III. CONTROL OF THE DISEASE

A. QUARANTINE

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

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

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

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

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

B. ORCHARD SANITATION

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

1. PRUNING

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

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

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

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

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

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

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

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

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* Private communication

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

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

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

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

2. ERADICATION OF THE ASCIGEROUS STAGE

a. Introduction

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

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

b. Preliminary Investigation.

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

(i) Methods and Materials

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

A. Copper sulphate 0.5% dipped for 1 minute.
B. Phenyl mercuric chloride 0.1%, dipped for 1 min.
C. Phenyl mercuric chloride 0.5%, dipped for 1 min.
D. Phenyl mercuric chloride 1.0%, dipped for 1 min.
E. Phenyl mercuric chloride 0.5%, dipped for 5 min.
F. Cyprex (N-Dodecyl quanidine acetate) 0.I% dipped for 5 minutes.
G. Cyprex (N-Dodecyl quanidine acetate) 0.5% dipped for 5 minutes.
H. Sodium dinitro-O-cresylate, 0.5%, dipped for 1 min.
I. Sodium dinitro-O-cresylate, 0.5%, dipped for 5 min.
J. Ammonium sulphate 1.0%, dipped for 5 minutes.
K. Lime Sulphur 2.0%, dipped for 1 minute.
L. Untreated control.

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

(ii) Results:

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

TABLE 27

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

Treat-ment No.	Treatment	40 days after treatment			62 days after treatment		
		% leaves with Pe-rithecia	Assess-ment of inciden ce of pe rithecia	Ripe asco-spores obser-ved	% leaves with pe-rithecia	Assess-ment of inciden-ce of pe-rithecia	Ripe asco-spores ob-served
A	$CuSO_4$ 0.5% 1 min.	100	+++++	Yes	100	+++++	Yes
B	PMC 0.1% 1 min.	72	+++	Yes	90	++++	Yes
C	PMC 0.5% 1 min.	54	++	No	60	++	Yes
D	PMC 1.0% 1 min.	4	+	No	8	+	No
E	PMC 0.5% 5 min.	4	+	No	6	+	No
F	Cyprex0.1% 5 min.	100	+++++	Yes	100	+++++	Yes
G	Cyprex0.5% 5 min.	100	+++++	Yes	100	+++++	Yes
H	DNOC 0.5% 1 min.	6	+	No	8	+	Yes
I	DNOC 0.5% 5 min.	6	+	No	6	+	No
J	NH_4SO_4 1.0% 5 min.	100	+++++	Yes	100	+++++	Yes
K	L. Sulphur 2.0% 1 min.	100	+++++	Yes	100	+++++	Yes
L	Control	100	+++++	Yes	100	+++++	Yes

+ - very few perithecia present and scattered

++ - few perithecia present, scattered or in groups

+++ - perithecia present, but clearly less than control

++++ - abundant perithecia, but less than control

+++++ - abundant perithecia.

(iii) Conclusions.

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

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

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

c. Field Trial

(i) Methods and Materials

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

Treatment A

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

The trees received 3 sprays of Bordeaux mixture (2½:2:100) which were applied on 18th September, 28th October 1959 and 3rd January 1960.

Treatment B

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

The trees were not sprayed.

Treatment C

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

Treatment C(a)

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

Treatment D

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

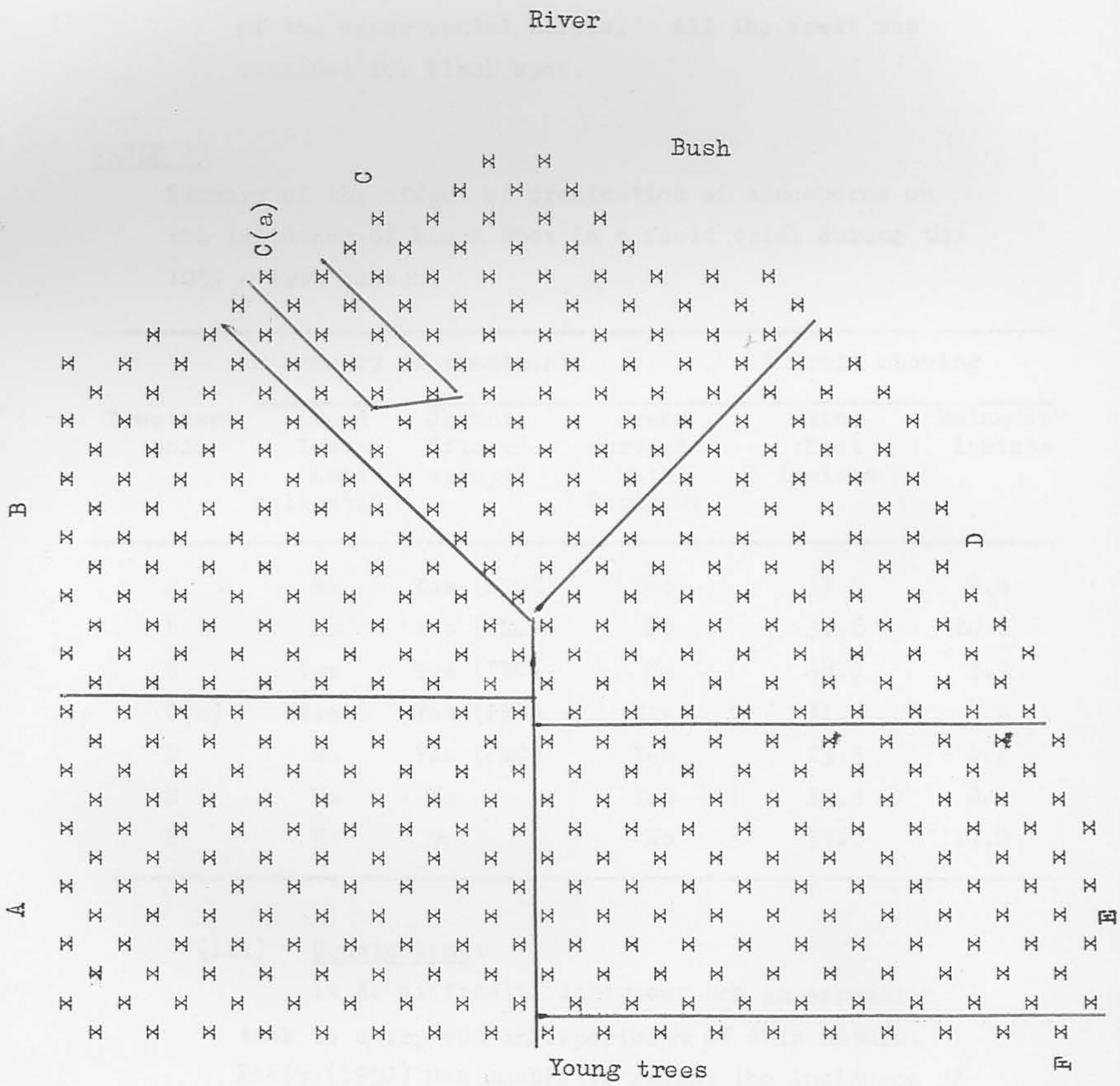
Treatment E

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

Treatment F

Unsprayed control.

Plan:



Legend:

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

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

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

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

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

" E. No floor treatment. Bordeaux on trees.

" F. Untreated control.

(ii) Results

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

TABLE 28

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

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

(iii) Conclusions:

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

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

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

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

Possible explanations for the negative results are:-

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

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

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

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

C. EVALUATION OF PROTECTIVE FUNGICIDES (HV)

1. INTRODUCTION

Mc Cleery (1939) indicated that the main infection period extends from blossoming until approximately 20 weeks later. This marked a major break-through on the black spot problem. Since then, control measures were aimed at protection of fruit against spore infection during the first 4 to 5 months after blossoming. Copper containing fungicides were superior to other fungicides (Kiely 1950; Wager 1952; Kotze 1961). Kiely (1950) found that "weak" home-made Bordeaux mixture (1½ lb commercial copper sulphate plus 1½ lb hydrated lime in 80 gallons water) gave satisfactory results under Australian conditions in a 3-spray programme. Stronger concentrations of Bordeaux mixture were also used, but both Kiely (1950) and Wager (1952) claimed undesirable effects on fruit quality. They also reported serious outbreaks of red scale (Aonidiella aurantii Maskell) after strong Bordeaux mixture sprays.

At the time when black spot research commenced at Letaba Estates, the official recommendation for black spot control by the Citrus and Sub-tropical Horticultural Research Station at Nelspruit, was 3 Bordeaux mixture (2½: 1¼: 100) applications, sprayed at 6 weekly intervals, and starting at petal-drop stage.

2. METHODS AND MATERIALS

a. Sites

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

b. Experimental layout

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

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

c. Spraying

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

d. Recording of results

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

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

e. Statistical Analysis of Results

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

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

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

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

f. Fungicides

(i) Preparation of Bordeaux mixture

Basically, the same method was followed for the preparation of Bordeaux mixture as recommended by Doidge (1910). "Standard Bordeaux" at Letaba consisted of 2½ lb commercial copper sulphate (snow) and 1½ - 2 lb high grade hydrated lime per 100 gallons of water. The copper sulphate was first dissolved in a small drum in enough water to bring the salt into solution. The copper sulphate solution was then poured into the big spray tank which was half filled with water. The lime was first mixed with water and while the spray tank was being filled after adding the copper sulphate, the lime suspension was slowly added. During the process the tank agitators were in operation to secure thorough mixing.

It was found that it is important to use fresh lime. It took about 2 lb of old lime to precipitate 2½ lb of copper sulphate, while 1 lb was sufficient when fresh lime was used.

The concentration of Bordeaux mixture will be given in an abbreviated form (e.g. Bordeaux 2½:1½: 100 will mean 2½ lb copper sulphate and 1½ lb lime, mixed in 100 gallons water).

(ii) List of fungicides:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

High grade hydrated spray lime

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

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

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

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

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

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

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

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

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

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

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

3. FIELD EXPERIMENTS

a. Copper fungicides

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

(i) Methods and Materials

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

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

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

(ii) Treatments and Results

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

TABLE 29

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

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

(iii) Conclusions

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

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

b. Organic fungicides

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

(i) Methods and Materials

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

(ii) Treatments and Results

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

TABLE 30

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

Treat-ment code	Treatments Material per 100 gallons water	Mean Actual % infected fruit	Inverse Arc sine trans-formation.
A	Bordeaux $2\frac{1}{2}$:2:100 + Alboleum $\frac{1}{2}$ gal.	13.35	21.18
B	Captan 2 lb	59.04	50.23
C	Captan 2 lb + Urea 5 lb	66.54	54.72
D	Captan 2 lb + PMC $\frac{1}{4}$ lb	41.35	39.62
E	Pomersol (TMTD) 2 lb	61.52	51.78
F	Dithane Z78 2 lb	35.36	35.95
G	Dithane M22 2 lb	24.93	29.83
H	Nirit 2 lb	86.96	69.10
I	Brestan 2 lb	74.16	60.40
J	Cyprex $1\frac{1}{2}$ lb	59.85	50.78
K	Phygon XL $\frac{3}{4}$ lb	68.73	56.12
L	Unsprayed Control	72.48	58.48
	Least significant difference	(p=0.o5)	8.83
		(p=0.01)	11.85

(iii) Conclusions

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

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

c. Organic fungicides (Second series)

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

(i) Methods and Materials

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

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

(ii) Treatments and results

TABLE 31

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

Treatment code	Material	Quantity per 100 gallons water	Actual % infected fruit	Arc sine transformation
A	0-3818-B	2 lb	59.1	50.8
B	Phaltan 50% WP	2 lb	68.6	56.0
C	Captan 50% WP plus PMC	1 lb ¼ lb	35.5	35.9
D	Crag	1½ pints	75.5	63.4
E	Hyamine	2 pints	51.9	46.6
F	Unsprayed control	-	75.4	61.8
	Least significant difference (p=0.05)			15.11

(iii) Conclusions

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

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

d. Copper fungicides plus oil and PMC

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

(i) Treatments and Results

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

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

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

TABLE 32

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

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

(ii) Conclusions

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

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

e. Evaluation of Dithane Z 78

(i) Methods and Materials

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

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

TABLE 33

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

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

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

(ii) Results

TABLE 34

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

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

TABLE 35

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

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

(iii) Conclusions

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

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

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

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

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

(i) Methods and Materials

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

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

(ii) Treatments and Results

TABLE 36

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

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

(iii) Conclusions

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

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

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

4. DISCUSSION

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

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

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

The copper fungicides give good results even when sprayed at comparatively long intervals, but there are also disadvantages, which make it desirable to find another type of fungicide to use against black spot.

The main disadvantage of copper fungicides are:

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

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

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

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

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

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

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

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

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

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

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

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

D. TIMING OF PROTECTIVE SPRAYS

1. INTRODUCTION

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

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

2. INVESTIGATIONS

a. Spray Intervals

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

(i) Method and Materials

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

(ii) Treatments and Results

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

TABLE 37

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

Code	Treatments:	Actual Dates of Spraying							Mean % fruit with Blackspot	Inverse arc-sine transformation
A	Bordeaux Mixture 2½:2:100	16/9/59	1/10/59	16/10/59	31/10/59	16/11/59	4/12/59	31/12/59	10.8	19.1
B	- do -	16/9/59	-	16/10/59	-	16/11/59	-	31/12/59	19.5	26.2
C	- do -	16/9/59	-	-	31/10/59	-	-	31/12/59	14.1	21.9
D	Bordeaux Mixture Alboleum ½ gallon	16/9/59	1/10/59	16/10/59	31/10/59	16/11/59	4/12/59	31/12/59	4.9	12.7
E	- do -	16/9/59	-	16/10/59	-	16/11/59	-	31/12/59	22.3	28.1
F	- do -	16/9/59	-	-	31/10/59	-	-	31/12/59	14.3	21.8
G	Captan 2 lb	16/9/59	1/10/59	16/10/59	31/10/59	16/11/59	4/12/59	31/12/59	58.6	50.5
H	- do -	16/9/59	-	16/10/59	-	16/11/59	-	31/12/59	65.1	54.7
I	Captan 1 lb + Dithane Z 78 1 lb	16/9/59	-	16/10/59	-	16/11/59	-	31/12/59	61.2	51.8
J	Unsprayed Control	-	-	-	-	-	-	-	66.9	55.4

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

(iii) Conclusions

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

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

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

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

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

b. Timing of Copper sprays and Dusting.

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

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

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

(i) Methods and Materials

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

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

(ii) Treatments and Results

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

TABLE 38

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

Treat-ment code	Material per 100 gallons water	Date of application	Mean % fruit infected	Arc-sine transformation
A	Perenox 1 lb 14 oz + Alboleum ½ gall.	16/11/60) after 29/11/60) long 23/12/60) rains 5/ 1/61)	0.5	4.0
B	Same as A	29/10/60) 5/12/60) 24/ 1/61)	1.5	6.7
C	Same as A	29/10/60) 16/11/60) 29/11/60)	8.1	16.2
D	Perenox 4 lb + Alboleum ½ gall.	3/11/60	10.0	18.3
E	Perenox/Sulphur dust	11/10/60) 29/10/60) 24/11/60) 23/12/60) 15/ 1/61) 31/ 1/61)	3.8	11.1
F	Untreated control	-	21.4	27.5
			(p=0.05)	3.75
			(p=0.01)	5.12

(iii) Conclusions

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

During the course of the experiment 12 ozs metallic copper and nearly 1½ lb sulphur were applied per tree in the dust treatment, compared with the normal orchard spraying (treatment B) where 4½ oz metallic copper and 1$\frac{1}{5}$ pint of Alboleum were used. The standard treatment was significantly better than dusting (p=0.05).

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

All treatments were superior to untreated control.

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

c. Spraying of young Navel trees

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

(i) Methods and Materials

The experiment was laid out on 8 year old Navel orange trees, using 3-tree plots, replicated 5 times per treatment. Perenox was used throughout at 1 lb 14 oz per 100 gallons water.

TABLE 39

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

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

(ii) Results

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

TABLE 40

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

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

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

Analysis of Variance:

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

(iii) Conclusions

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

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

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

d. Spraying of Young Valencia trees

(i) Treatments

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

(ii) Results

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

TABLE 41

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

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

Analysis of Variance

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

Analysis of Variance (contd)

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

Least significant differences:

Treatment means for all pickings: (i.e. 7.9 12.4, 31.7 and 34.9) (p=0.05) 4.35
(p=0.01) 6.09

Means of all treatments for each picking: (i.e. 10.2, 13.4 and 41.6) (p=0.05) 2.88
(p=0.01) 3.87

(iii) Conclusions

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

3. DISCUSSION

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

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

E. LOW VOLUME SPRAYING

1. INTRODUCTION

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

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

Investigations on low volume spraying were done in steps:

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

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

(3) To evaluate different materials.

2. COVERAGE

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

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

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

3. DOSAGE PER UNIT

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

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

a. Treatments

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

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

TABLE 42

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

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

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

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

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

b. Results

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

TABLE 43

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

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

c. Conclusions

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

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

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

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

4. EVALUATION OF FUNGICIDES

a. Treatments

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

b. Results

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

TABLE 44

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

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

c. Conclusion

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

5. DISCUSSION

It is possible to apply low volume (high concentration) spraying with success for the control of black spot. It would be advisable, however, to carry out further research on materials and different applicators. Preliminary trials carried out with a "Kinkelder" low volume machine showed that some modifications are necessary to this type of applicator before it can be used successfully in citrus.
Entomologists are rather sceptical about the control of red scale (*Aonidiella aurantii*) with low volume spraying. Few of the smaller growers can afford to have a low volume machine for black spot control only, but this is no problem to the bigger growers.

F. AERIAL SPRAYING

1. INTRODUCTION

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

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

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

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

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

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

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

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

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

2. PRELIMINARY TRIALS

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

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

a. Methods and Materials

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

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

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

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

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

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

1. Three rows sprayed from the air with copper-in-oil (Shell) diluted with oil to contain ½ lb metallic copper in the form of copper oxychloride per gallon.
2. Three rows of trees were sprayed with Perenox, Alboleum and water mixture, made up to contain ½ lb metallic copper and ¼ gallon of oil per gallon of spray mixture. Due to mechanical trouble and physical problems the above mixture was replaced from the second application onwards, with Colloidox, a colloidal copper fungicide. This fungicide was mixed with water to contain ⅓ lb metallic copper per gallon of the final spray mixture.
3. Three rows of trees in the middle of the plot were sprayed by hand, using Perenox at 2 lb per 100 gallons plus ½ gallon of Alboleum.

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

b. Results

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

TABLE 45

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

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

c. <u>Discussion and Conclusions</u>

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

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

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

3. <u>EXPERIMENTS WITH SPRAYBOOM AND ROTARY ATOMIZERS</u>

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

a. <u>Methods and Materials</u>

(i) <u>Equipment</u>

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

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

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

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

(ii) Droplet Size

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

(iii) Assessment of coverage and droplet size

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

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

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

(iv) Assessment of Penetration

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

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

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

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

b. <u>Treatments</u>

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

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

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

A plan of the treatments as given below:

PLAN

Plot 239	Plot 241	Plot 243	Plot 245	Plot 247
Boom 2 runs / C3	Atomizer 2 runs / C1	C5 / Conventional ground spraying.	C4 / Boom 3 runs	Atomizer 3 runs / C2

Legend:

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

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

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

TABLE 46

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

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

ж Parathion included with black spot sprays.

TABLE 47

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

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

c. Results

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

TABLE 48

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

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

d. Discussion and Conclusions

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

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

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

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

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

G. CONTROL OF BLACK SPOT AFTER INFECTION

1. INTRODUCTION

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

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

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

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

2. CONTROL, SHORTLY AFTER INFECTION

a. Methods and Materials

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

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

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

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

b. Results

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

TABLE:49

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

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

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

c. Conclusions

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

Although this must be regarded as a pilot experiment and it was further hampered by fruit-drop, the results indicate that by postponing the application of the fungicide to 4½ days after the commencement of an infection period complete control of black spot was not achieved. Ascospores were caught during the entire period of rain which lasted approximately 40 hours. It is therefore possible that infection that took place towards the end of that period was arrested by the fungicide, while the earlier infection was too far advanced at the time of dipping. On the other hand, the effect of the oil is obscure. Oil is known to penetrate plant tissue (Ebeling, 1959) and to have fungicidal properties under certain circumstances (Laville, 1960). The apparent control achieved in treatments A, B and C is probably due to the action of the oil.

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

3. CONTROL AFTER THE ANNUAL INFECTION PERIOD

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

a. Oil in January

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

(i) Methods and Materials

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

The treatments were as follows:-

Treatment A. Alboleum 2 gallons per 100 gallons water

Treatment B. Alboleum 1 gallon per 100 gallons water

Treatment C. Bordeaux mixture 2½: 2 per 100 gallons water

Treatment D. Unsprayed control.

About 15 gallons of spray mixture was applied per tree.

Date of application: 13th January 1960.

(ii) Results

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

TABLE: 50

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

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

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

b. Oil in April

(i) Methods and Materials

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

Treatment A. Alboleum 2 gallons per 100 gallons water

Treatment B. Alboleum 1 gallon per 100 gallons water

Treatment C. Unsprayed control.

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

(ii) Results

TABLE: 51

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

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

(iii) Conclusions

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

c. Oil with and without other fungicides

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

(i) Methods and Materials

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

The treatments were:

Treatment A. Alboleum 2 gallons per 100 gallons water

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

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

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

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

Treatment F. Unsprayed control

Two spray applications were carried out viz:

6th June 1960 and

1st August 1960.

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

(ii) Results

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

TABLE: 52

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

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

(iii) Conclusions

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

The role of oil on disease development is discussed later.

d. Cyprex and oil

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

(i) Methods and Materials

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

Treatment 1. Cyprex $1\frac{1}{2}$ lb plus 1 pint Alboleum/ 100 gallons water.

Treatment 2. Cyprex $1\frac{1}{2}$ lb plus $\frac{1}{2}$ pint Alboleum/ 100 gallons water.

Treatment 3. Unsprayed control.

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

(ii) Results

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

TABLE: 53

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

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

There were no significant differences between the treatments.

4. DISCUSSION

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

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

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

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

H. EVALUATION OF CHEMOTHERAPEUTANTS

1. INTRODUCTION

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

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

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

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

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

2. INTRODUCTION OF CHEMOTHERAPEUTANTS THROUGH HOLES

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

a. Methods and Materials

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

The chemicals used in the different treatments, were:-

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

B. Ditto A, but applied through holes.

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

D. Ditto C, but applied through holes.

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

F. Ditto E, but applied through holes.

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

H. Untreated control.

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

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

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

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

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

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

b. Results

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

TABLE 54

The incidence of black spot and "melanose" approximately $2\frac{1}{2}$ months after application of chemotherapeutants to the tree stems.

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

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

TABLE 55

Mean percentage fruit showing black spot and "melanose" lesions $14\frac{1}{2}$ months after chemotherapeutants were applied through holes and bark of the tree stems.

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

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

c. Discussion and Conclusions

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

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

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

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

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

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

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

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

3. PRELIMINARY EXPERIMENTS WITH FOLIAR APPLICATION OF CHEMOTHERAPEUTANTS

a. Materials

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

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

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

Ciba 113: An experimental systemic fungicide.

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

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

Gibrel: Contains 0.88% Potassium gibberellate

Griseofulvin: A wettable powder containing 50% active material.

8-Hydroxyquinoline benzoate.

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

Nickel Chloride: An experimental fungicide

P.P 645: An experimental fungicide.

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

Phenyl mercuric chloride: containing 40% active material.

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

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

Salicylic Acid.

Urea: Commercial Urea with low biuret content.

b. Treatments and Results

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

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

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

TABLE 56

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

Treatments			Mean % fruit with Black Spot Lesions.
No.	Materials	Rate of applications	
A	Grisiofulvin	1,000 p.p.m.	67.1
B	Grisiofulvin plus Perenox	200 p.p.m. 7,000 p.p.m. (Cu)	38.6
C	Gibrel	50 p.p.m.	50.2
D	Acti-dione	150 p.p.m.	41.0
E	Acti-dione	2 p.p.m.	49.7
F	Nickel chloride	1,000 p.p.m.	60.9
G	Copper quinolate	2,000 p.p.m.	57.4
H	Unsprayed control	–	82.1
		(p=0.05)	18.9

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

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

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

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

TABLE 57

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

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

(p=0.05) 18.6

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

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

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

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

TABLE 58

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

Treatment			% Fruit with black spot lesions	Inverse arc sine transformation
No.	Material	Rate of application		
A	($FeSO_4 \cdot 7H_2O$	0.05 %		
	($MgSO_4 \cdot 7H_2O$	0.05 %		
	(Urea	0.50 %	23.1	28.60
	(Salicylic acid	0.05 %		
B	Ciba 113	0.10 %	19.9	26.45
C	Dimecron	0.20 %	26.2	30.65
D	8-Hydroxyquinoline benzoate	0.10 %	12.9	18.87
E	P.P. 645	0.25 %	6.1	13.15
F	Perenox (Metallic Cu)	0.10 %	1.8	7.55
G	Perenox, plus	0.075%	0.4	2.47
	Phenyl mercuric chloride	0.01 %		
H	Untreated control	-	18.2	24.90
			(p=0.05)	8.54

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

c. Discussion and Conclusions

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

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

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

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

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

I. POST-HARVEST CONTROL OF BLACK SPOT

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

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

1. SCREENING TRIALS

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

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

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

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

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

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

TABLE 59

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

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

(p=0.05) 17.8

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

3. EVALUATION OF ALBOLEUM

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

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

The different treatments were as follows:

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

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

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

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

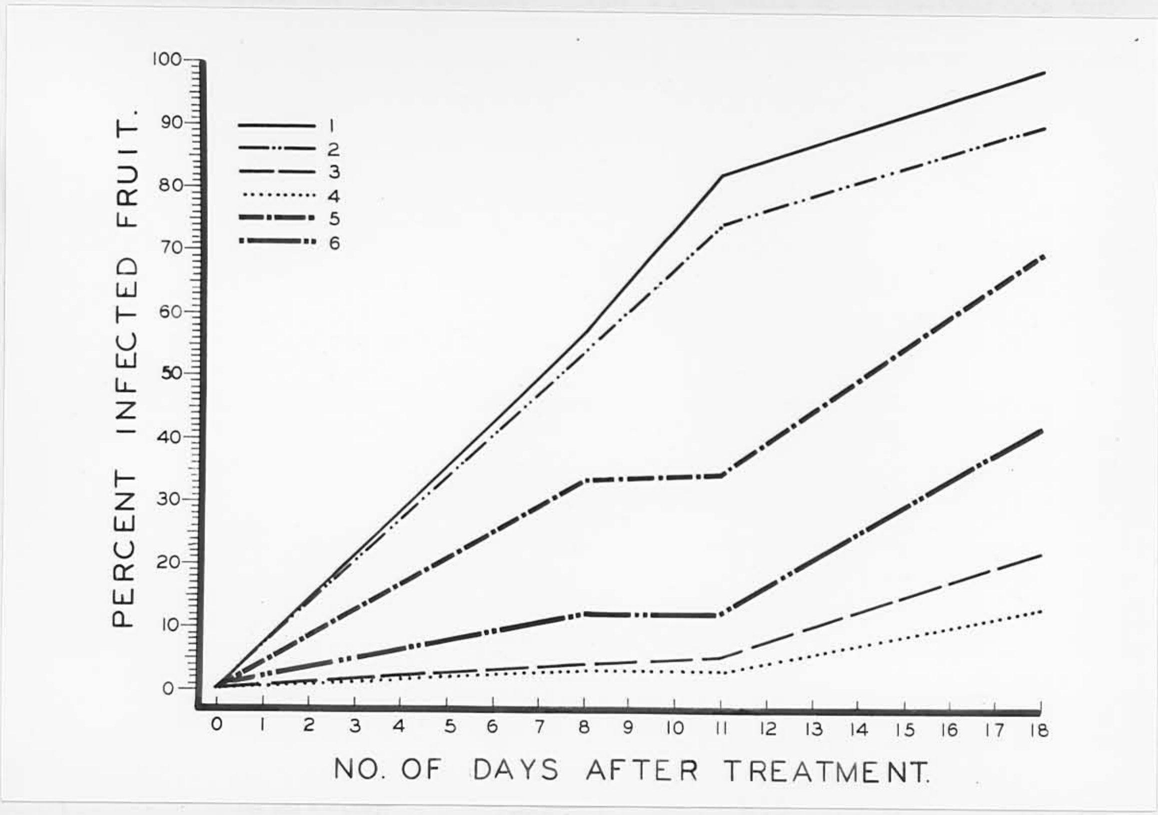

FIG. 6. Graphic representation of the effect of Alboleum on the post-harvest control of black spot.

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

4. MINERAL, VEGETABLE AND ANIMAL OILS

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

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

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

TABLE 60

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

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

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

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

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

5. DISCUSSION

When the respiration process is stopped, fruit will ferment and undesirable products will be formed like alcohols, aldehydes and other toxic materials. The sugars, acids, vitamins and flavour are also destructed (Kalmar 1960). Presumably, the oil also interferes with the respiration of *G. citricarpa*. The fungus is not killed by the oil, because it was readily isolated from the fruit rinds long after the oil had been applied. Laville (1960) reported that mineral oils penetrate to the inter-cellular spaces of orange rind tissues, but vegetable oils enter the inter- and intra-cellularly. Whether this explains the difference in effectiveness between the mineral oils and oils from other origins, may become clear after extensive studies on the host- parasite relationship of the black spot disease.