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.

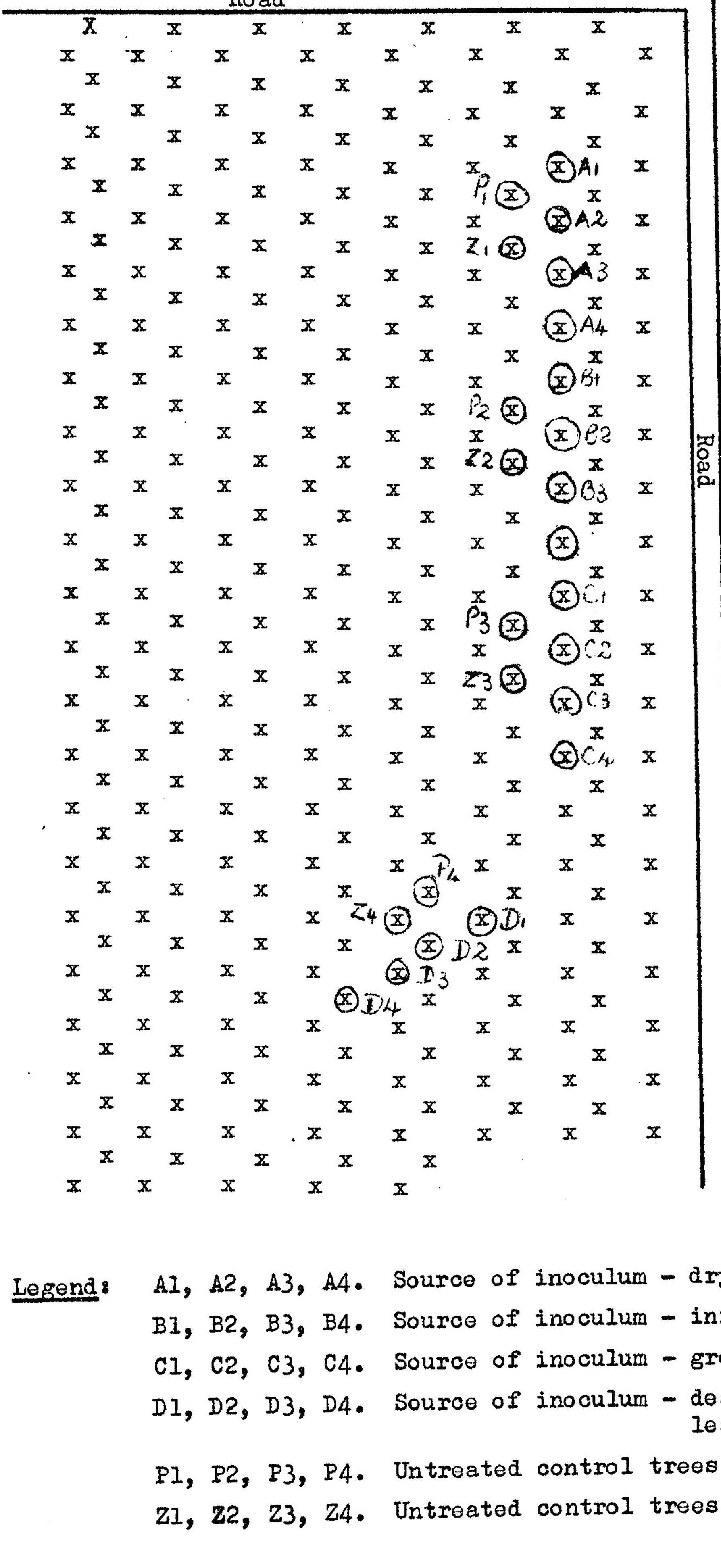
Before this experiment commenced all out-of-season fruit and as much dead wood as possible were removed from the experimental trees.

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).

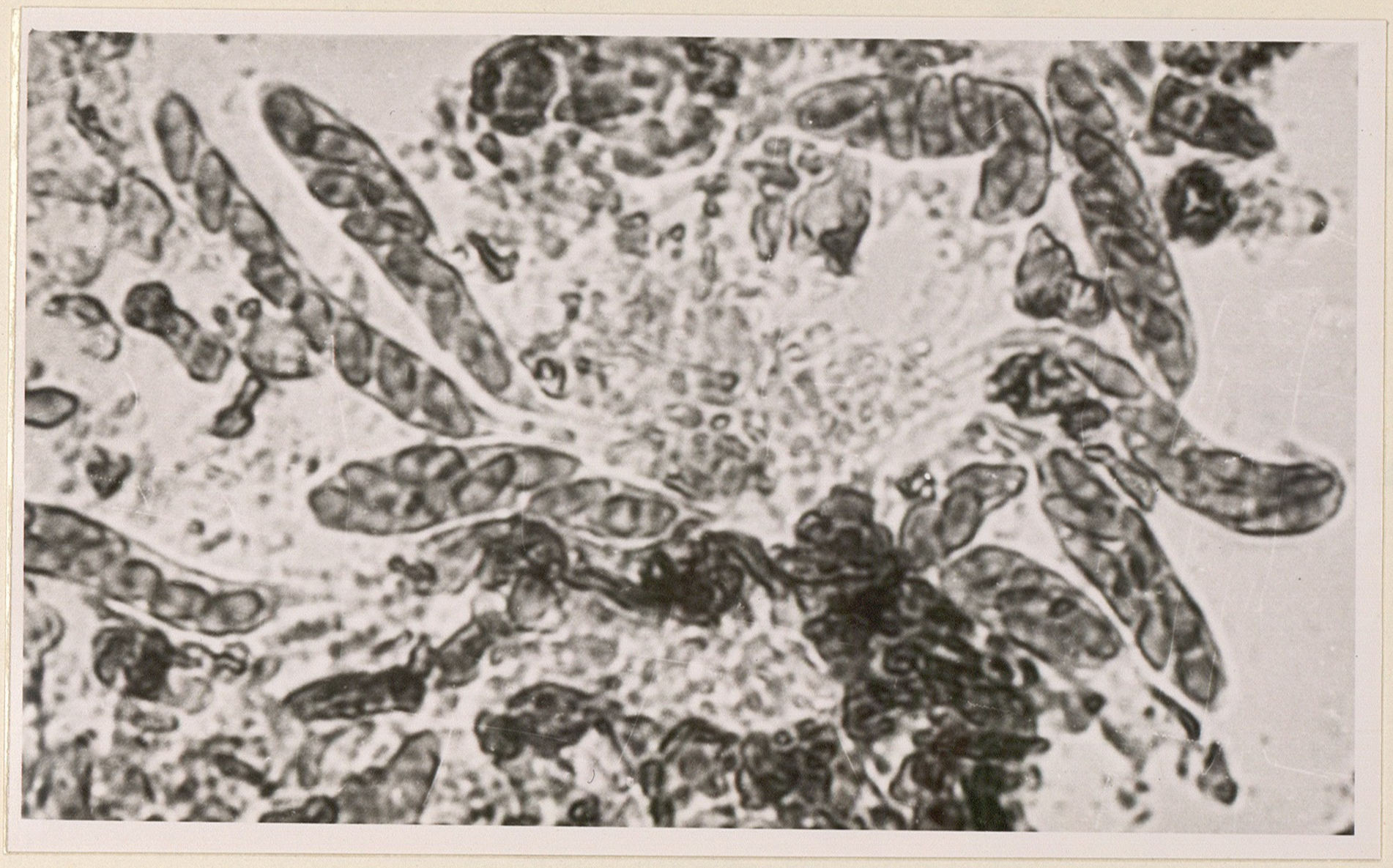


PLATE 2. Asci with ripe ascospores of G. citricarpa after pressing the perithecium between two glass-slides.

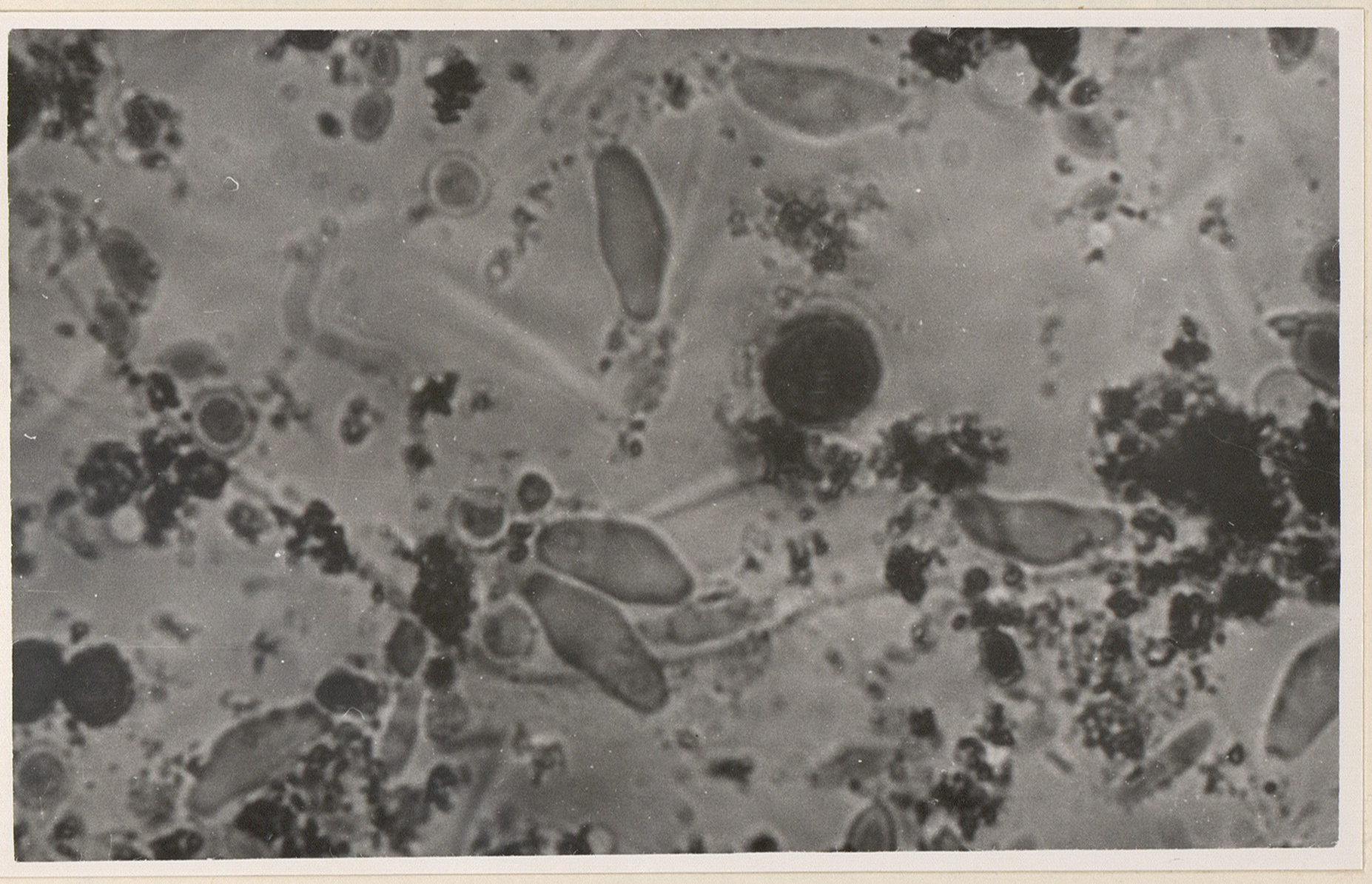


PLATE 3. Ascospores of G. citricarpa, caught with a Hirst spore trap on vaseline-coated glass-slides.

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.

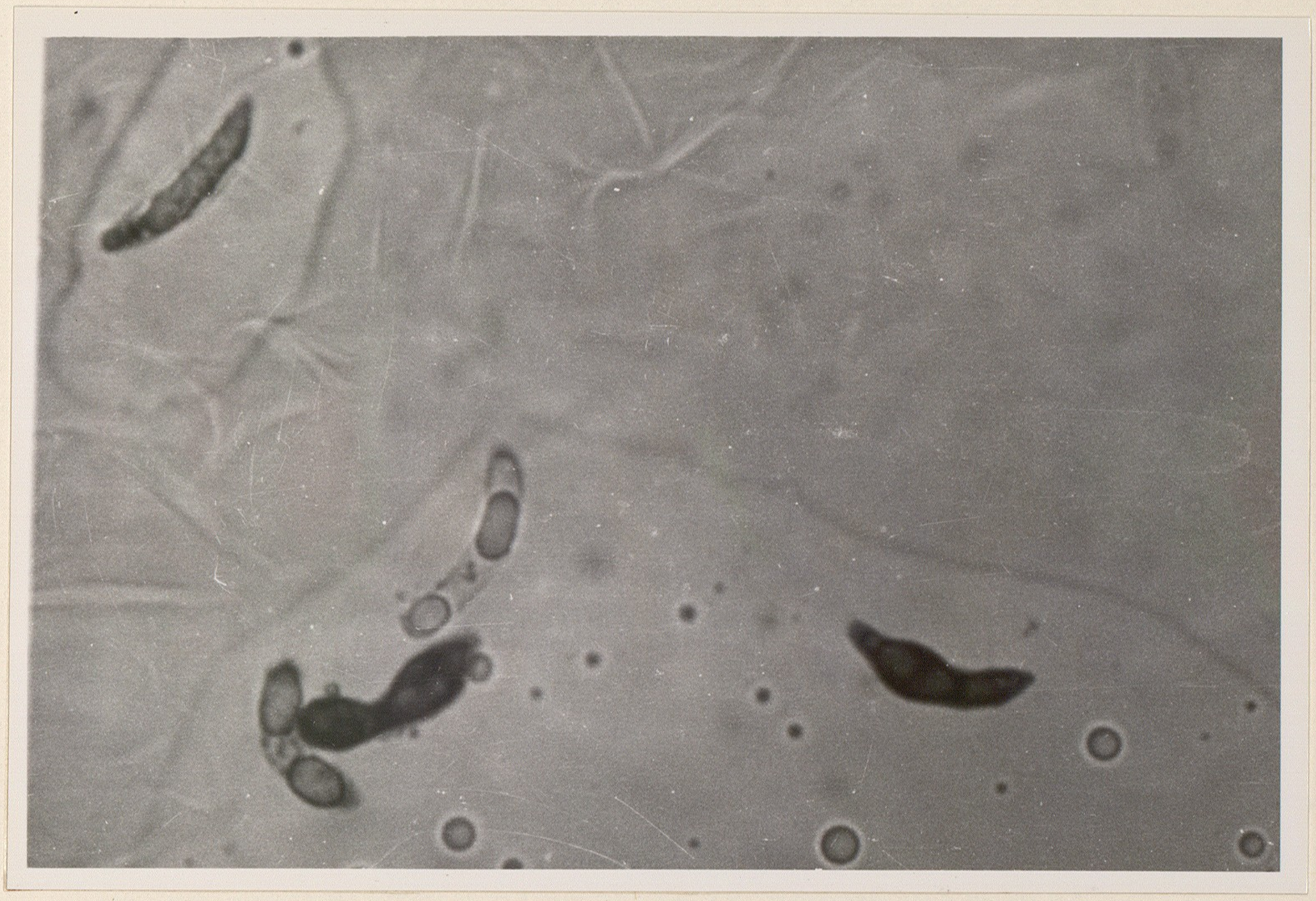


PLATE 4. Malformed ascospores of G. citricarpa.

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.

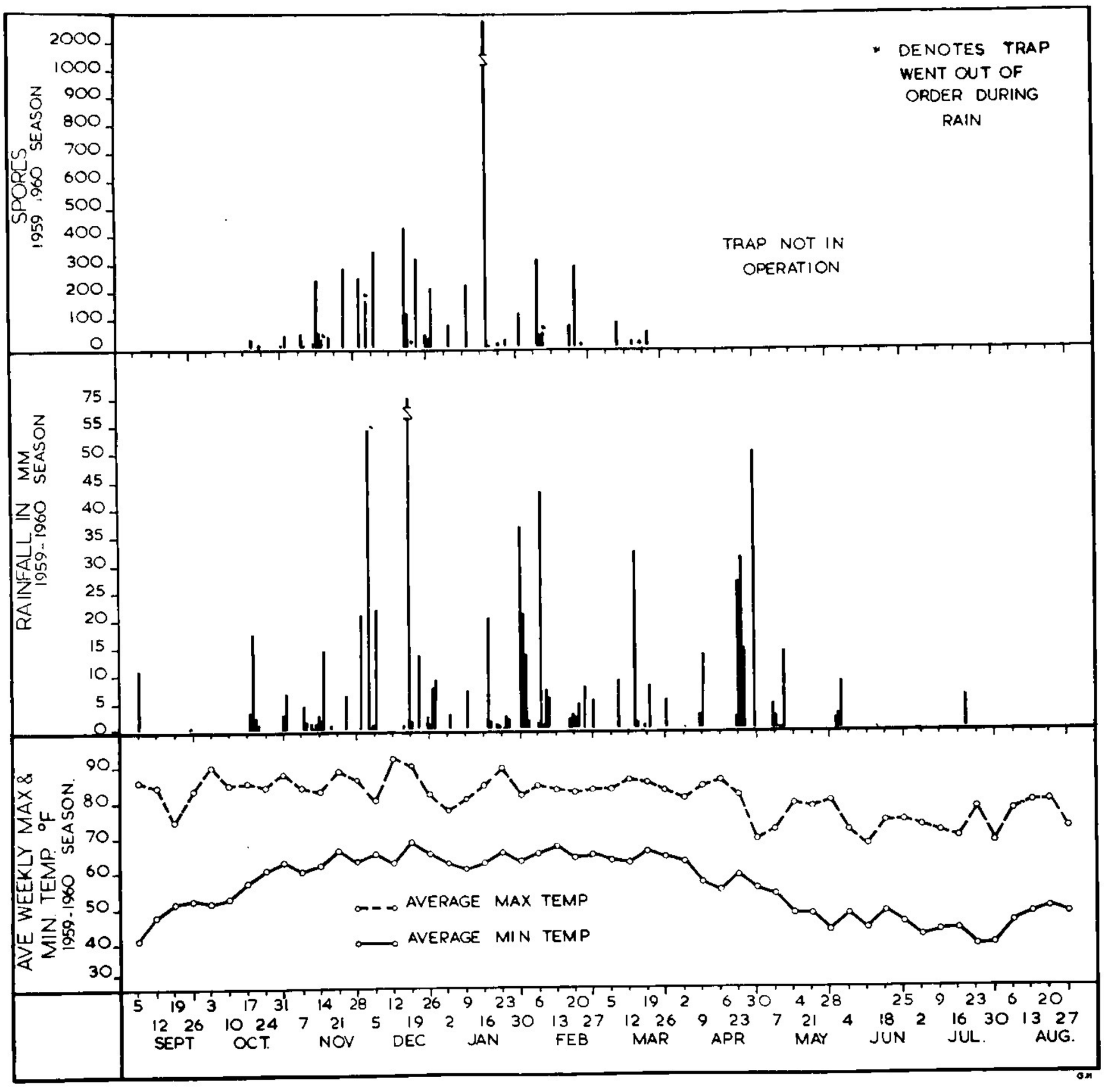


FIG. 1. Graphic representation of the ascospore discharges of G. citricarpa, rainfall and average weekly maximum and minimum temperatures during the 1959-1960 citrus season at Letaba Estates.

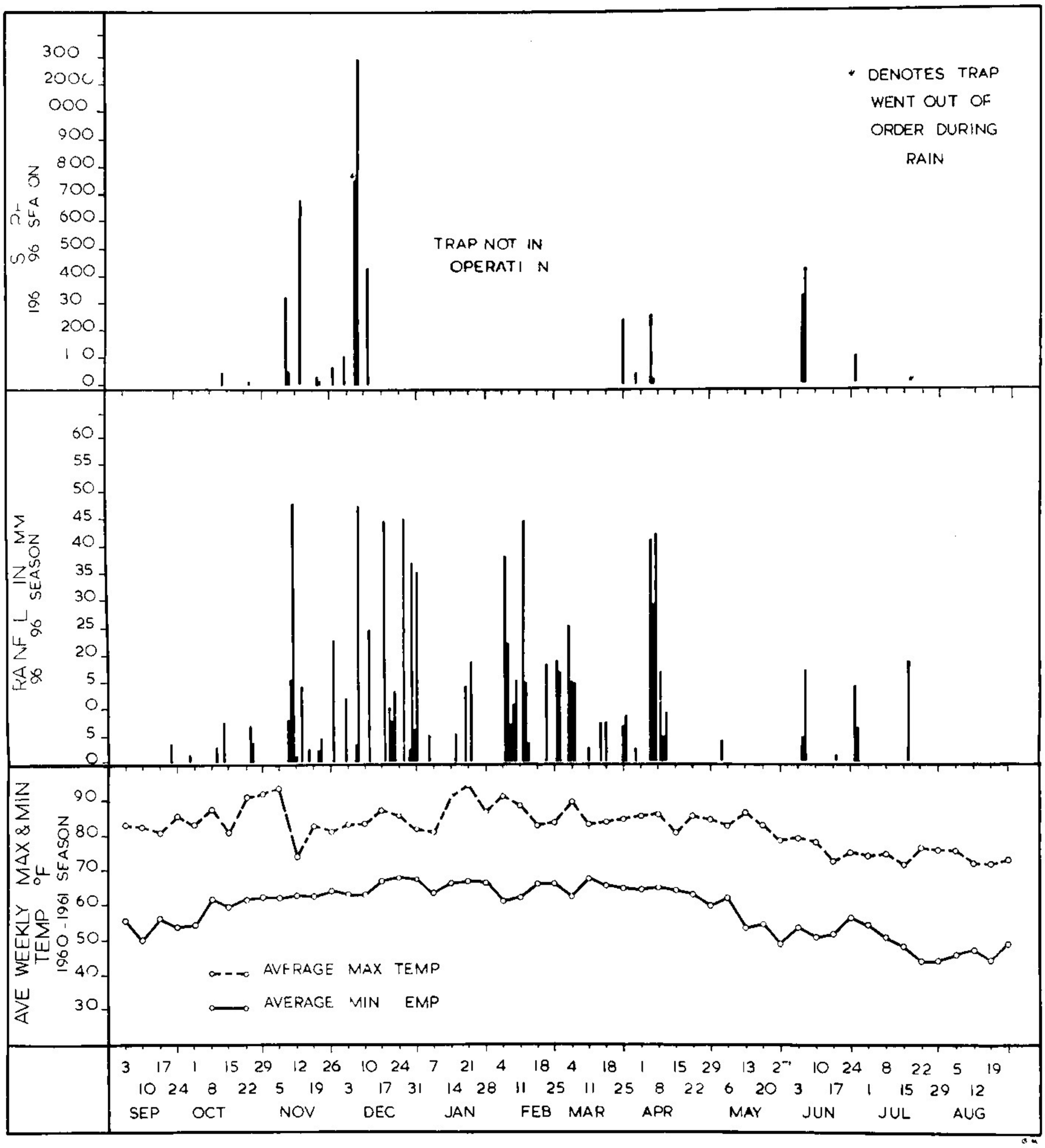


FIG. 2. Graphic representation of ascospore discharges of G. citricarpa, rainfall and average weekly maximum and minimum temperatures during the 1959-1960 citrus season at Letaba Estates.

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.

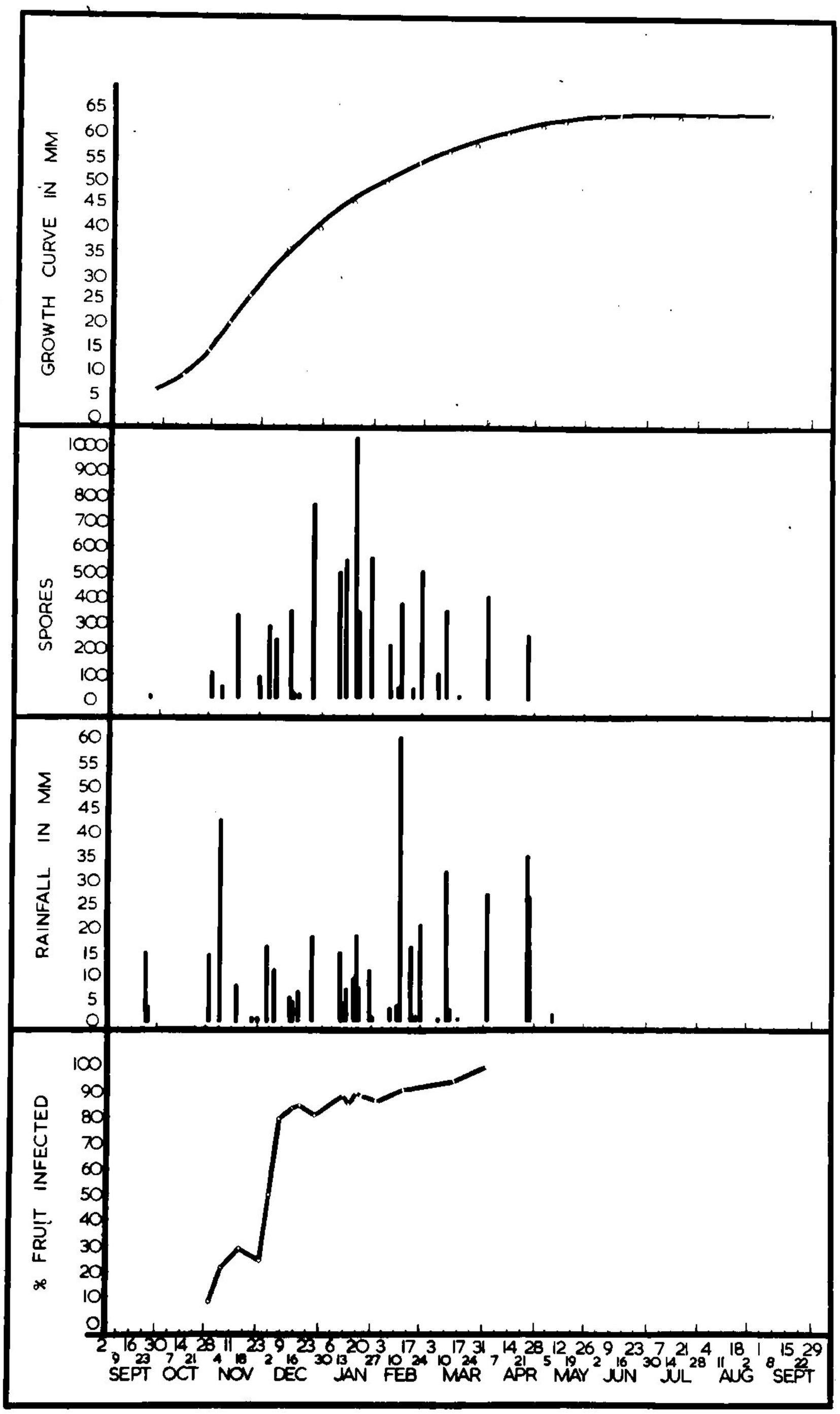


FIG. 3. Graphic representation of Valencia orange fruit growth, ascospores trapped, rainfall and progressive increase of disease incidence during the 1961-1962 citrus season at Letaba Estates.

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.

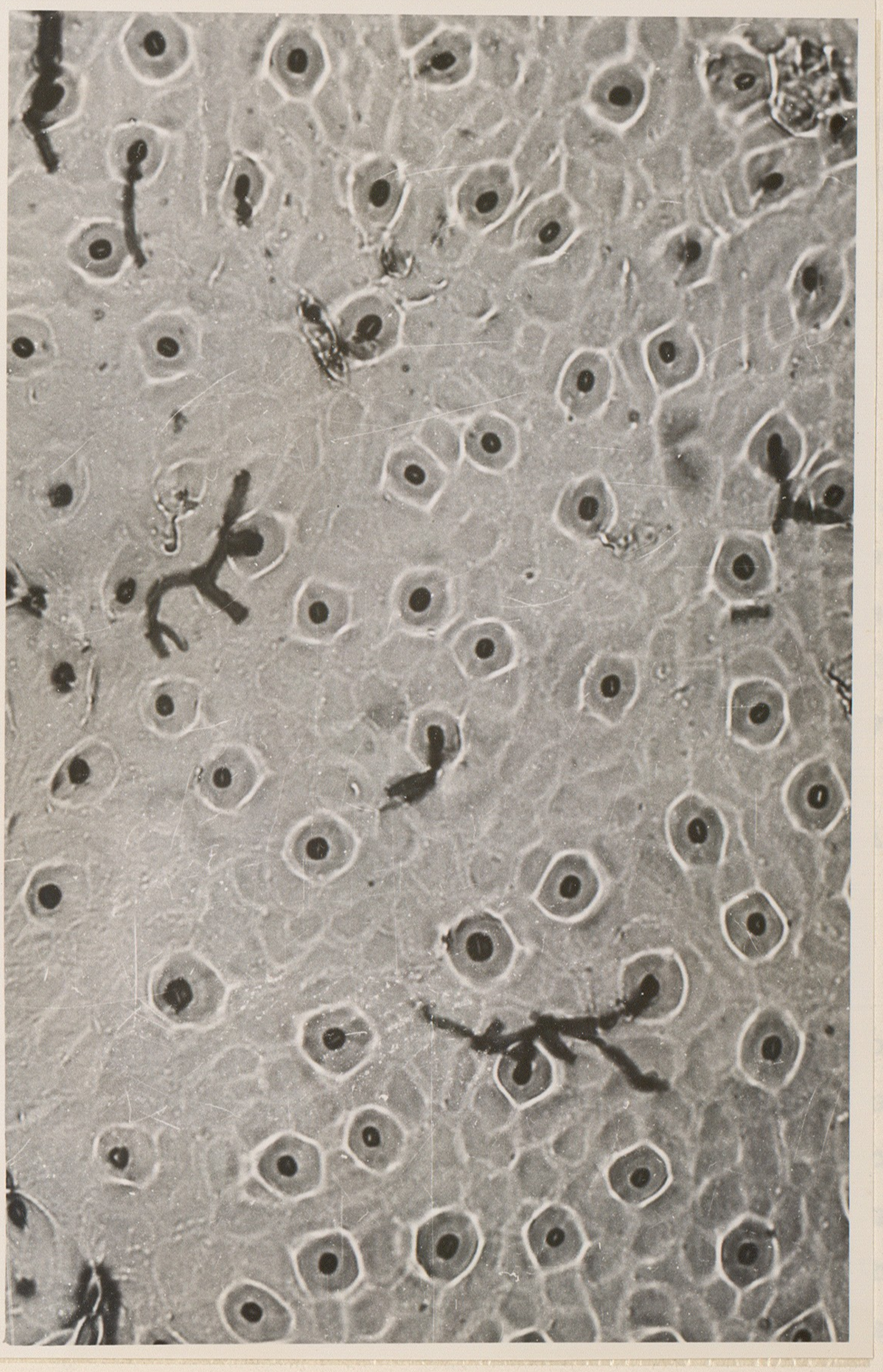


PLATE 5. Mycelium penetrating stomata of a citrus leaf.

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.

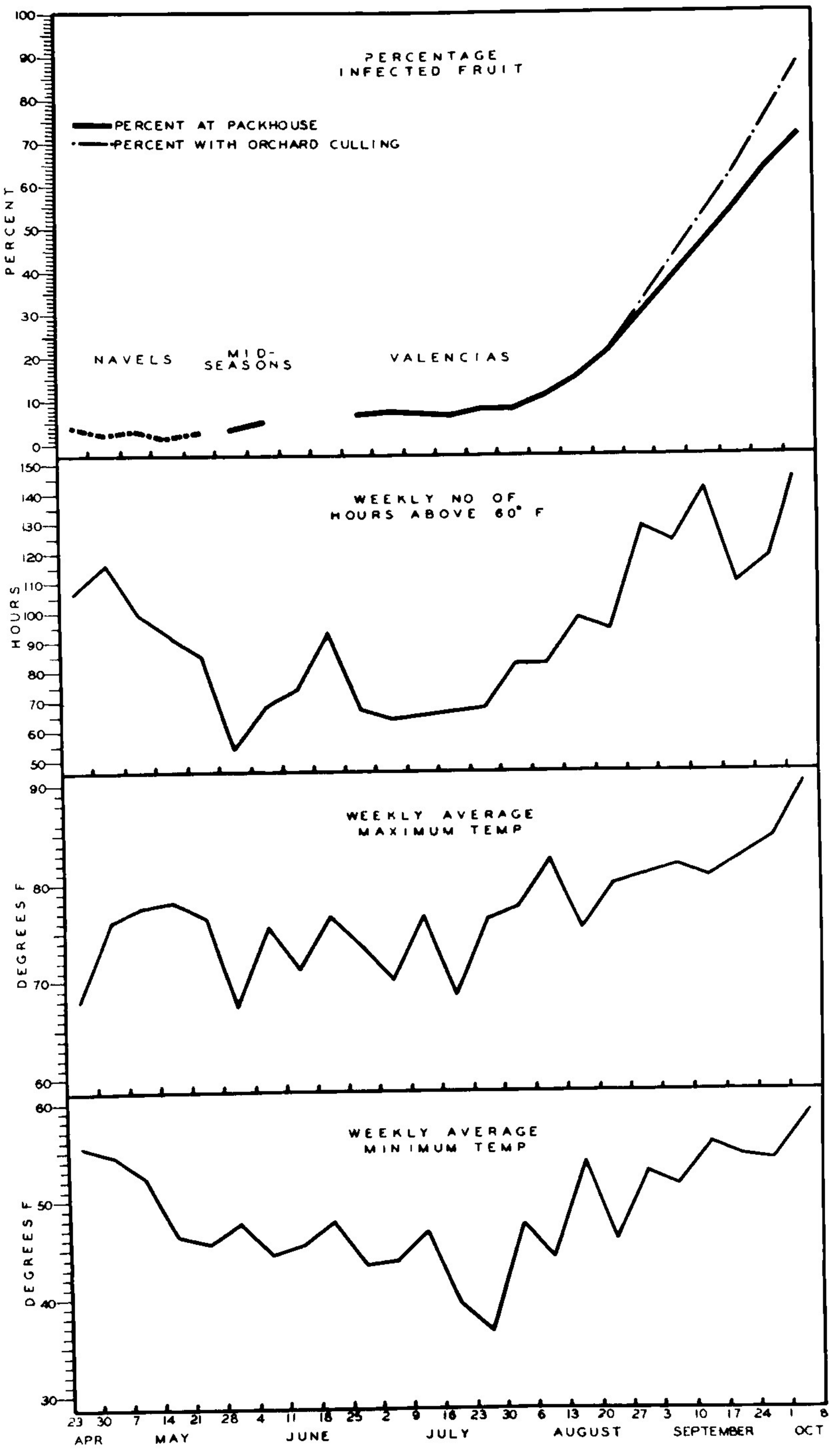


FIG. 4. Graphic representation of the increase of black spot during the 1959-1960 citrus picking season at Letaba Estates. The weekly no. of hours above 60°f as well as the average weekly maximum and minimum temperatures are given.

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.

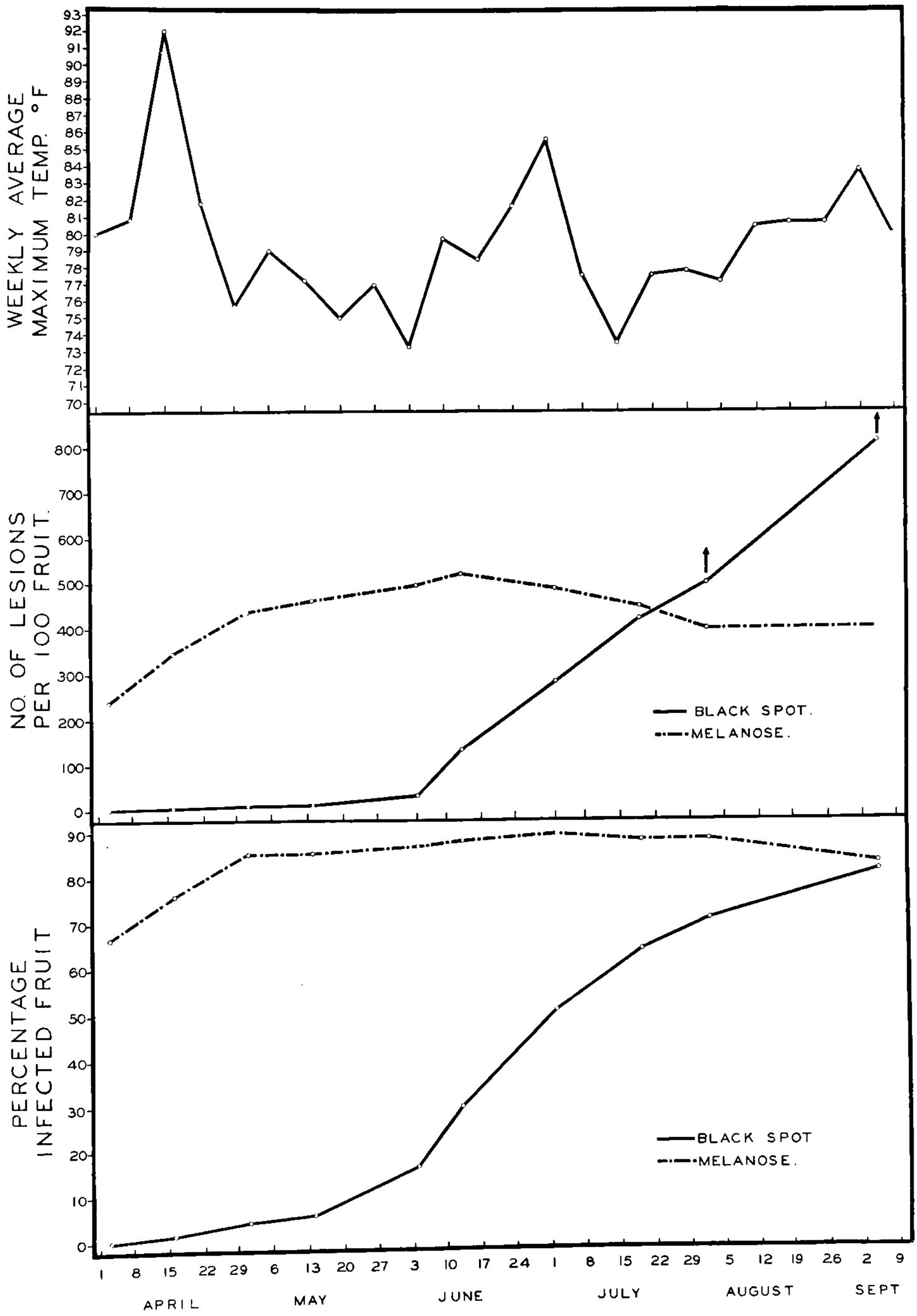


FIG. 5. Graphic representation of the weekly average maximum temperatures, the numbers of black spot and "melanose" lesions which developed during the season and the percentages of fruit with black spot and "melanose" lesions during the 1961-1962 citrus season at Letaba Estates.

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.