by aircraft for the control of Bollworm (*Heliothis armigera* Hübner) and Thrips (*Scirtothrips aurantii* Faure) has been done for years in South Africa, no information exists on the control of a disease such as black spot by aircraft.

The basic principle of crop protection is to achieve 100% coverage of the susceptible parts of the plant at the lowest cost. It follows then that in general one has to distribute the chemical as equally as possible in the smallest particles.

a. Methods and Materials

Initial trials with a motorized fog generating machine (T I F A) were abandoned, as the droplets were too small to settle on the trees under climatic conditions at Letaba.

Spraying by aircraft was hereafter investigated. Since no information was available in this country on disease control in citrus from the air, numerous fruitless trial runs were carried out.

A Piper Super Cub aircraft with 46 nozzles (Spraying System Inc.) on a 30 foot boom was used for the first spray trial. The nozzles were turned forward so that the orifices faced the direction of flight, but at a slight angle downward to prevent the spray from blowing back on to the nozzles and aircraft. At a speed of 80 to 85 m.p.h. and a pressure of 35 lb per square inch, droplets varying between 50 and 300 microns were produced.

After several trial runs it appeared that 5 to 8 gallons per acre provided a fairly good coverage of citrus leaves and fruit in an old orchard.

In order to see whether black spot could be controlled from the air, a simple trial was laid out as follows:-

Three rows of trees on two opposite sides of an old Valencia orange orchard were chosen for aerial spraying, while 3 rows in the centre of the plot were sprayed by hand in the conventional manner. The treatments were:

1. Three rows sprayed from the air with copper-in-oil (Shell) diluted with oil to contain ½ lb metallic copper in the form of copper oxychloride per gallon.

2. Three rows of trees were sprayed with Perenox, Alboleum and water mixture, made up to contain ½ lb metallic copper and ¼ gallon of oil per gallon of spray mixture. Due to mechanical trouble and physical problems the above mixture was replaced from the second application onwards, with Colloidox, a colloidal copper fungicide. This fungicide was mixed with water to contain ⅓ lb metallic copper per gallon of the final spray mixture.

3. Three rows of trees in the middle of the plot were sprayed by hand, using Perenox at 2 lb per 100 gallons plus ½ gallon of Alboleum.

In the two aerial treatments, the aircraft sprayed each row 3 times. At approximately 2 gallons per acre per flight it means that 6 gallons per acre were applied. Drift and overlapping occurred which were impossible to control. It was estimated that approximately 12 gallons of spray mixture were sprayed over the centre row. The aircraft flew at a height of approximately 3 feet above the tree tops.

b. Results

On 16th August 1961, 5 trees in each row (15 trees per treatment) were harvested and examined for black spot.

TABLE 45

Summary of results in a trial where aerial spraying was compared with conventional spraying for the control of citrus black spot, during the 1960 - 1961 season.

Treatment	Average percentage fruit infected in row			Row mean	Arc sine trans formation
	1	2	3		
Copper-in-oil (Aerial)	5.7	6.0	4.7	5.5	13.1
Perenox + Alboleum followed by Colloidox	44.4	50.1	38.4	44.3	42.2
Hand spraying	11.6	11.2	11.3	11.4	19.8
				(p=0.05)	3.5

c. Discussion and Conclusions

This was not a true randomized block experiment, (as randomization of blocks was not possible) but it was treated as such in the statistical analysis. It is extremely doubtful, however, whether block randomization would have made a big difference because black spot had always been very severe throughout this plot in the past. No apparent differences existed between the different rows in each treatment despite the fact that the centre rows received a higher dosage of spray material from the air.

The copper-in-oil treatment was outstanding and indicates that black spot can be controlled from the air successfully. The other aerial treatment was a failure, but this must have been due to an inferior product. In another low volume experiment it was revealed that Colloidox is an inferior product for the control of black spot.

It would be uneconomical to spray 6 to 12 gallons per acre from the air and further experimentation was deemed necessary.

3. EXPERIMENTS WITH SPRAYBOOM AND ROTARY ATOMIZERS

In view of the previous year's results on aerial spraying it was decided to evaluate copper-in-oil at lower dosage rates, for economic reasons. Better control of droplet size was also desirable.

a. Methods and Materials

(i) Equipment

A Piper Super Cub aircraft with a wing span of 35 feet was used for all the aerial spraying. The aircraft was fitted with 46 of Spraying Systems' nozzles. The orifices D4, D6 and D8 were used. When a high output was required the bigger orifices were used. The nozzles were again arranged so that the orifices faced forward, but directed slightly downward to prevent the spray from blowing back onto the nozzles and the aircraft.

Four "Micronair" rotary atomisers were fitted on the same aircraft, but when boom spraying was carried out, the atomizers were removed. During atomizer spraying the booms were removed in order that the supply pipes could feed the atomizers.

PLATE 7. Photograph shows how the rotary atomizers were mounted on the aircraft.

All the mechanical work and piloting was carried out by Multispray Limited, Grand Central Airport, Halfway House, Transvaal.

(ii) Droplet Size

By altering the setting of the atomizer blades and thereby altering the speed of rotation the droplet size was usually regulated. It was found that small droplets were usually lost in drift on hot days in which case the droplets were increased. With application of higher dosage rates the increased volume of spray mixture tended to slow down the atomizers and a finer setting of the blades was necessary to keep the atomizer speed constant.

(iii) Assessment of coverage and droplet size

Various methods were employed, such as white paper strips, paper treated with various dyes, or by using dyes in the spray mixture. Glass slides are useful for rough work, but are seldom better than the windscreen of a motor car or a white shirt!

The easiest method for more accurate work, is to coat glass slides with magnesium oxide (Norman and Britten, undated). When the slides were exposed to the spray, a clear droplet pattern was obtained. Each droplet formed a miniature crater in the magnesium oxide which could be measured. The crater diameter was factored by 0.87 to obtain the actual droplet diameter.

For boom spraying early in the mornings the droplets varied between 40 to 300 microns, with an average droplet diameter of approximately 110 microns. With the atomizers the droplets varied from 30 - 250 microns, but the average droplet size was approximately 100 microns. Under hot conditions or when spraying had to be done under windy conditions the droplet sizes were increased.

(iv) Assessment of Penetration

A citrus tree is densely foliated and penetration of the droplets to the "inner" fruits may be difficult.

In order to investigate this, 12 feet wooden poles with cross beams, 1 foot apart, were placed vertically in the citrus trees which were sprayed. Similar poles were also placed outside the orchard on an open spot in line with the poles in the trees. On each cross beam two filter papers (11 cm diameter) were placed. After the aeroplane passed once over (spraying a copper-in-oil mixture) the papers were carefully collected and analysed for copper by the Technical Department at Zebediela Estates.

This preliminary investigation indicated that there was very little variation in the quantity of copper deposited at the different levels, but outside the orchard more copper deposited on the higher levels.

Glass slides, coated with magnesium oxide indicated that more big droplets accumulated at 10 feet heights than at 3 feet when trees were sprayed, but distribution outside the trees was more even.

b. Treatments

Five plots (12½ acres) of old Valencia orange trees were used for this trial. There were five spray-treatments but because the area over which the trial was carried out, was large and variation in the disease incidence was likely to occur, it was decided to leave an unsprayed control plot for each treatment. The actual area sprayed (disregarding drift) was approximately 1⅓ acres per treatment.

The material used for all the plots sprayed from the air was Shell's copper-in-oil, mixed to contain ½ lb copper in the form of copper oxychloride per gallon. The standard treatment for comparison was conventional hand spraying, using Perenox at 2 lb plus Alboleum at the rate of ½ gallon per 100 gallons water.

It was endeavoured to apply 4 and 6 gallons per acre with the spray boom and also with the atomizers. The output per run for both spray-boom and atomizers was approximately 1½ - 2 gallons per acre. In order to achieve 4 and 6 gallons per acre 2 and 3 runs had to be made respectively over the same areas.

A plan of the treatments as given below:

PLAN

Legend:

C1, C2, C3, C4 and C5 - unsprayed control plots.

The swathes of the booms and the atomizers were taken as 66 feet. It was therefore necessary for the aircraft to pass over every 3rd row.

All the experimental plots, except the handsprayed plot, were sprayed with Parathion for the control of insect pests before the trial commenced. The differential treatments as applied are given in the tables below.

TABLE 46

Actual number of gallons of spray mixture per acre as applied on the different dates in the various treatments in an aerial trial 1961 - 1962.

Date of spraying	Gallons spray mixture applied per acre				
	Treatment 1 (Atomizer 2 runs)	Treatment 3 (Atomizer 3 runs)	Treatment 5 (Boom 2 runs)	Treatment7 (Boom 3 runs)	Treatment 9 Conventional
5/10/61	2.4	4.5	3.7	6.7	1,400 ⁂
10/11/61	3.0	5.2	4.0	6.0	1,200
7/12/61	4.0	7.3	3.8	6.5	1,200
3/ 1/62	3.5	7.0	3.7	6.4	1,400 ⁂
Total	12.9	24.0	15.2	25.6	5,200
Average	3.2	6.0	3.8	6.4	1,300

⁂ Parathion included with black spot sprays.

TABLE 47

Table showing average and total oil and copper applied per treatment in the aerial spray trial 1961 - 1962.

Treatment	Material applied per acre			
	Average per application		Total	
	Oil	Cu	Oil	Cu
1. Atomizer 2 runs	3.2 galls.	1.6 lb	12.9	6.45
3. Atomizer 2 runs	6.0 galls.	3.0 lb	24.0	12.0
5. Boom 2 runs	3.8 galls.	1.9 lb	15.2	7.6
7. Boom 3 runs	6.4 galls.	3.2 lb	25.6	12.8
9. Conventional	6.5 galls.	13.0 lb	26.0	52.0

c. Results

Records were taken during the last week of August 1962. Eight trees were taken at random near the centre of each treatment for harvesting and record purposes. As it was known that the incidence of black spot is higher in the upper portions of trees than lower down, results were taken from upper and lower sections of the trees, to see how aerial spraying affected the occurrence of the disease.

TABLE 48

Summary of results on the control of black spot where aerial spraying with rotary atomizers and spray booms with nozzles were compared with high volume handspraying.

Treatment	Section of trees	Mean % Fruit with black spot	Inverse arc sine transformation	
			Upper & Lower halves	Whole treatment
1. Atomizer - 2 runs	Upper half	15.15	22.39	
	Lower half	5.70	12.81	17.60
2. Untreated control - C1	Upper half	54.78	47.75	
	Lower half	17.90	24.88	36.31
3. Atomizer - 3 runs	Upper half	17.83	24.11	
	Lower half	8.78	16.41	20.26
4. Untreated control - C2	Upper half	43.38	41.14	
	Lower half	12.45	20.14	30.64
5. Boom - 2 runs	Upper half	6.81	14.90	
	Lower half	4.39	11.60	13.25
6. Untreated control - C3	Upper half	28.34	32.00	
	Lower half	10.18	18.00	25.00
7. Boom - 3 runs	Upper half	18.22	24.99	
	Lower half	10.51	18.55	21.77
8. Untreated control - C4	Upper half	69.28	56.54	
	Lower half	27.90	31.68	44.11
9. Conventional handspray	Upper half	5.96	12.96	
	Lower half	1.51	6.84	9.90
10. Untreated control - C5	Upper half	52.87	46.68	
	Lower half	27.98	31.44	39.06
		(p=0.05)	3.85	4.326
		(p=0.01)	5.08	5.754

d. Discussion and Conclusions

This was an extremely costly experiment, not only when one considers the operational charges and costs of materials, but a considerable amount of fruit was unexportable on account of the severity of the disease in the unsprayed control plots. Under these circumstances, an experimental design which would lend itself better to statistical analysis was hardly possible. A complex statistical analysis was applied. It is doubtful whether differences brought out by other methods of analysis would have been valid.

Despite all the statistical short comings, valuable information was revealed.

In all cases, the sprayed plots were significantly better than the unsprayed control treatments. Variation in the incidence of black spot between different treatments made a direct comparison risky, but conventional hand spraying appeared to have been superior to aerial treatments. It would have been surprising if this was not the case, because considerably more copper and oil had been applied in the hand-sprayed treatment. Furthermore, due to mechanical trouble the aerial sprays could not have been applied under ideal environmental conditions and drift losses were severe on some occasions. A portion of the material sprayed from the aircraft never reached the experimental trees at which it was aimed. Commercial spraying at Letaba Estates, where large areas can be sprayed from the air, should yield better results than those obtained in these experiments. Spraying with helicopters may overcome many of the drift problems.

Like other experiments, this one shows clearly that the incidence of black spot is more severe in the tops of trees than in the lower portions, at Letaba Estates.

In the light of results so far, aerial spraying seems to be more suitable for old trees where comparatively little spray material (copper) reaches the orchard soil. For young trees (between 3 and 15 years) where the rows are open, low volume spraying from the ground seems to have advantages over aerial spraying.

G. CONTROL OF BLACK SPOT AFTER INFECTION

1. INTRODUCTION

The latent nature of black spot infections was demonstrated by several workers (Kiely 1948; Simmonds, 1940; Wager, 1952 and Tokunaga and Yokohama, 1955).

The distinction between superficially placed spores which still have to function, and those which have already germinated and penetrated into the host tissue, presents fundamental differences in control methods. Fungicidal sprays which are applied during the first 5 months after the blossoming of citrus trees, are aimed at the prevention of infection. Where the fungus is still superficial, fungicidal treatments applied with the object of preventing infection proved to be reasonably effective. Where the fungus has already penetrated the host tissues, and remain dormant until the host reaches a certain stage of maturity, it is unlikely that a fungicide will succeed, unless it has systemic qualities.

Like most fungal disease of plants, commercial control measures against black spot is based on protection of the fruit with a fungicide to prevent infection. This is achieved by spraying a copper-containing fungicide, to which an emulsified oil is usually added. The role of oil is still obscure, but will be discussed separately.

A spray round at Letaba Estates is usually completed in 3 weeks. In practice it may happen that infection occurs at the beginning of a spray round and it is unknown what the effect of subsequent spraying will be on the disease development. It was also indicated that aerial spraying may be carried out in future. Aerial spraying is a quick operation. Spore trap results as well as records on climatic conditions can possibly be used to show when an infection period occurred. The question arises therefore, that if it is known that infection took place at a certain time, how long afterwards can infection be eradicated if at all?

2. CONTROL, SHORTLY AFTER INFECTION

a. Methods and Materials

For this experiment thousands of blossoms were covered on 4, 12 year old Valencia orange trees. Only 205 of these blossoms developed into young fruit.

Dead leaves under the trees were regularly examined for the presence of ripe ascospores. At the beginning of

December large numbers of ripe perithecia were found which indicated that considerable infection might occur under favourable climatic conditions. The paper bags were removed on 13th December just before a spell of rain. Rain commenced that evening and the trees remained wet for approximately 40 hours. Altogether 10.2 mm of rain were recorded for that period. All the fruits were placed in paper bags again as soon as they were dry. The fruits were then divided into lots of 52,52, 53 and 48 and each lot was dipped in a mixture of 2 lb Perenox plus ½ gallon Alboleum per 100 gallons water except the fourth lot as set out in Table 49.

After each lot of fruit had been dipped the paper bags were immediately replaced. In June 1962 all the paper bags were removed to allow the fruit to mature under natural conditions. No rain occurred from June until the fruits were harvested on the 15th August 1962, so that a possibility of infection during that period can be excluded. A large number of the fruits dropped.

b. Results

After an incubation period of 14 days at 23°C to 26°C the fruits were examined and all the black spot lesions were counted. The results are given below.

TABLE:49

Table showing the incidence of black spot after dipping fruit in a mixture of Perenox and Alboleum at different intervals after exposure to infection.

Treatment Code	Total Number of fruit		Period between onset of infection and dipping	% Fruit infected	Total No. of spots	Av .No. spots per fruit
	Treated	Survived				
A	52	32	114 hours	37.5	25	0.78
B	52	25	204 hours	48.0	37	1.48
C	53	30	18 days	63.3	64	2.13
D	48	31	Undipped	58.1	74	2.39
	DF = 3			X^2= 4.815		

The differences between treatments (% infected fruit) are regarded as significant.

c. Conclusions

This experiment was repeated 3 times but the periods of wetness were too short during the exposure time and no results were obtained in the other two experiments.

Although this must be regarded as a pilot experiment and it was further hampered by fruit-drop, the results indicate that by postponing the application of the fungicide to $4\frac{1}{2}$ days after the commencement of an infection period complete control of black spot was not achieved. Ascospores were caught during the entire period of rain which lasted approximately 40 hours. It is therefore possible that infection that took place towards the end of that period was arrested by the fungicide, while the earlier infection was too far advanced at the time of dipping. On the other hand, the effect of the oil is obscure. Oil is known to penetrate plant tissue (Ebeling, 1959) and to have fungicidal properties under certain circumstances (Laville, 1960). The apparent control achieved in treatments A, B and C is probably due to the action of the oil.

A study of eradication of G. citricarpa after infection certainly warrants close attention. Experiments on the times as described above, using various eradicative fungicides at short and long intervals after infection should yield information which may be of great importance in the control of this disease in future.

3. CONTROL AFTER THE ANNUAL INFECTION PERIOD

In South Africa trees are usually sprayed 3 or 4 times with a copper fungicide (with or without oil) from October to January, for the control of black spot. Kiely (1950) and Wager (1952) indicated that little or no control is achieved by spraying with copper after that period. Kiely (1950) observed however that the application of white spray oil, after a weak Bordeaux mixture programme, contributed greatly towards the control of black spot.

a. Oil in January

In view of Kiely's report on the effect of oil on black spot development after a weak Bordeaux mixture programme, and the observations made in the Letaba district, it was decided to investigate this aspect.

(i) Methods and Materials

A block of 38 year old Valencia trees, which received three Bordeaux sprays from September 1959 to 15th December 1959 was selected as an experimental site. The layout was based on a randomized block principle with 4 tree plots and 4 replications per treatment.

The treatments were as follows:-

Treatment A. Alboleum 2 gallons per 100 gallons water

Treatment B. Alboleum 1 gallon per 100 gallons water

Treatment C. Bordeaux mixture $2\frac{1}{2}$: 2 per 100 gallons water

Treatment D. Unsprayed control.

About 15 gallons of spray mixture was applied per tree.

Date of application: 13th January 1960.

(ii) Results

During the 3rd week of September 1960 all the fruit from 6 feet and lower was stripped and examined for the incidence of black spot.

TABLE: 50

Summary of results of an experiment to evaluate the effect of oil sprays, after a weak Bordeaux mixture programme.

Treatment		Mean percentage fruit with lesions	Arc sine transformation
Code	Material		
A	Alboleum (2g.)	18.1	25.1
B	Alboleum (1g.)	17.2	24.4
C	Bordeaux	17.9	24.7
D	Control -	17.3	24.5

The differences between the results of the various treatments were non-significant.

b. Oil in April

(i) Methods and Materials

Laboratory tests indicated that when fruits are dipped in oil-water emulsions after picking, black spot development was retarded. This led to another field experiment but this time the oil sprays were applied on 26th April. This experiment was carried out on old Valencia orange trees which received 4 copper fungicide sprays from October 1960 to January 1961. Black spot had always been severe on the fruit of these trees in the past. At the stage when this experiment commenced, black spot showed up on a very low percentage of the fruit.

Treatment A. Alboleum 2 gallons per 100 gallons water

Treatment B. Alboleum 1 gallon per 100 gallons water

Treatment C. Unsprayed control.

The layout was a simple randomized block with 9 replications of single tree plots per treatment.

(ii) Results

TABLE: 51

Percentage fruit infected on 28th September 1961 after spraying 1% and 2% Alboleum on 26th April 1961.

Treatment		Mean % fruit with black spot lesions
Code	Material	
A	Alboleum (2g.)	28.8
B	Alboleum (1g.)	37.6
C	Control	47.4
	(p = 0.05)	14.15

(iii) Conclusions

Although both 1% and 2% Alboleum reduced the incidence of black spot when compared with the unsprayed control 2% Alboleum gave significantly better control than the unsprayed control treatment.

c. Oil with and without other fungicides

A further evaluation of the effect of oil was undertaken. It was considered desirable to include some other treatments which may inhibit or prevent black spot development.

(i) Methods and Materials

This randomized block experiment was laid out on young Valencia trees, using single tree plots, replicated 6 times per treatment.

The treatments were:

Treatment A. Alboleum 2 gallons per 100 gallons water

Treatment B. Perenox 2 lb + PMC 2 oz + Alboleum 2 gallons per 100 gallons water.

Treatment C. Perenox 2 lb + PMC 1 oz per 100 gallons water

Treatment D. Perenox 2 lb + PMC 4 oz per 100 gallons water

Treatment E. Dithane M22 (special) 2 lb + Alboleum 2 gallons per 100 gallons water.

Treatment F. Unsprayed control

Two spray applications were carried out viz:

6th June 1960 and

1st August 1960.

Approximately 8 gallons of spray material was applied per tree on both occasions.

(ii) Results

Records were taken at harvesting during the last week of September 1960.

TABLE: 52

The effect of Alboleum with and without other fungicides on the control of black spot.

Treatment No.	Percentage fruit in black spot		
	Less than 5 spots per fruit	More than 5 spots per fruit	Total % black spot
A	12.6	7.6	20.2
B	12.5	6.9	19.4
C	17.3	8.2	25.5
D	29.5	9.7	39.2
E	18.2	8.1	26.3
F	26.7	26.4	53.1
		(P = 0.05)	9.0

(iii) Conclusions

All fungicidal sprays reduced the incidence of black spot. The addition of Dithane M22 (Special) or PMC plus Perenox gave no better results than Alboleum without additives. The control afforded by the Perenox plus PMC treatments and Perenox plus PMC plus Alboleum is of interest but considerable leaf and fruit drop occurred. This type of phytotoxicity was particularly severe in treatments B and D. A considerable amount of spray injury occurred on the fruit in treatment D and also to a lesser extent in treatments B and C. It was extremely difficult to differentiate between newly developed black spot lesions and spray injury so that the results of these treatments are somewhat unreliable and exaggerated.

The role of oil on disease development is discussed later.

d. Cyprex and oil

Claims were made that Cyprex, when applied with small quantities of oil will retard or prevent symptom development.

(i) Methods and Materials

The following treatments were applied in a randomized block experiment, using 38 year old Valencia trees which received 4 Bordeaux mixture applications from September 1959 to January 1960. Each treatment consisted of 10 single tree plots.

Treatment 1. Cyprex 1½ lb plus 1 pint Alboleum/ 100 gallons water.

Treatment 2. Cyprex 1½ lb plus ½ pint Alboleum/ 100 gallons water.

Treatment 3. Unsprayed control.

These sprays were applied on 27th July 1960 when 3 samples of 100 fruits, picked at random from the experimental trees showed an average of 4.8% infected fruit.

(ii) Results

During the 3rd week of September 1960 all fruit up to 7 feet from the ground was stripped and examined for black spot. An average of 3 orchard boxes per tree were examined.

TABLE: 53

Results on the control of black spot after spraying Cyprex plus Alboleum shortly before harvesting.

Treatment No.	Mean percentage fruit with black spot lesions	Arc Sine Trans-formation
1	16.5	23.8
2	12.9	20.3
3	20.0	25.9

There were no significant differences between the treatments.

4. DISCUSSION

Between the time of infection and the time when disease symptoms appear, there is a latent period which may last several months. Protective sprays during that period have no effect on the disease incidence. Applications of Alboleum and PMC shortly before picking gave encouraging results. PMC was very phytotoxic under certain circumstances. Further investigations with similar fungicides are necessary.

In years when temperatures during May and June are high, sprays with Alboleum (or similar products) may be used to retard lesion development. It was found that oil retards the colouring of fruits but this is not a great disadvantage where Valencia oranges are involved. On Navel oranges at Letaba where the fruits are usually ripe before the rinds are yellow, oil sprays may delay colouring considerably. According to Riehl, et al (1958) mineral oil sprays retarded

the transpiration of citrus for many weeks. Our own results showed that oil can also reduce the crop.

Govindaswamy (1959) showed that oil reduced spore germination of several fungi and inhibited mycelium growth. Calpouzos et al (1959) claimed that oil inhibited the mycelium of Mycosphaerella musicola inside banana leaves. The fungus was not killed by the oil however. Oil sprays were so effective against Sigatoka disease of bananas that it replaced copper sprays.

Oil seems to affect both the host and the parasite and growers should bear this in mind. The judicious use of oil prior to picking may contribute considerably towards the control of black spot.

H. EVALUATION OF CHEMOTHERAPEUTANTS

1. INTRODUCTION

Sueda (1941) was the first worker to claim that "the black spot fungus of citrus" spread in a systemic manner and that the fungus spread to new citrus plants through infected grafts. Leaves which grew out of grafts became infected by the movement of the fungus in the host tissues.

Sueda also stated that fruits became infected by the movement of mycelium from infected tissues. Only a translated summary of Sueda's report was seen, in which the causal organism was not directly mentioned. Tokunaga and Yokohama (1955) confirmed however, that Sueda's studies were carried out on Phoma citricarpa Mc Alp. They also disclosed that this fungus was isolated from citrus flower stalks, receptacles and ovaries.

Schüepp (1960) who strongly supported the "systemic infection theory", suggested that the control which is obtained in practice with copper fungicides is due to penetration of copper through the epidermis or by changing the "physiological state of the citrus plant". Although the possibility of chemotherapeutic action of copper cannot be ignored completely, it seems rather unlikely. According to Stoddard and Dimond, copper is fixed by woody cells and never moves to a great distance when injected into plants. Leaf analysis[≡] on citrus leaves at Letaba showed that penetration of copper into the leaves was poor in the case of conventional spray applications.

Apart from systemic infection by mycelium, it was established that there is a latent period after spore infection. This latent period lasts for several months. The possibility of control of the disease during this latent period with a suitable chemotherapeutant seems to be a promising field for investigation.

≡ Leaf analyses were carried out by the Technical Department, Zebediela Estates.

2. INTRODUCTION OF CHEMOTHERAPEUTANTS THROUGH HOLES

To attain systemic chemotherapy, the chemical must enter the plant so that translocation to the point of need can take place. Upward translocation seems relatively simple according to Howard and Horsfall (1959) but most compounds are translocated slowly downwards, if at all. Dr. Schüepp, in a verbal discussion suggested that chemicals should be introduced into the trees through boreholes. Stoddard and Dimond (1949) also referred to infection of chemicals through holes.

a. Methods and Materials

An experiment in which chemicals were introduced through holes in the stems of trees was conducted on 4 year old Valencia orange trees which had never received any sprays for the control of black spot. The experiment was laid out on a randomized block design, using single tree plots, replicated 5 times per treatment.

The chemicals used in the different treatments, were:-

A. Zinc sulphate 0.1% solution plus
Potassium permanganate 0.25% solution plus
Borax 0.25% solution.
These salts were dissolved in water and applied through the bark of stem (see below)

B. Ditto A, but applied through holes.

C. Sulphaquanadine 0.25% in water applied through bark of the stem.

D. Ditto C, but applied through holes.

E. Sulphanilimide 0.25% in water, applied through bark of stems.

F. Ditto E, but applied through holes.

G. Sulphanilimide 0.25% in acetone and water, applied through holes.

H. Untreated control.

In treatments A, C and E, a strip of absorbant cotton-wool, about 3 inches wide was placed round each tree stem at a height of 2½ feet above ground level. The cotton-wool was fixed round the stem with twine, but one end of the cotton-wool was loose.

The loose end was about 12 inches long. A sheet of plastic material 18 x 18 inches was placed round the cotton-wool and the two ends were sealed together to form a cilinder round the trunk. About 6 inches below the cotton-wool , the lower end of the plastic cilinder was fastened with a rubberband so that no water could leak through. The loose end of the cotton-wool was placed in the plastic reservoir and the appropiate solution was poured into the reservoir. After this all the cotton-wool was soaked in the solution and the top end of the plastic reservoir was tied with a rubber band. Through capillary action the chemical solution remained constant and ensured that the bark remained in contact with the chemotherapeutant in the cotton-wool. It was hoped that the chemicals would be absorbed through the bark and translocated to the fruit (see Plate 8).

In treatments B, D, F and G, four holes, $\frac{1}{2}$ inch diameter and 2 inches deep were drilled per tree. Into each hole one end of a one inch wide absorbent cotton-wool strip was loosely plugged. The plastic reservoir was then applied in the same way as above, so that the loose ends of the cotton-wool strips rested in the bottom of the reservoir.

Two litres of the chemotherapeutant solution was put into each reservoir. Every three weeks the old solutions were drained and replaced with fresh solutions.

This experiment was commenced in the 2nd week of June, 1960 and completed in September 1961 so that two season's crops were available for examination. A pre-treatment examination of fruit was carried out on 25 fruits per tree, on 12th June 1960 and the percentage of fruit showing lesions was recorded.

During the last week of September 1960, nearly $2\frac{1}{2}$ months after the experiment commenced, a random sample of 2 orchard boxes of oranges were picked from each tree and examined for black spot and "melanose".

b. Results

PLATE 8. Photograph shows how the plastic bags were placed round the tree stems for the introduction of chemotherapeutants through holes or directly through the bark.

TABLE 54

The incidence of black spot and "melanose" approximately 2½ months after application of chemotherapeutants to the tree stems.

Treatment No.	Mean percentage fruit with black spot		Mean percentage fruit with "melanose"
	Before treatment	After treatment	After treatment.
A	3.3	21.1	2.0
B	4.5	24.0	2.7
C	2.8	16.5	1.9
D	2.9	20.3	3.1
E	5.0	25.0	2.2
F	3.1	19.0	2.3
G	4.2	21.4	1.8
H	5.7	19.6	2.5

A statistical analysis showed that there are no significant differences between treatments.

TABLE 55

Mean percentage fruit showing black spot and "melanose" lesions 14½ months after chemotherapeutants were applied through holes and bark of the tree stems.

Treatment No.	Mean percentage fruit with	
	Black spot	"Melanose"
A	90.6	2.6
B	74.8	3.1
C	87.7	2.7
D	78.6	4.1
E	84.3	2.3
F	81.7	2.9
G	88.1	3.0
H	82.0	2.9

The difference between treatments were statistically non-significant. All the treatments applied in the way described here, failed to control black spot and "melanose". This failure might have been due to the inefficiency of the chemical, slow translocation, or to the method of application.

c. Discussion and Conclusions

It is accepted that black spot is less severe in young, vigorous growing, healthy trees than in old debilitated trees.

Leaf analysis[*] carried out on one year old leaves, picked from poor and healthy trees, indicated a deficiency in boron and potassium in the trees in poor condition and on which black spot was very severe. By supplying boron and potassium to the tree it might be possible to influence the susceptibility of the plant directly or indirectly.

In the case of treatments A and B, leaves showing typical zinc deficiency were tagged in June 1960. These leaves were examined occasionally and in August 1961, zinc deficiency symptoms disappeared almost completely on these trees. It is therefore not unreasonable to assume that some of the elements in treatments A and B reached the leaves. It made no difference to the incidence of disease, however. On the other hand, a tree will only respond to the application of an element in which there is a deficiency. The trees on which the experiment was carried out showed no deficiency in boron but a slight deficiency in potassium before the experiment commenced, according to leaf analysis.

This experiment was also repeated on old Valencia orange trees, but the results were also negative.

According to Rudd-Jones (1956) the translocation of sulphaguanadine is slow in some plants. It is conceivable that the negative results obtained with the sulphonamides were due to poor translocation but no evidence is available to substantiate this.

The distribution of chemicals is poor when they are introduced into a tree through boreholes (Stoddard & Dimond 1949). Zentmeyer and Horsfall, (1943) in injecting chemicals into elms for chemotherapeutic purposes against Dutch elm disease, observed that distribution tended to remain localized with certain chemicals, but with others such as boron, redistribution occurred extensively.

[*] Leaf analysis was carried out by the Technical Department, Zebediela Estates.

One obstacle in the use of chemotherapeutants is their brief period of activity inside the plant (Brian 1956). As the disease occurs mostly on the fruit, one would expect that a direct spray application of a candidate chemotherapeutant to the fruit would stand a better chance of succeeding than when applications are made to the roots and stems.

3. PRELIMINARY EXPERIMENTS WITH FOLIAR APPLICATION OF CHEMOTHERAPEUTANTS

a. Materials

Acti-dione (ferrated): Containing 57% active ingredients: Cycloheximide 2.26% w/w (Beta- 2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl glutarimide.
Ferrous sulphate 54.74%.

Acti-dione Concentrate: containing 4% w/w Cycloheximide (Beta- 2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl) glutarimide.

Alboleum: an emulsified light hydrocarbon oil, with an unsulphonated residue of 94%.

Ciba 113: An experimental systemic fungicide.

Dimecron: A systemic insecticide with fungicidal properties at high concentrations. Contains 2-chloro-2-diethylcar-bamoyl-methylvinyl-dimethyl phosphate as active material.

Ferrous sulphate: $Fe\ SO_4 . 7\ H_2O$

Gibrel: Contains 0.88% Potassium gibberellate

Griseofulvin: A wettable powder containing 50% active material.

8-Hydroxyquinoline benzoate.

Magnesium sulphate: $Mg\ SO_4 . 7\ H_2O$

Nickel Chloride: An experimental fungicide

P.P 645: An experimental fungicide.

Perenox: A wettable powder, containing cuprous oxide (50% metallic Cu).

Phenyl mercuric chloride: containing 40% active material.

Quinolate-20: A copper fungicide with eradicant action, containing 20% copper 8-hydroxyquinoleate

Sankyo Mercuric Bordeaux: containing 18% basic copper sulphate and 0.71% phenyl mercuric chloride.

Salicylic Acid .

Urea: Commercial Urea with low biuret content.

b. Treatments and Results

It was decided to carry out preliminary experiments in which various materials could be screened. As this was a new approach to the black spot problem, considerable information was necessary on materials, concentrations and times of application.

The first experiment consisted of 8 treatments with 4 replications of single tree plots. Treatments A, B, C, D and E (Table 56) were applied with a low volume applicator at approximately 1½ pints per tree per application. Treatments F and G were applied with a conventional high volume sprayer at 8 gallons per tree.

The dates of spraying were: 10/10/60, 9/11/60, 22/12/60 and 26/1/61. The fruits were harvested on 11th and 12th September 1961.

TABLE 56

Summary of treatments and results of a preliminary experiment to evaluate various chemotherapeutants.

Treatments			Mean % fruit with Black Spot Lesions.
No.	Materials	Rate of applications	
A	Grisiofulvin	1,000 p.p.m.	67.1
B	Grisiofulvin plus Perenox	200 p.p.m. 7,000 p.p.m. (Cu)	38.6
C	Gibrel	50 p.p.m.	50.2
D	Acti-dione	150 p.p.m.	41.0
E	Acti-dione	2 p.p.m.	49.7
F	Nickel chloride	1,000 p.p.m.	60.9
G	Copper quinolate	2,000 p.p.m.	57.4
H	Unsprayed control	-	82.1

(p=0.05) 18.9

All the sprayed treatments were significantly better than the unsprayed control except treatment A. This was rather unexpected.

Other experimental data indicated that considerable infection occurred during January and to a lesser extent in February. Results with some of the materials could probably have been better if a spray had been applied during February or March.

The following exploratory experiment was carried out to see what the effect on black spot is when a spray is applied after the main infection period. The experiment was laid out on 8 year old Valencia orange trees, with 3 single tree plot replications per treatment.

Only one spray application was carried out (15th March 1961). The experimental data is summarized below.

TABLE 57

Summary of treatments and results of a preliminary experiment to evaluate chemicals with chemotherapeutic action for the control of black spot, after the main infection period.

Treatments			% Fruit with Black Spot lesions
No.	Material	Rate of application	
A	8-Hydroxyquinolinebenzoate	1.0%	34.2
B	8-Hydroxyquinolinebenzoate	0.1%	47.1
C	Actidione plus Alboleum	0.01% 10.0%	37.8
D	Griseofulvin, plus Alboleum	0.1% 10.0%	49.5
E	Mercuric Bordeaux, plus Alboleum	0.025% (Cu) 0.001% (Hg) 1.0%)	29.7
F	Untreated control	-	59.2

(p=0.05) 18.6

Treatment E was applied at approximately 5 gallons per tree with a high volume sprayer. Treatments A, B, C and D were applied with a low volume applicator at approximately 3 pints per tree. Treatments A, C and E gave significantly better results than the unsprayed control. It was shown before, that oil retards the development of symptoms and Alboleum could therefore have contributed greatly towards the results in treatments C, D and E. 8-Hydroxyquinoline benzoate showed promise in retarding symptom development.

During the 1961-1962 season another preliminary experiment was carried out to evaluate chemotherapeutants for the control of black spot. This experiment was conducted in a 12 year old Valencia orchard.

Single tree plots were used, replicated 5 times. All sprays were applied with a conventional high volume applicator at 8 gallons per tree. Dates of application were: 10/11/61, 15/12/61 and 1/2/62.

The fruit was harvested and examined during the first week of September 1962.

TABLE 58

Summary of treatments and results of a preliminary experiment to evaluate chemicals with chemotherapeutic action.

Treatment			% Fruit with black spot lesions	Inverse arc sine transformation
No.	Material	Rate of application		
A	($Fe SO_4 \cdot 7H_2O$	0.05 %		
	($Mg SO_4 \cdot 7H_2O$	0.05 %		
	(Urea	0.50 %	23.1	28.60
	(Salicylic acid	0.05 %		
B	Ciba 113	0.10 %	19.9	26.45
C	Dimecron	0.20 %	26.2	30.65
D	8-Hydroxyquinoline benzoate	0.10 %	12.9	18.87
E	P.P. 645	0.25 %	6.1	13.15
F	Perenox (Metallic Cu)	0.10 %	1.8	7.55
G	Perenox, plus	0.075%	0.4	2.47
	Phenyl mercuric chloride	0.01 %		
H	Untreated control	-	18.2	24.90
			(p=0.05)	8.54

Treatments E, F and G were significantly superior to the unsprayed control treatment. Treatment G was significantly better than all the other treatments except F.

c. Discussion and Conclusions

The modes of action of chemotherapeutic chemicals against fungal disease may be numerous. The chemical may kill or inhibit the causal organism within the host. According to Stoddard and Dimond (1949), it is probable that 8-quinolinol benzoate kills the causal organism of Dutch elm disease (Ceratostomella ulmi) within the plant.

A chemotherapeutant may also inactivate or antidote toxins produced by the pathogen. It is known that plant pathogens produce toxic substances in diseased plants which are primary factors in pathogenesis (Gäumann 1954). Howard (1941) demonstrated this in the case of bleeding canker disease of maples. He suggested that chemicals which react with the toxin and antidote it, should be effective in combating the disease.

A chemical may prevent toxin formation by the pathogen but may otherwise not effect the fungus adversely (Stoddard and Dimond 1949).

Finally, there is the possibility that the host itself may become more resistant to disease by a chemical treatment. (Wain 1959).

In a chemotherapeutic study of the control of black spot, considerable basic research should still be done on the cause of symptom development. Experiments on time of application of 8-hydroxyquinoline benzoate, copper-mercury compounds and others, should be conducted. The latent period i.e. during February, March and April and even later seems to lend itself to chemotherapeutic treatment.

I. POST-HARVEST CONTROL OF BLACK SPOT

The major portion of Letaba's crop is exported through Capetown and Durban harbours. It may take more than a week to reach the ports by rail from Letaba. It often happened that considerable losses occurred due to development of black spot on the fruit in transit, especially during warm spells. These lesions originate from latent infections in the fruit rind. Lesion development can be prevented by cool temperatures, but cool railway trucks were not available during the period of investigation.

Christ (1959) showed that the development of black spot was suppressed after fruits had been dipped in a 5% sodium carbonate solution. Calavan (unpublished report 1959) indicated that black spot was inhibited by oil and waxes. He made the paradoxical conclusion that "at present there is no promise that fungicidal treatments will control black spot in warm infected fruits during the post-harvest period".

1. SCREENING TRIALS

Evaluation of chemicals for the control of black spot after harvesting commenced in 1959. The first step was to test a wide range of materials. Since these experiments had to be conducted during the busy harvesting season, methods of evaluation were simplified for the screening procedure. One hundred unsprayed ripe Valencia oranges without lesions were used per treatment. The experimental fruits were picked from the same trees on each occasion. The fruits were well mixed and afterwards divided into lots of 100 fruits before the treatments were applied. The fruits were stored in wooden boxes at room temperature after the different treatments had been applied. Records were taken at various intervals. The fruits in each treatment were examined individually and classified into three categories: 'clean' - less than five spots per fruit and more than five spots per fruit.

According to the results of these screening tests, the following treatments were ineffective for post-harvest control of black spot:-

Phenyl mercuric chloride (0.01% and 0.05%); Captan (0.5% and 1.0%); Zineb (0.2% and 0.5%); Maneb (0.5%); Cyprex (0.2% and 0.4%); Thiram (0.5%); Dichlone (0.1% and 0.2%); Copper sulphate (0.1% and 0.4%); Bordeaux mixture (4:2:100); Hydrated lime (1.0%); Urea (1.0%); Potassium permanganate (0.5%); Copper oxychloride (1.0%); Sodium carbonate (5% and 10%); Sodium sorbate (2.0%); 8-Hydroxyquinoline sulphate (0.5%); 8-Hydroxyquinoline benzoate (0.5%); Actidione ferrated (50 p.p.m. and 100 p.p.m.); Griseofulvin (1,000 p.p.m. and 3,000 p.p.m.); Pimaricin (1,000 p.p.m.) and Malonic acid (0.1%).

Two materials, viz. Malachite Green, and Alboleum afforded control and were investigated further.

2. EVALUATION OF SODIUM CARBONATE, MALACHITE GREEN AND ALBOLEUM.

On 21st July 1960, mature Valencia oranges were picked from unsprayed old trees. The fruits were mixed into 12 lots of 100 fruits each. There were 4 different treatments with 3 replications of 100 fruits per treatment. The fruits were stored in wooden boxes at 22°C to 25°C.

TABLE 59

Table showing the various treatments and percentages of fruit without black spot lesions, 9 days after the treatments had been applied.

Treatment		Time of Immersion	% Fruit without B. spot	Arc Sine Transformation
Material	Concentration			
Na_2CO_3	5%	15 minutes	52.5	46.4
Malachite Green	1%	5 minutes	76.1	60.7
Alboleum	5%	1 minute	93.8	75.6
Untreated control (Tap water)	-	5 minutes	52.1	46.2
			(p=0.05)	17.8

No differences were found between sodium carbonate and untreated control. The results of Christ (1959) were therefore not confirmed. Malachite Green stained the fruits badly. Alboleum gave promising results and an evaluation of higher concentrations was desirable.

3. EVALUATION OF ALBOLEUM

Small consignments of Valencia oranges treated with 0.5% Albolcum were sent to Capetown, but these treatments made little difference to the development of black spot. In view of these results and experimental data, it was decided to investigate the effect of higher concentrations of Albolcum.

The experimental fruits were picked from unsprayed, old Valencia orange trees on 9th August 1961. These fruits showed no symptoms at that stage. Each treatment consisted of 50 fruits, replicated four times.

The different treatments were as follows:

1. Untreated control (dipped in tap water for 5 mins.)
2. Albolcum 10%, dipped for 1 minute and washed in 0.2% Agral 90 for 1 minute.
3. Albolcum 10% dipped for 1 minute.
4. Albolcum 10% dipped for 5 minutes.
5. Albolcum 2% dipped for 1 minute.
6. Albolcum 2% dipped for 5 minutes.

After dipping, the fruits were left in the sun for 15 minutes to dry and afterwards stored in wooden boxes in a glass house. Direct sunlight was kept out by covering the glass house with hessian. The temperatures in the house during the storage period varied between 17°C and 37°C. The fruits were examined 8, 11 and 18 days after treatment. The results (percentage of fruit which developed black spot lesions) are presented graphically in Figure 6.

It is remarkable that treatment 4 (Albolcum 10% dipped for 5 minutes) reduced the disease incidence to 3% after storage of 11 days at most favourable temperatures for black spot development. There was little difference between treatments 3 and 4. Treatment 6 (dipped for 5 minutes in 2% Albolcum) was promising, especially because it is more practical and cheaper than the other oil treatments.

Fruits which were treated with Albolcum had an "oily" appearance. It is known (Laville-1960) that oil penetrates orange rind tissues. If the control of black spot by oil is due to some action of oil inside the tissue, removal of excess oil on the fruit surface should not make much difference. In treatment 2 (Figure 6) it is shown that the removal of excess oil resulted in very poor control.

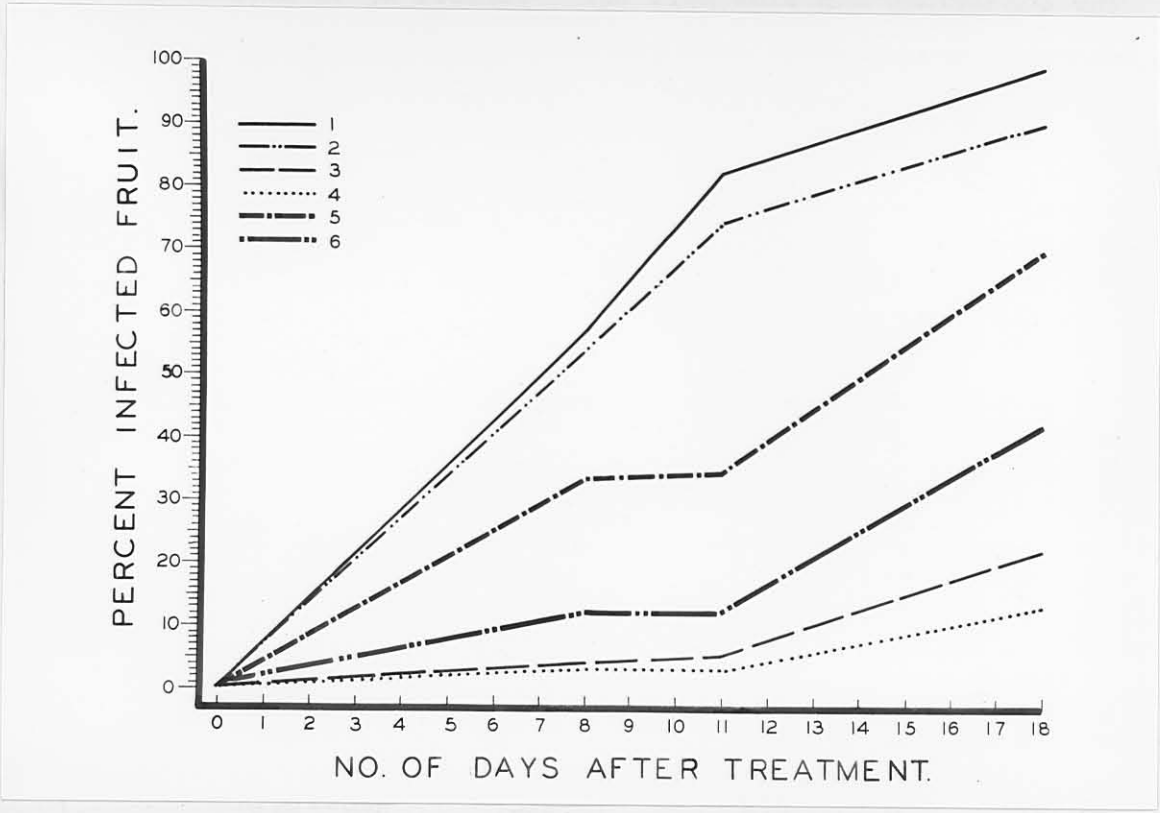

FIG. 6. Graphic representation of the effect of Alboleum on the post-harvest control of black spot.

1. Untreated control.
2. Alboleum 10% dipped for 1 minute and washed in 0.2% Agral 90 for 1 minute.
3. Alboleum 10% dipped for 1 minute.
4. Alboleum 10% dipped for 5 minutes.
5. Alboleum 20% dipped for 1 minute.
6. Alboleum 2% dipped for 5 minutes.

4. MINERAL, VEGETABLE AND ANIMAL OILS

The previous experiment showed that dipping in Alboleum (which is a mineral oil) inhibited black spot development almost completely. There may be two major objections against the use of a mineral oil.

1. It may be undesirable for health reasons where the orange peel is used for human consumption.
2. The fruits become insipid.

It was decided to evaluate some mineral, vegetable and animal oils. This experiment was conducted at Letaba Estates in 1962 in collaboration with Mr. H.T. Brodrick ≠. Unsprayed ripe Valencia oranges were used. Each treatment consisted of three lots of 50 fruits. The oils were all emulsified and diluted to a 5% emulsion. The fruits were all dipped for 3 minutes in the appropriate emulsions and afterwards stored in wooden boxes at room temperature. (26° - 30° C).

TABLE 60

Summary of effect of different oils on the post-harvest control of black spot.

Treatment	Rate of Application	Mean % Fruit with symptoms 12 days after treatment.	Arc Sine transformation
Groundnut oil	5.0%	49.3	44.7
Sunflower oil	5.0%	59.3	50.4
Seal oil	5.0%	50.6	45.4
Alboleum	5.0%	4.7	12.4
JF 1383 (derivative of Alboleum)	5.0%	4.0	10.9
Untreated control		42.6	40.5
		(p=0.05)	13.8
		(p=0.01)	19.6

It is obvious from the above results that the two mineral oils only controlled black spot. These fruits were insipid after 3 weeks.

The fruits in the other treatments retained their flavour (except Seal Oil) but the incidence of black spot was high.

≠ Mr. H.T. Brodrick, Plant pathologist, African Explosives and Chemical Industries Limited.

5. DISCUSSION

When the respiration process is stopped, fruit will ferment and undesirable products will be formed like alcohols, aldehydes and other toxic materials. The sugars, acids, vitamins and flavour are also destructed (Kalmar 1960). Presumably, the oil also interferes with the respiration of *G. citricarpa*. The fungus is not killed by the oil, because it was readily isolated from the fruit rinds long after the oil had been applied. Laville (1960) reported that mineral oils penetrate to the inter-cellular spaces of orange rind tissues, but vegetable oils enter the inter- and intra-cellularly. Whether this explains the difference in effectiveness between the mineral oils and oils from other origins, may become clear after extensive studies on the host- parasite relationship of the black spot disease.

IV. SUMMARY AND CONCLUSIONS

Black spot was observed for the first time at Letaba Estates in 1946 and reached epidemic proportions eight years later.

The disease inflicted serious losses. At one stage the annual losses were estimated at R200,000 at Letaba Estates.

Other workers showed that dead leaves, infected fruit and green leaves with lesions are sources of inoculum. Investigations showed that dead twigs, on the tree or on the orchard "floor" can be an important source of inoculum.

Strong evidence was obtained that ascospores are the most important source of infection. Perithecia on dead leaves ripened rapidly during the summer months, but slowly during winter. Relatively few ripe perithecia were found from July to the end of October. From October onwards the perithecia ripened rapidly.

The seasonal discharge of ascospores were ascertained with a Hirst spore trap which operated in the citrus orchards. Relatively few ascospores were trapped before November, but large numbers were recorded from November onwards.

Under laboratory conditions ascospores were ejected to a distance of 1.2 cm. Low temperatures did not arrest the discharge of ascospores.

Ascospores were only ejected when the perithecia were wetted. In the absence of rain ascospores were never trapped. Although water is an essential requirement for ascospore discharge, spores were not trapped with every rain. The amount of rain did not seem to play an important role in spore liberations. Spores were never trapped during flood irrigations.

Numerous malformed ascospores were observed towards the end of a discharge period.

Ascospores germinated after approximately 15 hours and usually formed appressoria, but not always. Under laboratory conditions ascospores germinated and penetrated citrus leaves through stomata. Mycelium of *P. citricarpa* also penetrated through stomata under artificial conditions. Valencia orange fruits were successfully inoculated with pycnidiospores and ascospores.

Predictions of infection periods were made by examining perithecium development on dead citrus leaves. These predictions were highly successful during the 1961-1962 season.

The infection period lasted from the beginning of November until February. The period from blossoming to end-October was relatively infection-free. The fruit apparently became resistant to infection after February. The incidence of black spot increased rapidly when the fruits reached peack maturity and the temperature increased.

The incidence of black spot was higher in the upper portions of the trees than the lower portions. The incidence of black spot was higher on those fruits that received the most sunlight.

A period of drought during the first four months before harvesting increased the severity of black spot.

Eradication of inoculum on the orchard "floor" gave negative results.

Short spray intervals did not always give better results than long intervals. The success of a spray programme depended on how close a spray had been applied before an infection period.

A few bound copper fungicides gave better results than Bordeaux mixture.

When copper fungicides were used, applications of calcium arsenate had no effect on early maturity of Valencia oranges. Copper fungicides also caused unsightly blemishes on the fruit.

Various organic fungicides were evaluated but only Zineb (Dithane Z-78) Maneb (Dithane M-22) and PMC (Phenyl mercury chloride) showed promise. Dithane Z-78, used in a programme gave satisfactory control, provided a good copper fungicide was used for the last spray application in December and January. The addition of spray-oil to Dithane Z-78 improved the overall control. In a combined spray with Dithane Z-78 calcium arsenate was as effective on early maturity as where calcium arsenate was used alone. Dithane Z-78 caused no blemishes.

Satisfactory control was achieved with a copper-sulphur dust.

Low volume spraying was successful in controlling black spot, when suitable materials were used.

Aerial spraying with copper-in-oil and applied with a fixed wing aircraft gave encouraging results.

When copper plus oil was applied 4½ days after infection only partial control was achieved.

Sprays with an emulsified mineral oil shortly before fruit ripening reduced the incidence of black spot at picking time.

Several fungicides with chemotherapeutic action were evaluated. The results obtained with chemotherapeutants were not nearly as good as those obtained with copper fungicides.

Mineral oils, used as dip treatments, gave almost complete control of post-harvest development of black spot. Fruit treated with mineral oils, often developed a bad flavour. Vegetable oils did not affect the flavour of the fruits, but did not control black spot.

At Letaba, symptoms which are popularly called "melanose", appear on the fruit of all citrus varieties. These symptoms are similar in description to melanose caused by Phomopsis citri. These particular symptoms at Letaba appeared nearly 16 years ago, at approximately the same time when black spot was observed. It was easier to isolate Phoma citricarpa from these lesions, than from any recognised black spot lesion. Isolations were made from "melanose" symptoms on fruit which came from a farm where black spot had never been observed in the past. P. citricarpa grew out of 85% of these isolations but no cultures of Phomopsis citri were obtained. Melanose symptoms were observed on fruit which had been inoculated with pycnidiospores and ascospores of G. citricarpa.

According to Wager (1953) melanose infections take place shortly after petal-drop and the fruit becomes resistant to infection within a few weeks after petal-drop. It was shown that "melanose" symptoms were caused by infection several months after petal-drop at Letaba.

It was several times observed that Navel orange trees in poor condition will show a high incidence of black spot symptoms, but very little "melanose". The healthy trees showed a high incidence of "melanose", but no black spot.

Although time did not permit a thorough study of the "melanose" phenomenon at Letaba, there is considerable evidence so far that "melanose" (or at least some of the symptoms) is caused by G.citricarpa.

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