PHYSIOLOGICAL STUDIES WITH *GUIGNARDIA CITRICARPA* KIELY

by

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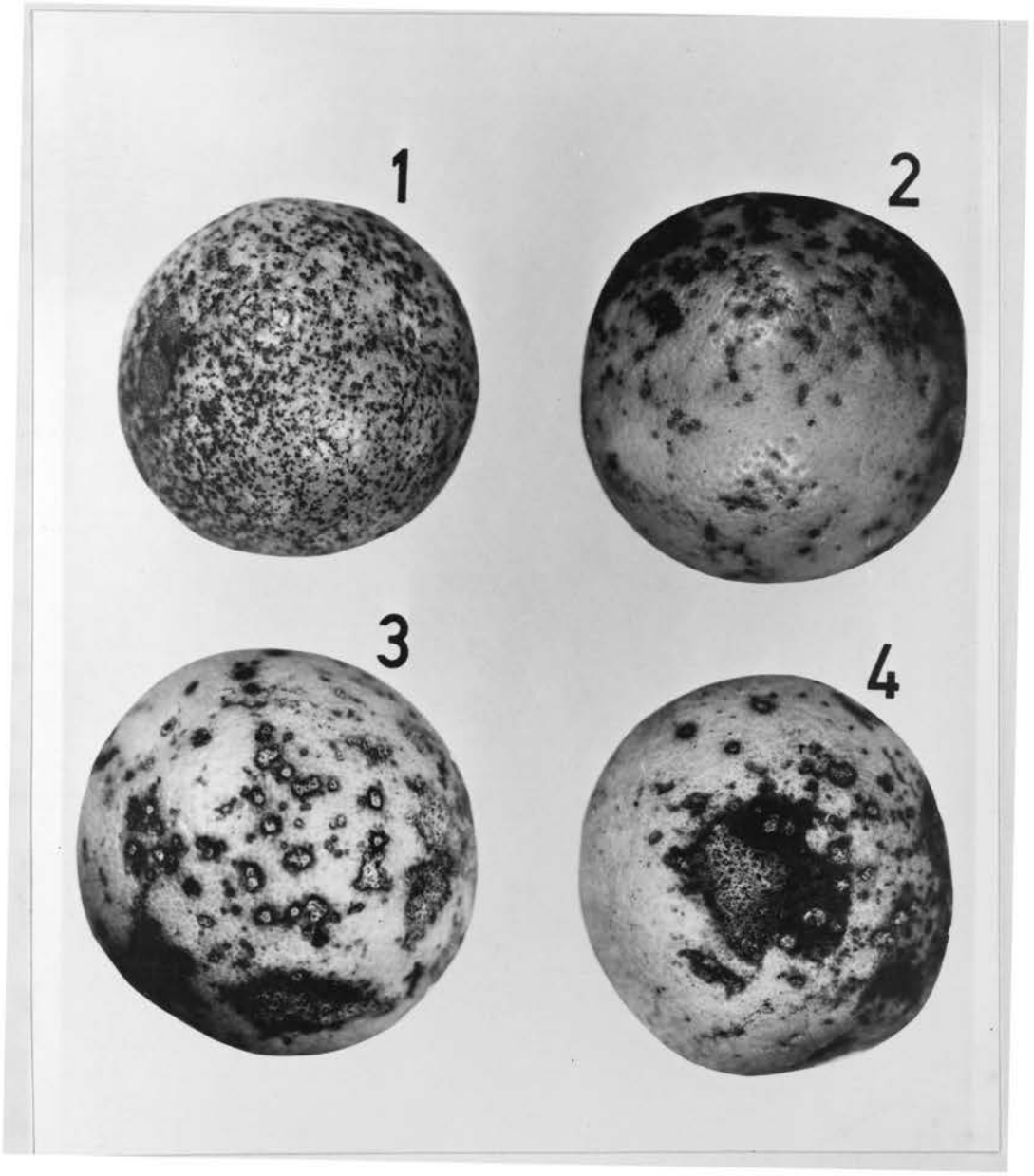
INTRODUCTION

Citrus black spot caused by Guignardia citricarpa Kiely, is the most serious fungal disease affecting citrus in the Republic of South Africa. The disease is also reported from Swaziland, Rhodesia and Mozambique and in different countries overseas, notably in Australia and also in China, Japan, Formosa, India, Philippine Islands, Java, Brazil, Argentine and the U.S.S.R. The disease is most serious on Valencia oranges but may also cause serious losses on grapefruit, lemon and on early maturing oranges as well. It is also reported on citron, Jaffa orange and kumquat and in China on mandarins. At present, this disease costs the South African citrus industry over $1\frac{1}{2}$ million rand to control annually. Notwithstanding the application of preventative measures, losses frequently result due to post-harvest development of the symptoms on export citrus in transit from the packhouses to the ports, prior to shipment overseas.

Infection occurs on young leaves and fruit after which there is usually a protracted latent period. With fruits this may vary from two to ten months depending on the cultivar and prevailing environmental conditions. Symptoms are seldom seen on leaves and it is only after the leaves have fallen that the perfect form or perithecial stage of the fungus develops. Infection by Guignardia citricarpa results in four different types of lesions on the fruit (Figure 1). Infection depends on prevailing environmental conditions and the

Figure 1 - Appearance of various black spot lesions on Valencia fruits

1. Speckled blotch
2. Freckle spot
3. Hard spot
4. Virulent spot



stage of fruit development. Evidence indicates that a non-pathogenic isolate of G. citricarpa exists latently on a number of hosts but does not play a role in the epidemiology of black spot disease. Although the two isolates are morphologically ~~similar~~ they can be distinguished in culture. The isolate non-pathogenic to citrus, is fast-growing and forms perithecia in culture, while the citrus pathogen is slow-growing and only forms pycnidia and spermagonia in culture.

The primary source of infection results from ascospores ejected from mature perithecia on dead leaves while pycnidiospores, arising from pycnidia on fruit lesions, serve as the secondary source of infection. Penetration occurs through the epidermis by means of an infection peg from the appressorium, after which the fungus remains latent as a knot of mycelium directly below the cuticle. Failure of the fungus to develop further, immediately after infection, was stated to be due possibly to an unfavourable nutrient balance in the young fruits or to inhibitory substances in the peel. Secondly, the activation of the pathogen and symptom development at a later stage, was thought to be due to the effect of various physical factors or that certain factors inhibiting fungal growth in the peel were nullified at some stage.

In South Africa black spot control is based on fungicidal protection of young, developing fruits

from ascospore infection. Previously, copper fungicides were used almost exclusively but recently have virtually been entirely replaced by organic fungicides viz. dithiocarbamates. The main reason is that copper has a tendency to darken rind injuries and also results in a black stippling, which together cause fruits to appear unsightly and in severe cases, unsuitable for export. Secondly, copper interferes with the action of arsenate sprays applied to Valencias to hasten maturity. Thirdly, after repeated spray applications, copper may accumulate in the soil, and at low soil pH's could become toxic to the tree. Notwithstanding this however, copper foliar sprays are still applied to citrus in South Africa as a corrective measure for copper deficiency, since soil applications of copper appeared ineffective. As copper accentuates blemish, the more extensive the original blemish the greater the severity of copper markings on the fruit.

Economic importance

In South Africa in the northern and eastern citrus-growing areas of the Transvaal and in Natal, nearly 3.4 million orange trees (viz. approximately 29 percent of the total citrus) are protected annually from citrus black spot. Losses from this disease would be considerable if control measures were not applied, considering that in certain seasons over 60 percent loss of fruits for export

Table 1 - The number of orange trees+ in various citrus-producing areas of the Republic of S.A., protected annually against citrus black spot disease

Citrus black spot area	Number of orange trees	
	* Navels and midseasons	** Valencias
Northern Transvaal	481,336	1,054,483
Eastern Transvaal	828,431	867,805
Natal	84,565	143,856
Totals	1,394,332	2,066,144

* 2.5 fungicidal sprays

** 3.5 fungicidal sprays

Valencias, which are more severely infected by G. citricarpa than early-maturing cultivars, need greater fungicidal protection than the latter

+The 1968 tree census compiled by the S.A. Co-operative Citrus Exchange (S.A.C.C.E., 1968). Only 6-year-old and older trees (disease-prone) were included in this survey

has been recorded from unsprayed Valencia orchards. Kiely (1948) stated that in Gosford, Australia, this disease resulted in losses ranging from 100,000 to 140,000 rand per annum and in certain seasons these losses could be quadrupled. Kotzé (1963) calculated losses annually from black spot on a large citrus estate in the Republic of S.A. comprising 192,000 trees, at 167,400 rand or 87.3 cents per tree. He mentions that in spite of the amount spent on control, 10.1 percent of the crop was nevertheless rejected for export as a result of this disease. This is probably representative of the situation occurring in the citrus industry in a very wet season. If Kotzé's figures were used as a basis, and adjusted to 126 cents per tree to comply with present day standards, losses totalling 4 million rand annually may be expected. However, in a normal season and using organic fungicides, which have largely replaced copper fungicides in the past four years, costs involved for citrus black spot control are estimated at approximately 1.5 million rand per annum (Table I). These figures are based on those given by Mc Onie and Smith (1964), Kotzé (1963) and the South African Co-operative Citrus Exchange Report (1968). From Table I the approximate total quantity of fungicide sprays applied in one season to Navel/midseason and Valencia trees respectively were calculated at 118,750,000 and 246,215,550 litres. These figures are based firstly, on an average of 34.05 l of fungicide spray per tree per application and secondly, on an average of 2.5 and 3.5 spray rounds for

Navels/midseasons and Valencias respectively (Table 1). The average cost of Mancozeb applications at rates of 0.68 to 0.91 kg per 455 l, was calculated at 1.81 rand per 455 l. This figure was obtained by adding average application costs viz. 83 cents to the cost of material viz. 98 cents per 455 l. Costs involved in controlling this disease in the Republic of S.A. amount to approximately 1,452,000 rand per annum.

Apart from the costs involved in the application of control measures, further losses may be incurred when symptoms develop on the fruit in transit from the packhouses to the ports prior to shipment. An entire consignment could be rejected for export while the costs involved in repacking these fruits may be extremely high.

Indirect effects resulting from the use of copper fungicides for the control of this disease are two-fold. Firstly, copper fungicides, which were the mainstay in the black spot areas during the past decade, have a deleterious effect on the external quality of the fruits. Reduction of fruit injuries due to wind or insects therefore, would considerably lessen the incidence of copper blemish. In Florida copper sprays applied for melanose control, caused by Phomopsis citri Faure, resulted in the formation of corky lesions and excessive blackening of fruit blemish (Knorr, Suit & Du Charme, 1957; Pratt, 1958). These authors stated that the later copper sprays were applied

in the season, the greater the accentuation of rind blemish. In South Africa, copper sprays applied for citrus black spot control, were shown to accentuate existing blemishes and also resulted in severe stippling on the fruits. Mc Onie (1966) mentioned that in the 1964/65 season, when rind blemishing was very severe, rejection of fruits for export from an orchard at Nelspruit, varied from nearly 40 percent for conventional spraying to as high as 70 percent when the concentration of copper was increased three fold (viz. speed-spraying). The importance of pre/disposing factors for copper blemish cannot be over-emphasised.

The effect of fruit blemish alone was reported by Bedford (1943) who stated that wind caused greater losses than any other major insect pest of citrus. Allright (1939), during the same period, mentioned that if all the orchards had been protected effectively against wind, the windbreaks would have increased the total returns for the 1938/39 season's crop by about 10 percent as a result of having less blemished fruit.

According to Smith (1958) copper toxicity in the soil may become a problem with the continued use of copper fungicides. He mentioned that in Florida, 15 years of continuous application of copper as fertilizers and sprays has led to a serious decline in the productivity of citrus trees. Various workers have reported that copper is not easily leached from the soils. Bryan (1957) reported that over

898 kg of copper oxide per Ha have accumulated in certain soils in Florida. In the citrus black spot areas of the Republic approximately 34 kg of metallic copper ~~is~~ are applied per Ha annually (equal to 337 kg metallic copper per Ha over a ten year period). Fortunately copper toxicities become manifest at relatively low pH's which are not normally encountered in citrus soils in the Republic of S.A. According to Mc Onie et al (1964), soil analyses taken from black spot areas indicated that there was no immediate danger at that time. However, the hazard attendant ~~with~~ on the continued use of copper fungicides cannot be ignored and is one of the factors contributing to the change to organic fungicides in recent years. Notwithstanding the accumulation of copper in soils in certain citrus areas of the Republic, within two years of changing to organic fungicides, many citrus trees in these areas showed symptoms of copper deficiency which was verified by leaf analyses (du Plessis, 1968). Once copper is in the soil it becomes unavailable to the citrus tree under normal conditions of soil pH. The only alternative is the annual application of copper as a foliar spray whereupon the problem of copper blemish on the fruits will have to be contended with once more.

Physiologic forms of *Guignardia citricarpa* Kiely

Two fungal isolates associated with citrus black spot disease were recognised by Mc Onie (1964) who described

the one as Guignardia sp (non-pathogenic to citrus) and the other as Guignardia citricarpa or the citrus pathogen.

Kiely (1948) reported the occurrence of latent infections of G. citricarpa in nine different plant families in Australia. Wager (1952) reported finding perithecia of G. citricarpa on Smilax sp. Schüepp (1961) mentioned that he found this fungus on dead leaves of species of Lagerstroemia, Royena and Combretum. Kotzé (1963) found perithecia (similar to those on citrus leaves) on dead rose leaves.

Mc Onie (1964) after making extensive investigations, found that apart from the citrus pathogen G. citricarpa, a Guignardia sp was found to occur on a wide host range and according to him, played no part in the epidemiology of citrus black spot disease. It appeared that although the perithecia and ascospores of this fungus and that of G. citricarpa were identical, there were various physiological and pathogenic differences which made it possible to distinguish between these two fungi. Ascospores of Guignardia sp from perithecia on leaves of various hosts inoculated onto citrus fruits gave negative results. Secondly, he found that when grown on a wide range of agar media, Guignardia sp was mainly ascocarpic and formed colonies which were darker coloured, more luxuriant and faster-growing than those of G. citricarpa. The latter fungus only produced pycnidia and pycnidiospores but never formed fertile

perithecia in culture.

A similar situation was reported by Luttrell (1948) for Guignardia bidwellii (Ell) Viala and Ravaz. He identified three distinct physiologic races differing in morphology, growth in culture and in their pathogenicity on bunch grapes, muscadine grapes and Virginia creeper. He mentioned that in view of the intergradations in morphology of these fungal types, that they should be regarded as belonging to the species Guignardia bidwellii having three forms, separated on the basis of pathogenicity.

According to the prevailing rules of botanical nomenclature (Bisby, 1953), the species name common to the two forms has to be based on the form first described as the species irrespective of the systemic rank in which it was described. On this basis, G. citricarpa Kiely, being the oldest species name, would automatically be retained for both the citrus pathogen and the isolate non-pathogenic to citrus. From the evidence presented, the writer regards these as two forms of the same fungus, and for the sake of clarity, the citrus pathogen will be referred to as G. citricarpa (a) and the so-called Guignardia sp, as G. citricarpa (b).

Factors affecting symptom development on the fruit

According to Mc Onie (1967), after infection

occurred in summer, the fungus appeared to remain latent, for from two to ten months as a tiny knot of mycelium between the cuticle and the epidermis. Latent infection by the fungus was shown by pure culture methods and histologically, to be present in the rind of symptomless fruits prior to maturity (Kiely, 1948; Kotzé 1963 and Mc Onie, 1963). The arrest in the development of the fungus in the rind at this stage was thought by Mc Onie (1963) to be due possibly to two factors viz. the presence of inhibitory substances or an unfavourable nutrient balance in the young fruit.

Kiely (1949) stated that black spots developed rapidly as the rind of the fruit reached maturity and began to colour. He also mentioned that where regreening occurred in Valencias, later in the season, there was a reduction in symptom development. Wager (1952) was unable to confirm this in his experiments and found that black spot development was equally severe on immature fruit as on mature fruits. He also found that colouring green fruits with acetylene in laboratory studies had no apparent effect on lesion development on the fruits. He observed too, that out-of-season fruits, which developed in autumn, frequently showed severe symptom development while still green and long before maturity.

Symptom expression on the fruit was thought by various workers to be induced by temperature, radiation (sun) or a water deficit. According to Kiely (1948) and Kotzé

(1963), it appeared from field observations that the disease was particularly severe on the warmer or sunny side of the tree (i.e. the north-western aspect in the Southern Hemisphere). Kiely (1949) stated that peak development of this disease on the fruit occurred at approximately 30°C. Wager (1952), in laboratory studies, showed that temperatures of 27°C to 30°C resulted in the highest numbers of lesions on experimental fruits. Kotzé (1963) reported that in the 1959/1960 season at Letaba, the time of the year during which fruit lesions increased sharply coincided with the period of increased temperature (i.e. in late Winter and Spring). Kotzé (1961) stated that an abnormally hot autumn led to the early development of symptoms despite a good control programme. When cooler conditions set in during winter, black spot development was arrested. Smith (1962) mentioned that low temperatures effectively reduced fruit symptom development. He showed that fruit lesions were almost completely suppressed by storing fruits below 21°C.

Kiely (1948) observed that pycnidia developing on fallen leaves on the orchard floor, occurred mainly on that side exposed to the sun's radiation. Kotzé (1963) reported that significantly more diseased fruit occurred on the upper compared ~~to~~ with the lower halves of Valencia trees. However, Kiely (1948) found no differences in disease incidence in a vertical plane in citrus trees in Australia. Contrary to the above reports, Calavan (1959) reported a higher disease incidence on the lower compared ~~to~~ with the upper tree halves in

a small experiment at Nelspruit. Kotzé (1963) found that increased disease development on the upper branches was more pronounced in older trees with sparsely foliated tops. Calavan (1960) mentioned that fruits on the periphery of the citrus tree had more lesions and latent infections than fruits towards the centre. Kotzé (1963) confirmed these results in his experiments, where outside fruits had significantly more lesions than inside fruits. Calavan (1960) stated that more lesions developed on the exposed compared to the shaded areas of the peel of infected oranges. The above workers have either strongly suggested or intimated that sunlight may be an important factor in inducing fruit symptom development. However, it is uncertain from these results and observations whether this is a temperature or light effect or due to some other factor.

According to Kiely (1948), there was a definite correlation between disease incidence and tree age. He found that young trees up to seven and eight years of age produced fruits free of symptoms although latent infections were shown, by pure culture methods, to be present in the rind. Wager (1952) however, reported that in several cases young trees of three and four years, interplanted in old infected orchards, showed severe fruit symptom development. One may speculate that these conflicting results may be due possibly to differences in the inoculum load in the respective orchards.

Mc Onie (1963) and Kotzé (1963), from bagging experiments and spray trials, showed that fruits apparently acquired a resistance at a later stage of development (i.e. after January/February). However, Mc Onie (1963), cultured rind pieces and showed that infection had in fact occurred after January, but did not give rise to symptoms on the fruit. One may speculate as to whether this suppression of fungus development is due to a physiological change in the fruit or due to an environmental factor. Kiely (1948), from inoculation experiments, showed that the duration of latency was independant of the time of inoculation. Symptoms developed after approximately the same time in all the inoculation treatments.

Observations were made on the effect of drought on this disease. Kiely (1949) observed that hot, dry winds at the time of fruit maturity, increased fruit symptom development. Kotzé (1963) found that wilting citrus trees during the winter months prior to picking increased the incidence of black spot.

Wager (1952) reported that the disease frequently occurred first on neglected, weak and debilitated trees and persisted even when the condition of the tree was improved.

Various workers have reported a correlation between reduced respiration in the fruit and the arrest of symptom development in the rind. Kotzé (1963) obtained effective control of the post-harvest phase of the disease by dipping

fruits in mineral oil. Because the fungus was readily recovered from treated fruits he speculated that the effect of the oil was through its ability to impair fruit respiration and hence lesion development. Seberry, Leggo and Kiely (1967), applied various wax emulsions and non-ionic detergent coatings to harvested Valencia fruits and the marked decrease in symptom development they obtained was attributed to the reduction in respiration effected by these treatments.

Physiological changes in the fruit rind

Bain (1958) followed the metabolic activities in Valencia oranges at 20°C, from early fruit set through three stages viz. cell division, enlargement and maturation, respectively. The rate of respiration per fruit increased during the first two stages (as much as ten-fold during the cell enlargement stage) but decreased during the maturation stage. Sinclair (1961) reported a gradually declining respiration rate as citrus fruits matured. He mentioned however, that certain workers in Australia had reported an increased respiration rate immediately after harvest primarily in early maturing cultivars (viz. Navels and midseasons).

The effect of temperature on the respiratory activity of Valencia fruits after harvest was reported by Haller, Rose, Lutz and Harding (1945). The respiratory

quotient (R.Q. = ml CO_2/ml O_2) was close to unity at low temperature ranges, 1.0+ at 25°C (and above) and 1.5 at 38°C. According to Sinclair (1961) further analyses (besides gas exchange measurements) are needed to understand the various reaction mechanisms taking place in the citrus peel.

The effect of pathogenic fungi on fruit respiration nas been reported. Ethylene emanating from Penicillium digitatum was found to result in a marked increase in the respiration of harvested fruits (Sinclair, 1961). A single orange with green mould caused the described response in as many as 500 fruits. Alternaria citri had the same effect but to a lesser extent.

It appears, from the available information, that light may have an effect on the respiration rate in citrus fruits. Sinclair (1961) stated that the methylene blue reducing ability of flavedo extracts was catalyzed by sunlight and artificial light. There was a correlation between methylene blue reducing activity, ascorbic acid content and increased respiratory rate in the fruit. This effect was greater in the flavedo as compared with the albedo. ?

Bain (1958) made extensive studies on developing Valencia fruits in Australia and reported a marked increase in the percentage moisture in the flavedo from fruit-set (October) until April (i.e. four months prior to maturity). A maximum was reached at maturity after which the value remained constant. Sinclair (1961) reported the existence of

an equilibrium between the water in the tree and in the fruit. Daily changes in fruit volume occurred in relation to transpiration from the leaves. Water and solutes were shown to be transported in the fruit by the vascular bundles confined primarily ~~to the albedo~~. One may speculate that in drought conditions the amount of moisture drawn from the fruit is directly related to the degree of foliation of the tree.

According to Sinclair (1961) analyses of orange peel showed a low concentration of citric acid and high concentrations of both malic and oxalic acids. Changes in quantity of these organic acids at various stages of fruit development were noted by Rasmusen (1964). Concentrations of both malic and oxalic acids in peel tissue, reached a peak during autumn and decreased to a low level thereafter (i.e. young peel contained more organic acids than mature ~~fruits~~ peel). Sinclair (1961) mentioned that calcium and magnesium occurred in high concentrations in the peel, and formed various salts with the organic acids leading to relatively high pH's. The pH of peel sap, 5.0 or greater, was much higher than that of the corresponding juices of the pulp (viz. pH approximately 3.0).

Frean (1964) found that Guignardia citricarpa isolates grew well in artificial liquid medium when hesperidin was used as the carbon source. According to Sinclair (1961) hesperidin, a flavourless glucoside ($C_{28}H_{34}O_{15}$) occurred

abundantly in oranges and other citrus fruits and could be isolated readily from chopped citrus peel by methanol extraction. Webber and Batchelor (1943) showed, in transverse sections of Valencia rind, that masses of needle-shaped hesperidin crystals were lodged intercellularly in the tissue. It appeared that hesperidin occurred largely in young fruits. Hendrickson and Kesterson (1961) stated that in Florida the optimal fruit size (diameters) of Valencias for the recovery of hesperidin was 1.6 to 2.2 inches which is approximately equivalent to the fruit size in late Summer and Autumn.

The distribution of nitrogen in various portions of mature Valencia oranges was investigated by Sinclair (1961). He found that the peel and the juice of oranges contained almost equal amounts of nitrogen i.e. 40 to 45 percent of total N in the fruit. Observations in the changes in nitrogen content in the peel were made by Bain (1958) during the developmental stages of Valencia fruits. In the early stages, most of the nitrogen in the fruit was contained in the peel. In Summer, during the period of rapid growth, the nitrogen content of the peel rose sharply and then fell during Autumn. Thereafter, up to maturity, increases in nitrogen content of the rind was slow.

All citrus fruits possess oil which is contained in numerous oil sacs in the rind. It was established that more oil occurred in Valencias than in Navels and at the stylar-end compared with the stem-end of the fruits. Secondly,

the yield of oil from fruits at different stages was directly correlated with the surface area of fruits up to maturity. At maturity there was a rapid increase in oil content, the extent of which depended on prevailing physiological and climatic factors (unspecified by the authors) and which varied from season to season (Bartholomew and Sinclair, 1946).

According to Bernard (1961) oil may be separated into two factors, the terpenes and terpenoids, of which 15 and 37 constituents were identified in each respectively. Braverman (1949) mentioned that the main terpene in citrus oils was d-limonene ($C_{10}H_{16}$) which made up 90 percent of the total constituents in citrus oil.

Growth and sporulation studies in fungi

Lilly and Barnett (1951) outlined various principles regarding growth and reproduction in fungi. Firstly, and quite obviously, growth must precede sporulation. A second principle is that growth and reproduction may depend on considerably different external conditions, which are usually narrower in limits in reproduction than in the growth stage.

The effects of various external conditions on fungi in culture will be reviewed with particular emphasis on those factors considered important in the growth, sporulation and symptom development of *Guignardia citricarpa* such as light, temperature, light and temperature interactions,

osmotic pressure and antifungal factors in the host tissue.

Cochrane (1958) mentioned that in light-growth studies the majority of workers reported that fungi were either unaffected or inhibited and only few stated that light increased growth. Blastocladiella emersonii was one of the few fungi studied where light stimulation of growth was confirmed. Different workers have shown that the effect of light on the fungus could be exerted through the medium. Yusef and Allam (1967) reported that light appeared to favour the break-down of substances in the synthetic medium beneficial to mycelial growth. According to Weinhold and Hendrix (1963) exposure of potato dextrose broth to light made the medium inhibitory to the growth of various fungi tested. Hawker (1950) made a general statement to the effect that low light intensities usually have little influence on growth whereas light of high intensity, sunlight, may inhibit growth or have a lethal effect. Only preliminary light experiments were conducted on Guignardia citricarpa in culture by Schüepp (1961). He stated that light appeared to have no effect on the growth and sporulation of this fungus. However, the results are questionable since he failed to control temperature and humidity factors.

The majority of reports on the effect of light have been concerned more with reproduction than with growth in fungi. Cochrane (1958) reported that light may either have no effect or it may be stimulatory or inhibitory to

sporulation. He refuted the phrase "stimulatory effect" and added that there was evidence to show that sporulation was initiated by a check in mycelium growth. The effect of light on various fungi with regard to reproduction, morphology, pigmentation and phototropic response have been reviewed by Marsh, Taylor and Bassler (1959). Recent studies by Knox-Davies (1965) and Carlile (1965) have also shown the influence of light on the production of pycnidia.

Interaction of temperature and light on sporulation was mentioned by Lilly and Barnett (1951). Higher temperatures replaced, in part, the beneficial effects of light in the case of Phoma urens and other fungi tested. They stated that since the sporulation was the same whether light or temperature was the stimulus, this meant that these stimuli probably brought about the same or equivilent changes in the physiology of the fungus.

Temperature studies on the growth of G. citricarpa were made by Wager (1952). He measured the diameters of colonies on agar medium and reported inconsistent results which he described as being due to the irregular outline of these colonies. Cochrane (1958) stated that the most satisfactory measurement of fungal growth is by determination of dry weight of mycelium. He mentioned that in certain cases a direct correlation was obtained between radial growth on agar and dry weight in liquid medium.

According to various workers, citrus fruits contain an antifungal factor in the peel. El- Toshby and Sinclair (1964) found that peel extracts resulted in significantly greater inhibition of Phomopsis citri in culture than that from the fruit endocarp. Unfortunately their technique was faulty and contaminants were noticed on the culture plates. Murdoch and Allen (1960) demonstrated the anti-microbial properties of orange peel oil and d-limonene (which comprises 90 percent of citrus oil) against a yeast in liquid culture. According to Kiely (1948) orange peel extracts stimulated germination of pycnidiospores of G. citricarpa in culture. The inducement of germination of Penicillium digitatum spores by orange rind components was reported as well, by Davis and Smoot (1965).

As osmotic changes occur in the peel during ripening (Bain, 1958) and since this coincides with the period of progressive symptom development on the fruit, studies were conducted to determine whether a correlation exists between the two. According to Cochrane (1958) osmotic pressure may have a great effect on the rate and amount of growth in fungi. Lilly and Barnett (1951) stated that difficulties in evaluating osmotic pressure effects upon fungi are due to the fact that cell membranes are permeable to other compounds in addition to water and in making calculations it must be assumed that an indifferent semipermeable membrane separates solutions of different concentrations. Cochrane (1958) mentioned that osmotic pressure studies are complicated by

the possible non-osmotic effects of the solute. Wainwright and Mc Veigh (1967) found that increased growth of Neurospora crassa occurred with increased osmotic pressure with the addition of polyethylene glycol "400" to the medium. They made no mention that this compound could possibly serve as a source of carbon to the fungus in culture media.

As little was known regarding the physiology of the fungus, i.e. from the onset of infection until the symptoms appear on the fruit, research was aimed at determining the effects of certain environmental and physiological factors both on the fungus and on fruit symptom development.

Previously it was found that the greatest disease development on the fruit occurred at temperatures ranging from 27° to 30°C. Field observations also showed the highest disease incidence on fruits on the warmer side of the tree i.e. north-western aspect in the Southern Hemisphere. In addition, the symptom development was more rapid on fruits at the end of Winter and early Spring when the temperatures rose sharply. Outside fruits and fruits on the top branches showed more lesions than inside fruits and those on lower branches, and secondly the part of the peel exposed to light was more infected than the shaded areas of the fruit. An hypothesis was formulated to the effect that besides temperature, light has an effect on symptom development on the fruit as it may trigger off sporulation. It was previously

shown that the pathogen in its latent form is situated superficially in the fruit i.e. just below the cuticle. From this, it was postulated that light has a direct effect on symptom development. Also that alternating the light and dark conditions approximating those in a packhouse, would increase symptom development on picked fruits.

Various workers reported the presence of an antifungal factor in citrus peel which was said to be due to citrus oil (d-limonene). As oil glands occur in the flavedo, it follows therefore that flavedo extracts would be more toxic to the fungus than extracts from the albedo. Limonene breakdown in the fruit is reported to occur in the presence of sunlight. Observations showed that black spot was more severe on fruits on the sunny side of the tree. As a result, an hypothesis was formed which stated that citrus oil (d-limonene) is inhibitory to the growth of G. citricarpa.

It was previously found that the percentage moisture in the peel increased from 70 percent at an immature fruit stage to nearly 80 percent when fruits reached maturity. As this coincided with the period of progressive increase of lesions on the fruit, it was postulated that osmotic pressure changes in the fruit peel resulted in conditions conducive to increased symptom development.

Blemish marks on fruits are rendered unsightly by copper sprays applied for black spot control. As copper accentuated blemish, the more extensive the original blemish

the greater the severity of copper markings on the fruit.

In recent years a continuous blemish on the side of the fruit resulted in serious export losses. It was postulated that wind was the major cause of blemish as orchards receiving insecticidal protection from thrips, Scirtothrips aurantii Faure, and red mite, Panonychus citri McG. still showed a large percentage of fruits with side blemish. Previously it was shown that resulting blemish on citrus fruits was often out of all proportion to the initial injury. This was attributed to the toxic effect of citrus oil released from the oil cells in the rind following injury. It was noticed that different types of blemish resulted from the same type of injury to fruits at various states of development. An hypothesis was formed to the effect that the presence of citrus oil at an advanced stage of fruit development resulted in deep corky lesions on the peel.

In Florida it was previously stated that the later the copper sprays were applied in the season, the greater the darkening of fruit blemish. However, conflicting results were obtained by other workers in South Africa. An hypothesis was formed on the basis of the timing of copper sprays for citrus black spot control in relation to the extent of copper blemish and to the external fruit quality.

PROCEDURES

Isolates and isolation methods

Mc Onie (1964) recognised two different fungal isolates associated with citrus black spot disease. The one he described as *Guignardia citricarpa*, the citrus pathogen, and the other as *Guignardia* sp which was non-pathogenic to citrus. Both types were used in these experiments and referred to as *G. citricarpa* (a) and *G. citricarpa* (b), as shown in Table 2 as isolates 1, 2, 4, and 5, and isolate 3 respectively.

Table 2 - Source of isolates of *Guignardia citricarpa*

Isolate Number	Source
1	Hard spot lesion on a lemon fruit from Nelspruit, Eastern Transvaal
2	Speckled blotch lesion on a Valencia orange from Nelspruit, Eastern Transvaal
*3	Symptomless grapefruit from Swaziland
*4	Single ascospore from perithecia on decomposing Valencia orange leaves from Letaba, Transvaal
5	Hard spot lesion on Valencia orange from Nelspruit, Eastern Transvaal

*Isolates 3 and 4 were isolated by Mc Onie (Nelspruit) and Kotzé (Letaba) respectively, and isolates 1, 2 and 5 were isolated by the author.

Valencia oranges and lemons showing black spot lesions were collected from orchards in the Nelspruit area and cultures of Guignardia citricarpa were easily obtained from infected fruits. Small pieces (approximately two mm square) were cut from the infected rind and sterilized for one minute in a 1:4 sodium hypochlorite solution before being rinsed three times in sterile water. Other sterilants used were 0.1 percent mercuric chloride for ½ minute or 70 percent ethanol for one minute. These pieces were placed on potato dextrose agar (PDA) and incubated at a temperature of 27°C.

All stock cultures were maintained on potato dextrose agar slants in a desiccator ($CaCl_2$) at 6°C or kept at 25°C under sterile liquid paraffin. When transferred after six months, cultures were still viable.

Growth measurements

Cochrane (1958) stated that growth of fungi could be most satisfactorily measured by determining dry weights of the mycelium. This method was adopted in preference to determining the linear growth of fungal colonies on agar, which was quite unsuitable in these studies due to the irregular colony outline of Guignardia citricarpa (a). In all experiments the cultures were grown in 100 ml or 150 ml conical flasks each containing 50 ml of liquid medium. Results were taken after 10, 15, 20 and in some experiments

after 30 days. Mycelium weights were determined to the nearest 0.1 mg after the cultures were filtered through Gooch crucibles (porosity 4) and dried for 24 hours at 72°C.

Culture media

The basal synthetic medium used in these experiments was a follows:

Sucrose		20.00 g
$(NH_4)_2\ SO_4$		2.00 g
$Mg\ SO_4.7H_2O$		0.50 g
$K_2H\ PO_4$		4.70 g
$KH_2\ PO_4$		23.60 g
Fe^{+++}	2ml/liter*	0.10 mg
Mn^{++}		0.05 mg
Zn^{++}		0.10 mg
Inositol		5 mg
Thiamine		100 µg
Biotin		5 µg
Niacin		100 µg
Distilled water		1,000

*A stock solution of the trace elements was made (Lilly and Barnett, 1951) and two ml of this solution was used per litre of basal medium. The stock solution contained the following:

$Fe\ (NO_3)_3.\ 9H_2O$	723.5 mg
$Zn\ SO_4.\ 7H_2O$	439.8 mg

$MnSO_4 . 4H_2O$	203.0 mg
Distilled water	1000.0 ml

The mono- and dibasic potassium phosphate salts were used to buffer the medium. The initial pH of the medium after sterilization was 5.8 and the subsequent pH of the medium was always measured after growth.

The medium was chemically sterilized using beta-propiolactone (BPL). Rabie and Steyn (1964) stated that G. citricarpa in culture did not utilize the degradation products of BPL as a carbon source. The same procedure adopted by Frean (1964) was used, where a fifth of the total volume of medium was sterilized with BPL and the remaining volume was made up with sterile distilled water. Two ml of BPL per litre was used after which the medium was kept at 41°C for 24 hours to allow for the hydrolysis of the chemical sterilant.

In the sporulation studies the medium used was either potato dextrose agar (Difco) or basal synthetic medium plus 20 g agar per litre. Each tube contained either 10 or 15 ml of the agar medium. The former medium was sterilized at 15 lb pressure for 20 minutes and the latter was steam sterilized in the autoclave on three consecutive days. The pH of both media ranged from 5.6 to 5.8. The slant cultures were inoculated by aseptically transferring a small piece of mycelium from colonies on agar.

Cultivation methods

Slants of both basal synthetic medium and PDA, inoculated with G. citricarpa, were placed in polyethylene bags in an incubator at 27°C in the dark for five days for the initial fungal growth to take place before being subjected to the various treatments.

Mycelial inoculum was prepared by removing three globose colonies from a 12 day old shake culture and macerating the mycelium in 10 ml of sterile water with a MSE homogeniser for three minutes. After diluting to 30 ml with sterile distilled water, 0.1 ml of this suspension was used as inoculum for each flask.

Lesion counts on fruits

Counts of all lesions on fruits were taken after exposure to the various treatments for periods of 10 and 15 days. With virulent spot (Figure 1), a cork borer of two mm diameter (the approximate size of a freckle-spot lesion) was used to determine approximately, the number of lesions in the areas where lesions had coalesced.

Pycnidiospore counts

The black crust-like layer embedded with pycnidia

was removed from the surface of both slant cultures and flavedo discs. The crust was macerated in 0.5 ml sterile distilled water, left for 12 hours and haemacytometer counts of pycnidiospores were then made. Pycnidiospore counts were expressed in terms of numbers of spores per ml.

Sporulation assessments

Pycnidia

The area of the black crust-like layer in which the pycnidia were embedded was rated as follows:

1 = no crust-like layer

2 = < 25%

3 = 25 to 50%

4 = 50 to 75%

5 = 75 to 100%*

*Area of crust in relation to total area of peel disc or slant culture (Figure 2).

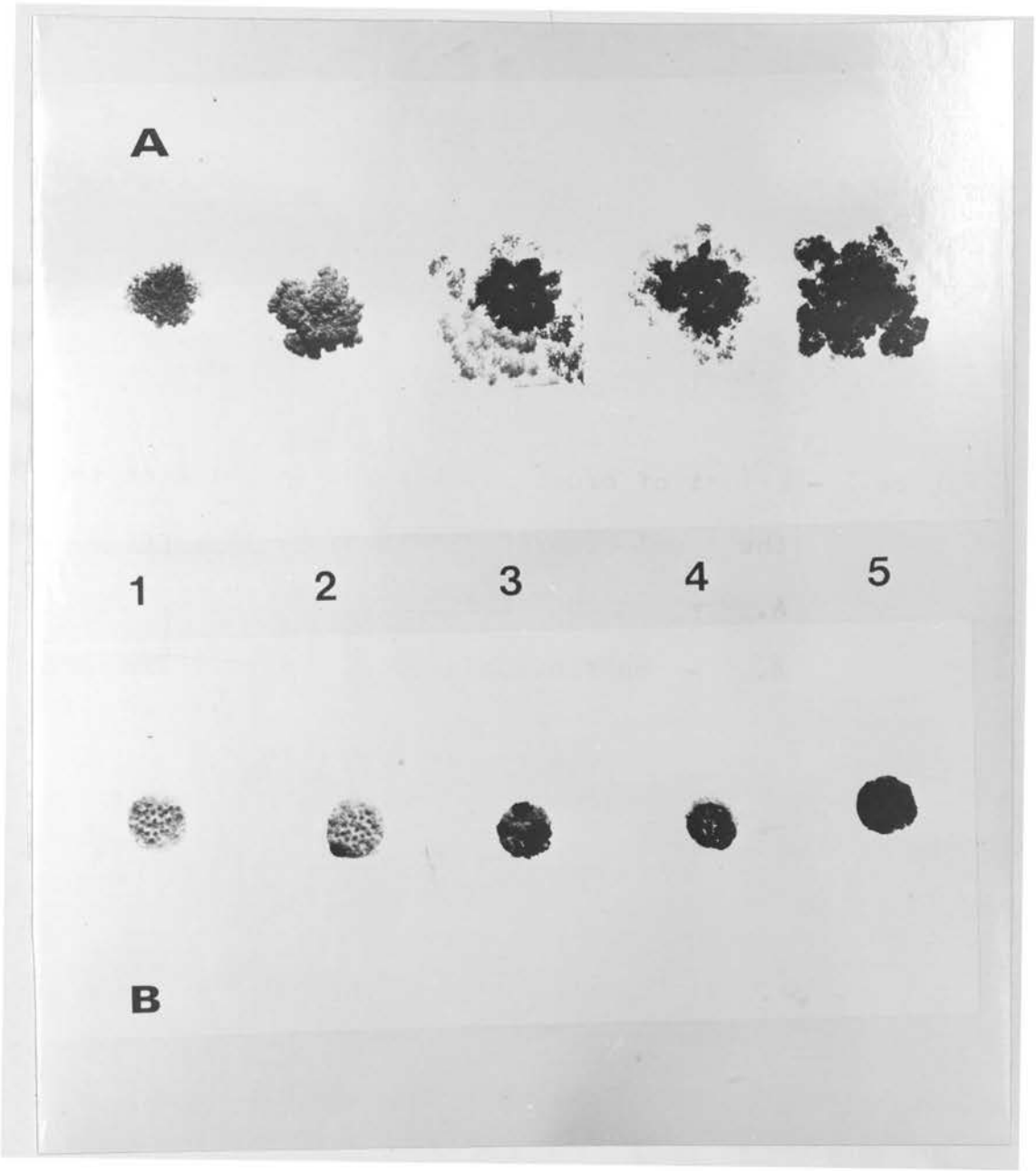
Spermatia

Due to the small size of the spermatia it was not practical to make exact counts. The spermatia in five of the smallest sub-divisions of the haemacytometer were counted to the nearest ten and expressed in terms of spermatia/ml. The four categories were as follows:

Figure 2 - Extent of crust development in relation to the total area of growth of G. citricarpa

A. - on PDA slants

B. - on flavedo discs



0 = no spermatia

* = maximum of 30×10^6

** = maximum of 100×10^6

*** = maximum of 200×10^6 spermatia per ml

Light treatments and temperature control

The light source comprised two 122 cm, 40 W day-light tubes mounted 76 cm above the cultures. The light intensity was measured using a Weston illumination meter and, depending on position, readings ranged from eight to twelve Lux units (Lux = number of Lumen/m^2). The three light treatments were as follows:

1. Continuous light for 24 hours per day.
2. Alternating light and dark. (Samples were changed from light to dark and vice versa, every 12 hours).
3. Continuous dark. (Light-proof tins were used and these were placed alongside the samples in the light in order to minimize possibile temperature differences between the treatments).

Polyethylene bags were used in all treatments to prevent the dehydration of cultures and fruits.

Experiments were conducted at two different

temperatures, viz. 20° and 27°C. The former temperature was obtained in a large cabinet fitted with a cooling unit while the higher temperature was maintained in a thermostatically-controlled room.

In order to ensure that the effects were not due to an increase in temperature inside the enclosed polyethylene bags in the light treatment a multipoint temperature recorder was used to give uninterrupted readings over a period of 20 days. Less than a degree centigrade difference was recorded between light and dark treatments over this period. Cochrane (1958) mentions that it is essential that determinations of the ambient temperature should be made in light experiments, as an increase in temperature may be expected in the light treatments unless precautions have been taken to overcome this.

In shake growth culture, four 15 W daylight tubes were mounted 46 cm above the flasks on the shaker bed inside a controlled environment incubator-shaker. The light intensity at culture level was 10 Lux units, the temperature was set at 27°C and the shaker speed at 65 rpm. Dark conditions were created by wrapping flasks in three layers of aluminium ~~tin~~ foil.

In the temperature experiment, flasks were placed in various incubators and briefly shaken every two days for the duration of the experiment.

Fruit treatments

Unsprayed mature Valencia fruits with latent infections of black spot were selected from ten trees at Nelspruit. After being wiped clean and existing lesions ringed, fruits were placed in polyethylene bags and sealed to prevent excessive loss of moisture. Forty fruits were randomly allotted to each treatment. Fruits were rotated twice daily in the light, to ensure uniform illumination.

In another experiment, fruit picked from the same trees as above, were tightly sealed into holes in a flat, light-proof box in such a way that one half of the fruit was exposed to the light while the other half was kept in the dark.

Rind discs

Circular discs of peel (seven mm in diameter) were cut with a cork-borer from the equators of 20 mature, naturally-infected Valencia fruits picked from unsprayed trees. Rind discs were surface sterilized in a 1:4 sodium hypochlorite solution for one minute and rinsed five times in sterile water. The discs were aseptically transferred to Petri dishes (five per plate) containing moist, sterile filter pads and the plates were sealed in polyethylene bags to prevent excessive moisture loss.

Fruit peel extractions

Valencia and Seville oranges were picked from the northern and southern sides (three fruits per side) of five trees in an orchard at Nelspruit. Samples were taken approximately every 30 days from January to July. The fruits were weighed and diameter measurements (two fruit axes at right angles to each other) were recorded. Fruits were wiped clean and the flavedo and albedo parts were carefully separated and weighed. These parts of the peel were macerated separately in a Waring Blendor for $\frac{1}{2}$ minute before the tissue was placed between filter pads, 5.7 cm in diameter, in an hydraulic laboratory press and subjected to a pressure of 275 bars for one minute.

In these experiments extraction from the peel was only successful if the peel was well macerated before being pressed. The extracts were transferred into screw-top bottles and sealed to prevent the volatile oils escaping, and then stored at -20°C. Braverman (1949) reported that oil is held under a pronounced turgor pressure within the oil glands situated in the flavedo. He also mentioned that the physical constants of citrus oils remained unchanged after storage for 20 months in sealed bottles, whether kept in cold storage or at room temperature.

Antifungal determinations

Macerated mycelium was treated with commercial d-limonene and peel extracts before being added to 10 ml aliquots of molten PDA and poured into Petri dishes. Three concentrations of d-limonene, viz. 0.25, 0.50 and 1.00 percent were tested. Albedo and flavedo extracts were applied at a concentration of 1.00 percent V/V. Control treatments with distilled water, were included for comparison. Immediately after pouring, the Petri dishes were well rotated to ensure a uniform distribution of both the test solution and the inoculum within the medium. The plates were then enclosed in polyethylene bags and incubated at 27°C for 5 and 10 days before colony counts were taken.

The flavedo and albedo extracts were sterilised with BPL according to the method described. Sterilization by filtration was tried initially but proved unsatifactory as the lighter terpene fractions remained on the filter pad.

Osmotic pressure determinations

An Osmometer, which measures the freezing point depression in terms of milliosmols[1], was used to determine the osmotic pressure [2] of flavedo and albedo extracts. Since theoretically the freezing point (Δ) of a molal solution of an unionized substance is 1.86°C and the osmotic pressure (OP) of such a solution is 22.4 atmospheres then:

$$(1)\ \Delta = \frac{1.858 \times \text{milliosmols}}{1{,}000}$$

$$(2)\ \text{OP} : 22.4 = \Delta : 1.86$$

$$\text{OP} = 12.04\ \Delta \text{ atmospheres.}$$

Averages were taken from three readings and the results expressed in terms of osmotic pressure in milliosmols.

Orchard experiments

Effect of wind, thrips and red mite

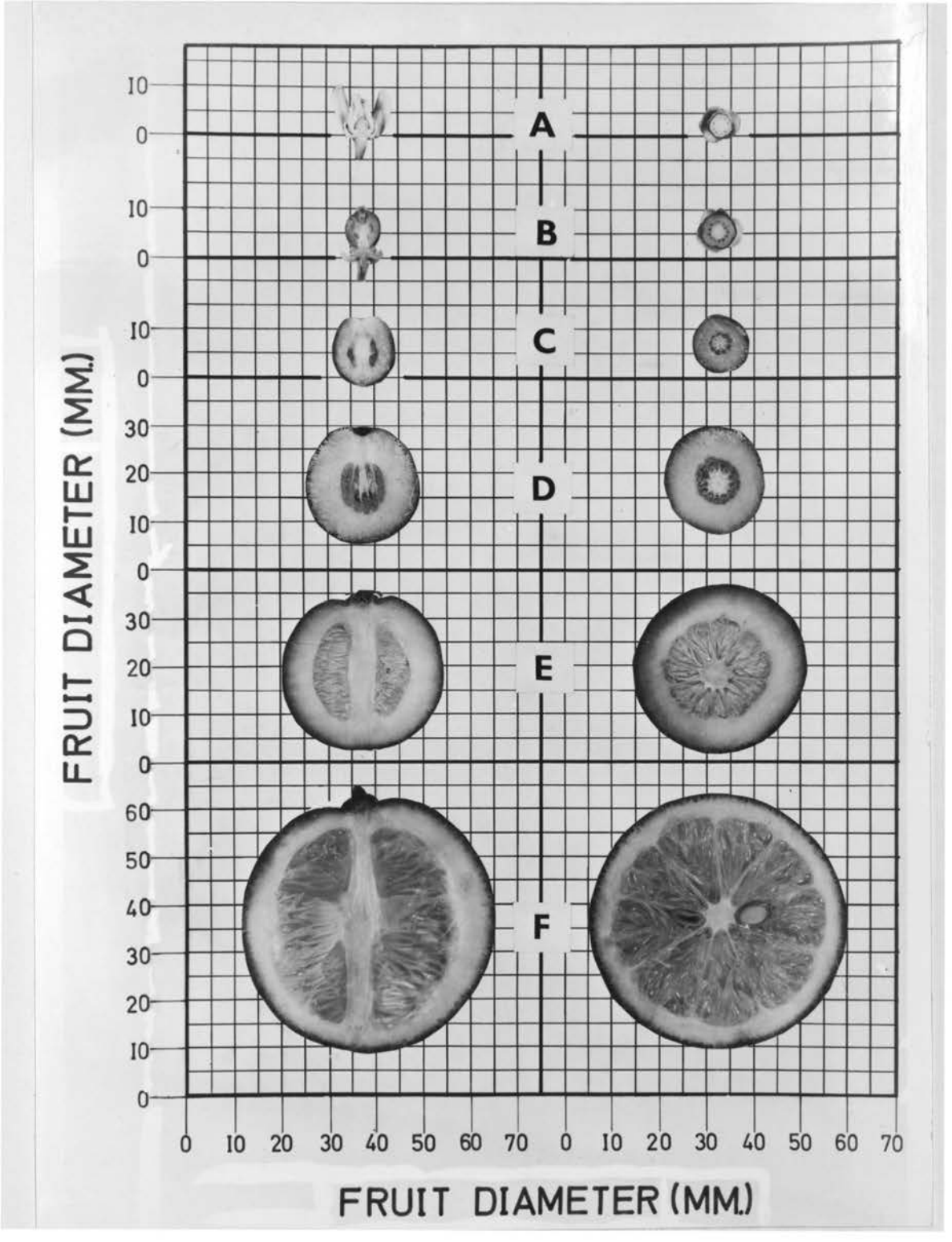
In the first experiment three possible primary factors causing side scars on citrus fruits, viz. wind, citrus thrips, Scirtothrips aurantii and citrus red mite Panonychus citri, were investigated. The experiments were conducted in the Nelspruit area in an orchard comprised of three-year-old Hamlin and Pineapple midseason cultivars planted alternately. This orchard was chosen because in previous seasons these oranges had been severely blemished. The various treatments were as follows:

(i) Wind. Trees were enclosed on all four sides by 10 foot high reed-thatch screens, while the rest of the orchard was left exposed to natural wind conditions (Figure 3).

(ii) Citrus thrips. Two spray applications of parathion 25 percent wettable powder at 1.35 kg/455 l, viz. 3 lb/100 gals water

Figure 3 - Vertical screens of 10 foot high reed-thatch built as a windbreak around young citrus trees

Figure 4 - Average fruit diameters (mm) in relation to the time of the year. A. September; B. and C. October; D. November; E. December and F. January



were applied at petal drop and six weeks later in certain treatments. Thrip infestations built up rapidly in those treatments not receiving parathion, especially on those trees inside the screens.

(iii) Citrus red mite. Effective control was obtained with kelthane 18.5 percent wp at 0.91 kg/455 l or 2 lb/100 gals water plus 0.5 percent summer oil emulsion.

In a second experiment the effect of wind damage was determined at various stages of fruit development. Trees were protected from wind by vertical screens, as described in (i) above. The trees were shaken by hand for five minute periods at different stages of fruit development (Figure 4 and Table 44). This was done to simulate the severe wind turbulence frequently encountered just prior to a Summer storm. These young orange trees were only 6 to 8 feet high, so shaking them manually was effective. Insecticidal sprays were applied for citrus thrip and red mite control.

The following forms of blemish were distinguished on fruits at harvest: (Figure 12, 1 - 5)

1. Thrip ring; characteristic ring at the stem-end
2. Continuous grey to brown blemish

Table 3 - Mature oranges classed into grades according to the extent of markings

Grade	Percentage of total area of the fruit affected
0	None
1	< 25
2	25 to 50
3	50 to 75
4	75 to 100

Table 4 - Average diameters of Valencia and Navel fruits at the time of mechanical injury

Date of injury	Average fruit diameters (mm)	
	Navels	Valencias
October	24	20
November	39	31
December	49	42
January	56	44

3. Broken, grey to brown blemish

4. Scratches and scabby lesions

5. Sunken scars and pits

These blemishes (except for 1. above) were classed into the various grades in Table 3.

Phytotoxic effects of citrus rind extracts

Comparisons were made of the possible injurious effects of citrus oils extracted from Valencia and Navel fruits at various stages of growth. The cold press method, as described by Bartholomew and Sinclair (1952) was used to extract citrus oils from the fruits. Peel discs (11 mm diameter) were cut with a cork-borer around the equator of each fruit. From three to eight discs were cut per fruit which were of three diameters (viz. 15, 25 and 35 mm). The oil was pressed from these discs and recovered with a micro-pipette. The oil obtained from 12 discs was used to treat approximately 100 fruits which were of the same diameters as described above. Positions were ringed on the equator of each fruit and each circle was randomly allotted a number from 1 to 3 (Table 49). One µml of extract was centrally placed in each ringed area. Treated fruits were protected from outside injury by enclosing each in an open-ended paper bag. Insecticides were applied for the control of thrips and mite infestations.

Mechanical injury

Valencia and Navel oranges were selected (10 fruits from each of five trees) from two orchards at Nelspruit. Using a marker pen, four positions were ringed on the equator of each fruit and each circle was randomly allotted a number from 1 to 4 (Table 47). At each fruit size a small metal tube of one mm internal diameter was used to mark the fruit in these numbered areas to a depth of one mm. The tube was enclosed in a rubber sleeve except for the tip of one mm.

These marks on the fruits were classed into the following grades:

1 = central area of circle cut into the rind, remained alive. 2 to 4 = central area dead, which later formed either a shallow depression (grade 2) or holes in the fruit two and four mm maximum in depth, grades 3 and 4 respectively.

Copper markings

The accentuation of fruit blemishes by copper fungicide applications was determined on three year old midseason and Valencia trees at Nelspruit and Alkmaar respectively, in the Eastern Transvaal Lowveld. Trees were planted on an exposed slope. The following materials were used:

Cu = Cuprous oxide (50 percent metallic copper) at 0.91 kg/455 l (Cu (.6) = 0.68 kg/455 l).

M = Mancozeb (80 percent active). Zinc complexed manganese ethylene bisdithiocarbamate, at 0.68 kg/455 l water.

I = parathion at 1.35 kg/455 l.

S = safener (slaked lime at 0.45 kg/455 l).

A light grade oil, spray-adjuvant, was included in the sprays at 0.25 percent for the first two applications and 0.5 percent thereafter (Table 51).

Two forms of copper injury on the fruits were recorded, namely, a blackening of the scar tissue where fruits were classed into grades 0 to 4 (Table 3) according to the extent of darkening of blemish marks and secondly, fruit stippling where fruits were graded as follows:

0 = no stipple

1 = faint, localized stipple

2 = faint, general stipple

3 = heavy, distinct stipple

The percentage exportable fruit for each treatment was determined from figures based on the standards set by the S.A. Co-operative Citrus Exchange in "Colour Prints for Blemish Standards", according to regulations under Section 43 of the Marketing Act, 1937.

Experimental design and statistical analysis

In experiments with light on fruit symptom development, the median or the middle item in an array, was calculated for each treatment and counts expressed as the number of lesions per fruit. If n (the number of fruit samples) was odd, then the median value was observation number (n+1)/2, while if n was even, the mean of observation numbers $^{n}/2$ and (n+2)/2 was taken.

In fruit blemish experiments the extent of injury was compared by using Friedmans' two-way analysis of variance by ranks, as described by Siegel (1956). This test determines whether it is likely that the samples came from the same population i.e. whether the rank totals (Rj) differed significantly. The statistic χ^2 was calculated as follows:

$$\chi_r^2 = \frac{12}{NK\ (k+1)} \sum_{j=i}^{k} (Rj)^2 - 3N\ (k+1)$$

N = number of samples

k = number of treatments

Rj = sum of ranks in the jth column

$\sum$ = sum of squares for ranks in all treatments.

If χ_r^2 is equal to or greater than χ^2 in Table C for a DF value = k-1, it implies that the sums of ranks for various treatments differ significantly. According to Reinach (1966) the LSD_{FR}, at the 0.05 level of significance,

can be calculated as follows:

$$LSD_{FR} = \sqrt{{}^{1}/_{12}\ NK\ (k+1)}\ .q\alpha\ dk\alpha$$

where q is the value (in studentized range tables) for k and α DF.

The Tables reflect figures rounded off to one or two decimals from the original analyses which were computed to four decimals. Under the circumstances the column and row averages can differ from the average of the values in the various cells.

RESULTS

Physiological studies

Light and temperature effects

Fruit symptom development

In the first experiment the effect of light and temperature was determined on the development of symptoms on unsprayed, mature Valencia fruits with latent infections of *G. citricarpa*.

The following treatments were used in a factorially arranged experiment:

(i) Continuous light, alternating light/dark (12 hourly) and continuous dark.

(ii) Two temperature levels viz. 20° and 27°C.

(iii) Two treatment periods viz. 10 and 15 days.

The experimental design was a randomized block and 20 fruits were allotted to each treatment. The experiment was repeated in July, August and September respectively, and each was regarded as a replicate.

Fruit lesion counts were taken and statistical analyses were applied to the calculated median values (described under Procedures). The $\sqrt{x+1}$ transformation was applied to the data to stabilise variance. Tukey's test of additivity (Snedecor, 1962) was applied to treatments

Figure 5 - Black spot lesions on fruits exposed to light and dark treatments after 15 days at 27°C A. Continuous dark B. Alternating light and dark C. Continuous light

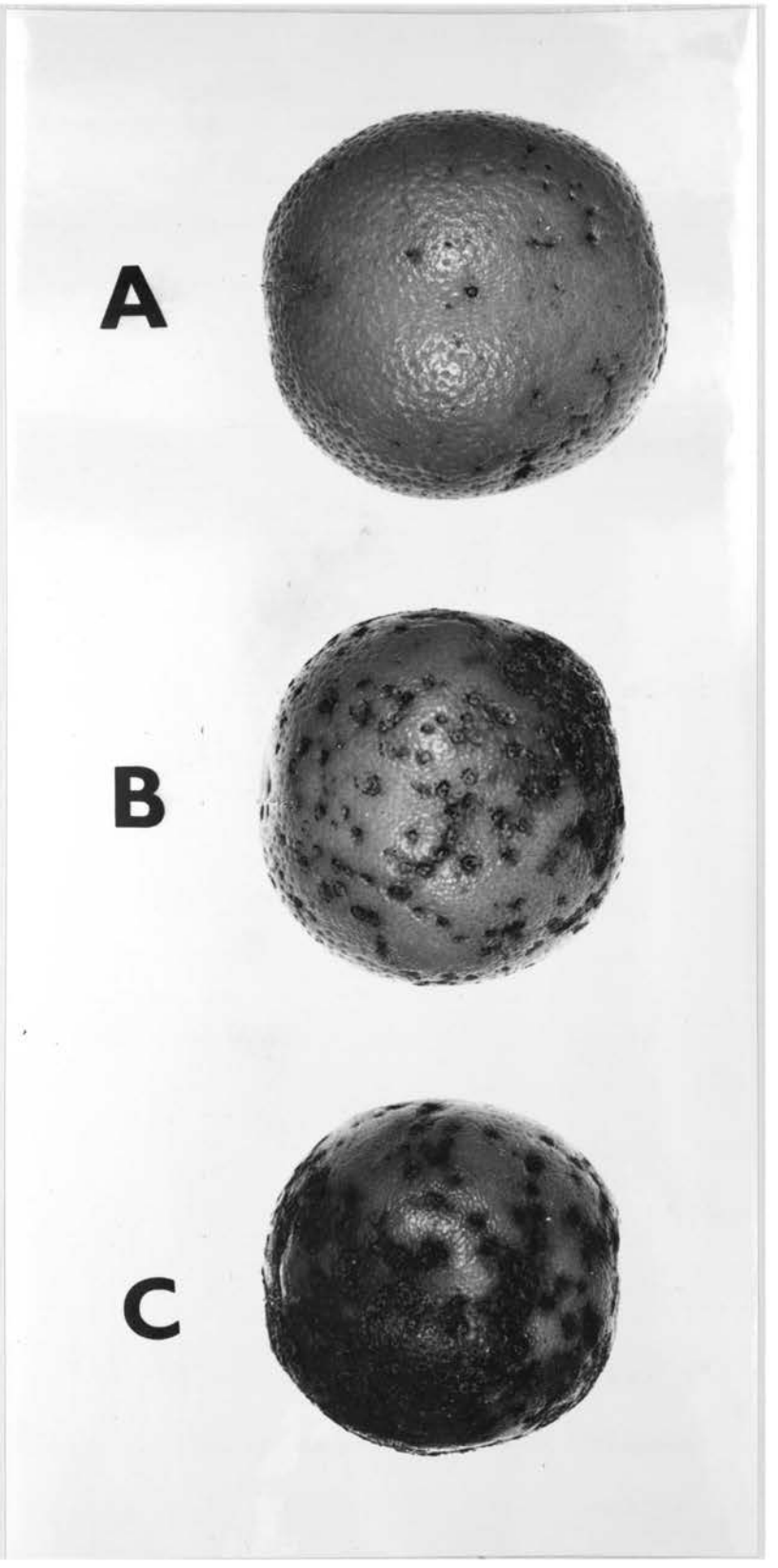


Figure 6 - The number of black spot lesions on fruits exposed to various light and temperature treatments for different periods

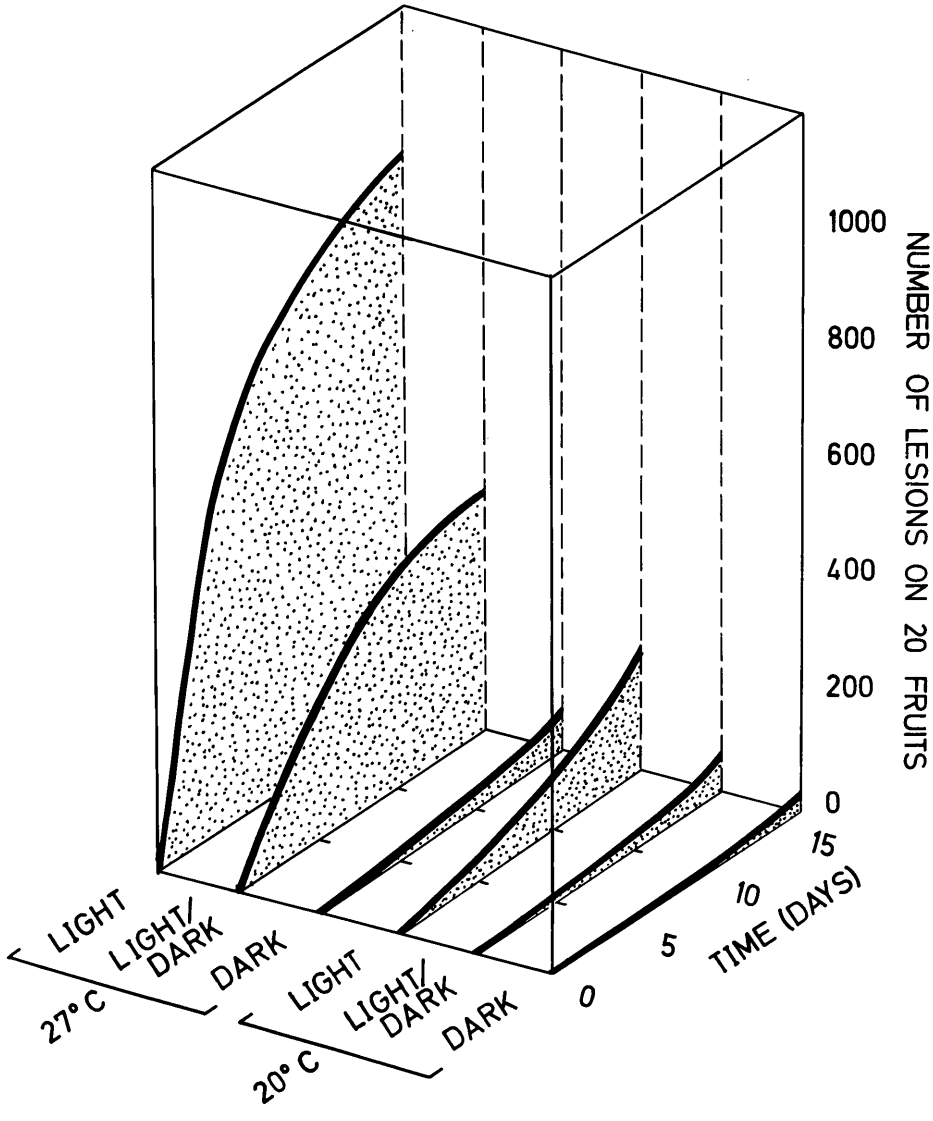


Table 5 - Average number of black spot lesions on fruits exposed to various light and temperature treatments for different periods

Treatments	Temperature (°C)				Average
	20°		27°		
	Period (days)		Period (days)		
	10	15	10	15	
Light	5.00	9.00	26.33	56.66	24.25*
Light/Dark	3.33	4.66	13.33	35.66	14.24*
Dark	1.33	2.00	2.66	4.00	2.50
Average	4.22		23.11*		

*Significantly different (P = 0.05)

Average of fruit lesion counts after 15 days was 18.66*, which differed significantly from that after 10 days i.e. 8.66

Table 6 - Analysis of variance

Source	DF	SS	MS	F test	P= 0.05	P= 0.01
Light(L)	2	45.81	22.91	38.83**	3.44	5.72
Periods(P)	1	9.77	9.77	16.56**	4.30	7.94
Temp.(Te)	1	44.86	44.86	76.03**	4.30	7.94
L x P	2	2.74	1.37	2.32	3.44	5.72
L x Te	2	15.68	7.84	13.29**	3.44	5.72
P x Te	1	3.30	3.30	5.59*	4.30	7.94
L x P x Te	2	1.26	0.63	1.07	3.44	5.72
Replicates	2	15.83	7.82	13.25**	3.44	5.72
Error	22	12.92	0.59			
Total	35	152.17				

Table 7 - Tukey's test of additivity

Source	DF	SS	MS	F test	f= 0.05	f= 0.01
Treatments	11					
Replicates	2	7.8200				
Error	22	12.9200				
Non-additivity	1	0.0728	0.0728	0.119**	4.32	8.02
For testing	21	12.8472	0.6117			

and replicates. Additivity was confirmed (Table 7), proving that the transformation was effective in stabilising variance.

The light source consisted of two 40 W daylight tubes. The light intensity at bench level varied from 8 to 12 Lux units (Lux = number of Lumen/m^2) depending on the positicn below the light source. Fruits were rotated regularly to ensure an even distribution of light. To prevent excessive moisture loss, fruits were enclosed in polyethylene bags for the duration of the experiment.

The results in Figures 5 and 6 and Tables 5, 6 and 7, show that continuous light resulted in significantly more fruit lesions than alternating light and dark, which in turn was significantly higher than the dark treatment. Secondly, raising the temperature from 20° to 27°C resulted in a significant increase in the number of black spot lesions on the fruits. Thirdly, the average fruit lesion counts were significantly higher in treatments after 15 days as compared with 10 days. Fourthly, results show that continuous light at 27°C was significantly better than any of the other treatment combinations.

Results given in Table 6 show that the three replicates differed significantly. Fruits picked in September showed a higher incidence of disease development than fruits picked in July and August.

Lesions which developed on the fruits were mainly "freckle spot" and in severe cases, "virulent spot" (Figure 1). First signs were the collapse of the flavedo into depressed spots up to two mm in diameter. At first the lesions were colourless but later the edge of the spot became reddish and the central area turned grey and became covered in pinpoint black pycnidia.

A second experiment was conducted to determine whether light has a direct or possibly an indirect effect on fruit symptom development.

There were two treatments namely, continuous light and continuous dark, applied simultaneously to opposite halves of mature, unsprayed Valencia oranges in the laboratory. The experiment was designed as a randomized block and 15 fruits were allotted per treatment. Fruits were fitted tightly into holes in a light-proof box in such a way that one half of each fruit was exposed to the light and the other half kept in the dark (see under Procedures). The temperature was kept at 27°C and fruit lesion counts were taken after 15 days. * Fruits retained their original turgidity as each fruit was separately enclosed in a polyethylene bag.

The experiment was repeated four times from July to September, and each was regarded as a replicate. The data, analysed statistically, is given in Table 9.

Figure 7 - Lesions on fruits after 15 days at 27°C

A. Halves of fruit in dark

B. Corresponding halves in light

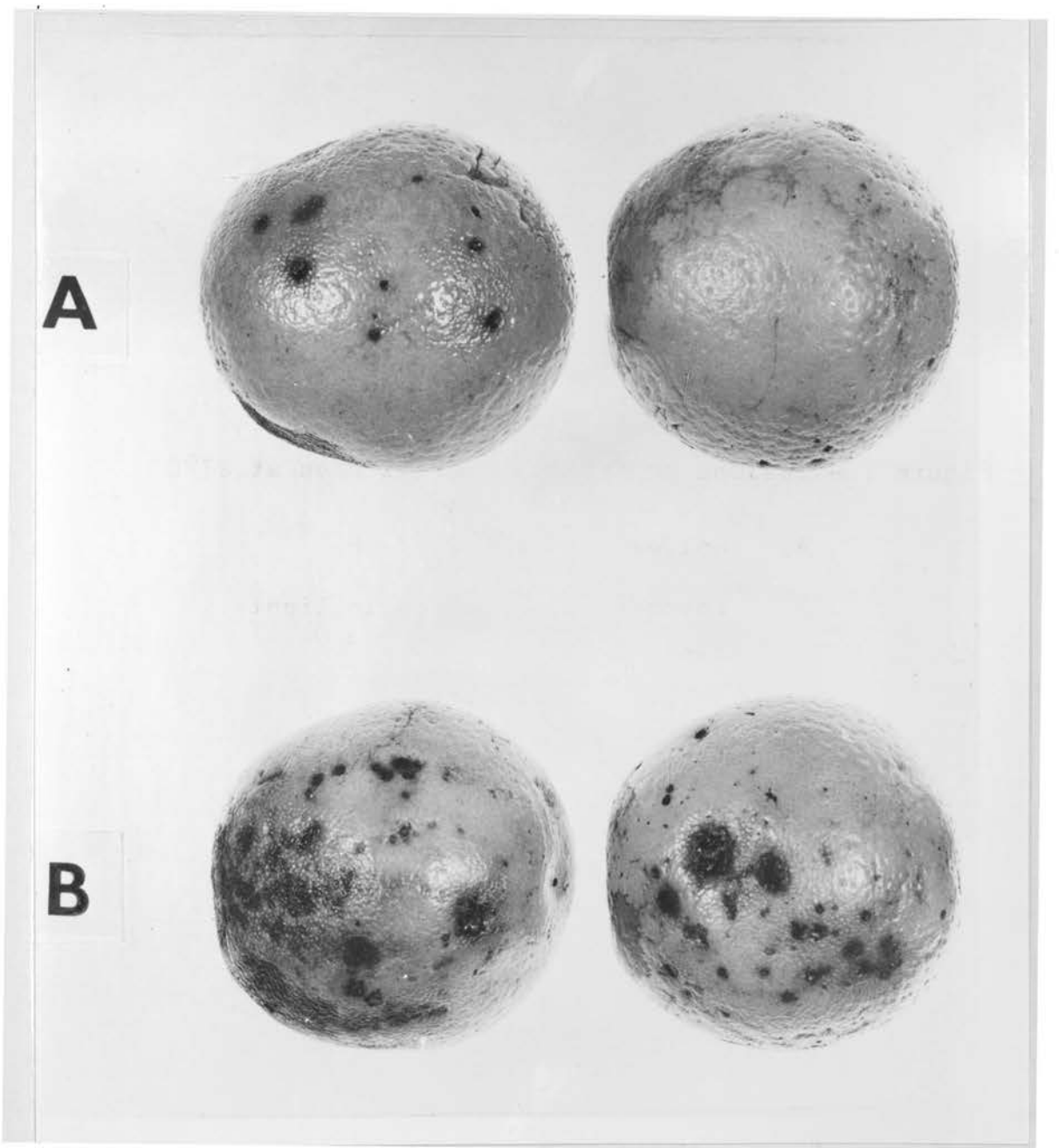


Table 8 - Total number of black spot lesions on opposite halves of Valencia oranges exposed to light and dark for 15 days at 27°C.

Replicates	Total number of lesions	
	Light	Dark
1	543	89
2	371	88
3	491	162
4	140	58
Average	386.25*	99.25

*Significantly different (P = 0.05)

Table 9 - Analysis of variance

Source	DF	SS	MS	F test	P= 0.05	P= 0.01
Treatments	1	164,738	164,738	13.79*	10.13	34.12
Replicates	3	66,439	22,146	1.85		
Error	3	35,847	11,949			
Total	7	267,024				

From the results it appears that light had a direct effect on fruit symptom development (Figure 7). Results from Table 8 show that significantly more black spot occurred on that half of the fruit in the light compared to the other half in the dark.

Sporulation on peel discs

Light and temperature effects were determined on the sporulation of G. citricarpa (a) on naturally infected, symptcmless pieces of cut rind.

In this experiment the following treatments were arranged factorially i.e. continuous light, alternating light/dark (12 hourly) and continuous dark; two temperature levels viz. 20° and 27°C and two treatment periods viz. 7 and 10 days.

Peel discs, seven mm in diameter, were cut with a cork-borer from symptomless areas around the equators of 20 mature, unsprayed Valencia fruits. After surface sterilization, the pieces were aseptically transferred to Petri dishes (five per plate) and sealed in polyethylene bags.

In preliminary experiments Colletotrichum gloeosporioides developed on the peel discs but this problem was overcome by allowing the pieces to dry out initially at 30°C for a period of 48 hours. Mc Onie (1962) reported

Table 10 - Average counts of pycnidiospores of G. citricarpa (a) from peel pieces of Valencia oranges exposed to various light and temperature treatments for different periods

Treatments	Temperature (°C)				Average
	20°		27°		
	Period (days)		Period (days)		
	7	10	7	10	
Light	1	27	42	370	110.00*
Light/Dark	18	78	152	194	110.50*
Dark	0	0	0	0	0
Average	31.00		189.50*		

*Significantly different (P = 0.05)

Average pycnidiospore count after 10 days was 167.25*, which differed significantly from that after 7 days (53.25)

Table 11 - Analysis of variance

Source	DF	SS	MS	F test	P= 0.05	P= 0.01
Light(L)	1	0.5	0.5	<1		
Temp. (Te)	1	50,244.5	50,244.5	24.31**	6.61	16.26
Periods(P)	1	25,992.0	25,992.0	12.58*	6.61	16.26
L x Te L x P Te x P L x Te x P	4	8,266.1	2,066.5			

Table 12 - Average percentages of flavedo discs with moderate to heavy crust development (* ratings 3, 4 and 5) of G. citricarpa (a) after different periods of various light and temperature treatments

Treatments	Temperature (°C)			
	20°		27°	
	Period (days)		Period (days)	
	7	10	7	10
Light	19.8	36.3	36.3	23.1
Light/Dark	26.4	29.7	13.4	29.7
Dark	6.0	0.0	13.2	9.9

* Ratings given in Figure 2 B

similar difficulties with this fungus in his experiments.

At first a greyish mycelial mat of the fungus G. citricarpa (a) was observed on the surface of the peel disc and after four days a black crust embedded with pycnidia developed, covering the entire surface of the piece of peel in severe cases. Sporulation assessments were made and discs were classed into ratings 1 to 5 according to the extent of development of the fungal crust on the peel disc. Results were recorded by three independent assessors. Pycnidiospore counts were made with a haemacytometer after the fungus crust was macerated in sterile water. (see under Procedures).

Since only zero counts of pycnidiospores were obtained in the dark, these counts were regarded as being significantly different from those of the two light treatments and were omitted from the statistical analysis (Table 11).

The results in Tables 10 and 11 show that there were no significant differences in pycnidiospore counts between continuous light and alternating light/dark treatments. Secondly, significantly more pycnidiospores were produced at 27° than at 20°C. Thirdly, spore counts were significantly higher after 10 days than after 7 days.

The assessment results in Table 12 show a higher percentage of peel pieces with fungus crust (containing

pycnidia) in the two light treatments compared with that in the dark. In the light at 27°C and in the dark at both 20° and 27°C, the percentage crust-development was higher after 7 than after 10 days. In the dark treatments there was no correlation between ratings for sporulation and pycnidiospore counts (Tables 10 and 12). Secondly, in certain light treatments peel discs with a low sporulation rating (viz. 2) gave high counts of pycnidiospores and vice versa.

Sporulation in culture

In the first experiment the effects of various light and temperature treatments were determined on the sporulation of G. citricarpa (a) isolates on PDA slant cultures. The following treatments were used in a factorially arranged experiment:

(i) Continuous light, alternating light/dark (12 hourly) and continuous dark

(ii) Two temperatures viz. 20° and 27°C

(iii) Three treatment periods viz. 10, 15 and 20 days

The experimental design was in the form of a randomized block. One slant culture was allotted to each treatment. The experiment was repeated twice, and each was regarded as a replicate. A $\sqrt{x+1}$ transformation was applied to the data in order to stabilize variance.

Table 13 - Average counts of pycnidiospores (x 10^6 per ml) from a Guignardia citricarpa (a) isolate on PDA after different periods of light and temperature treatments

Light treatments	Temperature (°C)						Average
	20°			27°			
	Period (days)			Period (days)			
	10	15	20	10	15	20	
Light	7.22	9.77	6.30	0	0.07	0.22	3.93*
Light/Dark	1.52	8.57	3.02	0	0	0.20	2.22*
Dark	0	1.45	0.05	0	0.20	0.22	0.32
Average	4.41*			0.10			

*Significantly different (P = 0.05)

Average pycnidiospore counts after 10, 15 and 20 days were 1.46, 3.35* and 1.67 x 10^6 spores per ml, respectively

Table 14 - Analysis of variance

Source	DF	SS	MS	F test	P= 0.05	P= 0.01
Light (L)	2	137.20	68.60	13.87**	3.59	6.11
Periods (P)	2	35.92	17.96	3.64*	3.59	6.11
Temp. (Te)	1	327.37	327.37	66.27**	4.45	8.40
L x P	4	7.92	1.98	< 1		
L x Te	2	151.62	75.81	15.35**	3.59	6.11
Te x P	2	36.04	18.02	3.65*	3.59	6.11
L x P x Te	4	12.02	3.01	< 1		
Replicates	1	25.91	25.91	5.24*	4.45	8.40
Error	17	84.01	4.94			
Total	35	818.01				

G. citricarpa (a) was grown on PDA slants for five days in an incubator at 25°C to promote initial fungal growth. The slants were enclosed in polyethylene bags to prevent them from drying out for the duration of the experiment viz. 20 days. Cultures were rotated regularly to ensure a uniform distribution of light in the light treatments. The light intensity measured at culture level was approximately 10 Lux units (see under Procedures).

Results were taken by scraping the cultures from the agar surface and macerating each in 0.5 ml sterile distilled water. After 24 hours counts were taken of pycnidiospores with a haemacytometer, and the results given in terms of 10^6 spores/ml.

Results from Tables 13 and 14 show that a significant increase in pycnidiospore production occurred in the light compared with the alternating light/dark treatment. Lowest counts were recorded in the dark treatment which differed significantly from both light treatments. Pycnidiospore counts were significantly higher at 20°C than at 27°C. The optimal sporulation period was 15 days where average spore counts differed significantly from those after 10 and 20 days.

Light and temperature interaction effects were significant (Table 14). Continuous light at 20°C and alternating light and dark at 20°C proved to be significantly better than any other treatment combination.

Results from Table 14 show that replicates differed significantly. This may be attributed to the fact that the inoculum load in the one experiment could have been greater than in the other.

In a second experiment the following treatments were arranged factorially viz. continuous light, alternating light/dark (12 hourly) and continuous dark; two media viz. PDA and basal synthetic agar (BSA) and three treatment periods viz. 10, 15 and 20 days.

The temperature was maintained at 20°C in all the treatments.

The experimental design was in the form of a randomized block and one slant culture was allotted to each treatment. The experiment was repeated twice and each experiment was regarded as a replicate. The data were transformed, $\sqrt{x+1}$ transformation, in order to stabilize variance.

The pH's of PDA and BSA were 5.6 and 5.8 respectively. Experimental details are the same as those described in the previous experiment. The method adopted for assessing numbers of spermatia is given under Procedures.

Results in Tables 15 and 16 show that continuous light resulted in a significantly higher production of pycnidiospores than alternating light/dark, which in turn

Table 15 - Average counts of pycnidiospores (x 10^6 per ml) produced by an isolate of G. citricarpa (a) on different media after different periods of various light treatments at 20°C

Treatments	Media						Average
	PDA			BSA			
	Periods (days)			Periods (days)			
	10	15	20	10	15	20	
Light	7.22	12.02	4.05	1.70	8.15	3.02	6.03*
Light/Dark	1.52	8.57	3.02	2.90	4.02	2.40	3.74*
Dark	0	1.45	0.05	0	1.25	0.07	0.28
Average	4.21*			2.49			

*Significantly different (P = 0.05)

Average pycnidiospore counts after 10, 15 and 20 days were 2.22, 5.72* and 2.10 (x 10^6 spores per ml) respectively

Table 16 - Analysis of variance

Source	DF	SS	MS	F test	P= 0.05	P= 0.01
Light (L)	2	80,332	40,166	30.14**	3.59	6.11
Periods (P)	2	40,600	20,300	15.23**	3.59	6.11
Media (M)	1	10,712	10,712	8.04*	4.45	8.40
L x P	4	16,134	4,033	3.03*	2.96	4.67
L x M	2	5,929	2,964	2.22	3.59	6.11
P x M	2	4,612	2,306	1.73	3.59	6.11
L x P x M	4	8,280	2,070	1.55	2.96	4.67
Replicates	1	15,006	15,006	11.26**	4.45	8.40
Error	17	22,654	1,333			
Total	35	204,259				

Table 17 - Assessments of the number of spermatia produced by Guignardia citricarpa (a) on different media after various periods of different light and temperature treatments

Period (days)	Temperature	Treatments					
		Light	Light/Dark	Dark	Light	Light/Dark	Dark
		PDA	PDA	PDA	BSA	BSA	BSA
(1) 10	20°C	*	***	-	-	-	-
15		*	***	-	-	-	-
20		*	**	-	-	-	-
10	27°C	***	***	-	-	*	-
15		***	***	***	*	-	-
20		***	***	***	-	*	-
(2) 10	20°C	-	-	-	-	-	-
15		-	-	-	-	-	-
20		-	***	-	-	-	-
10	27°C	-	-	-	-	-	-
15		**	***	***	-	-	-
20		**	***	***	-	-	-

(1) Replicate 1 and (2) Replicate 2

Assessment (Spermatia per ml)

-	=	0 spermatia
*	=	max. of 30×10^6
**	=	max. of 100×10^6
***	=	max. of 200×10^6

proved significantly superior to the dark treatment. Pycnidiospore counts were significantly higher on PDA compared with BSA. The maximum spore production was recorded after 15 days where the average count was significantly higher than that after 10 and 20 days.

From Table 17 results show a higher number of spermatia on PDA compared to BSA, and at 27° compared to 20°C.

In the next experiment, the effect of light and temperature conditions approximating those occurring in the packhouses at harvest, were determined on the sporulation of G. citricarpa (a) in artificial culture.

The following treatments were used in a factorially arranged experiment:

(i) Light at 27°C alternating 12 hourly with dark at 20°C; continuous light at 20°C and thirdly continuous light at 27°C

(ii) Three treatment periods viz. 10, 15 and 20 days

The experimental design was in the form of a randomized block with four replicates per treatment.

Two 40 W daylight tubes were used as the light source, and the light intensity at culture level was approximately 10 Lux units. Pycnidiospore counts and spermatial assessments were taken. Experimental details

Figure 8 - Average pycnidiospore production of *Guignardia citricarpa* (a) after various light and temperature treatments for different periods

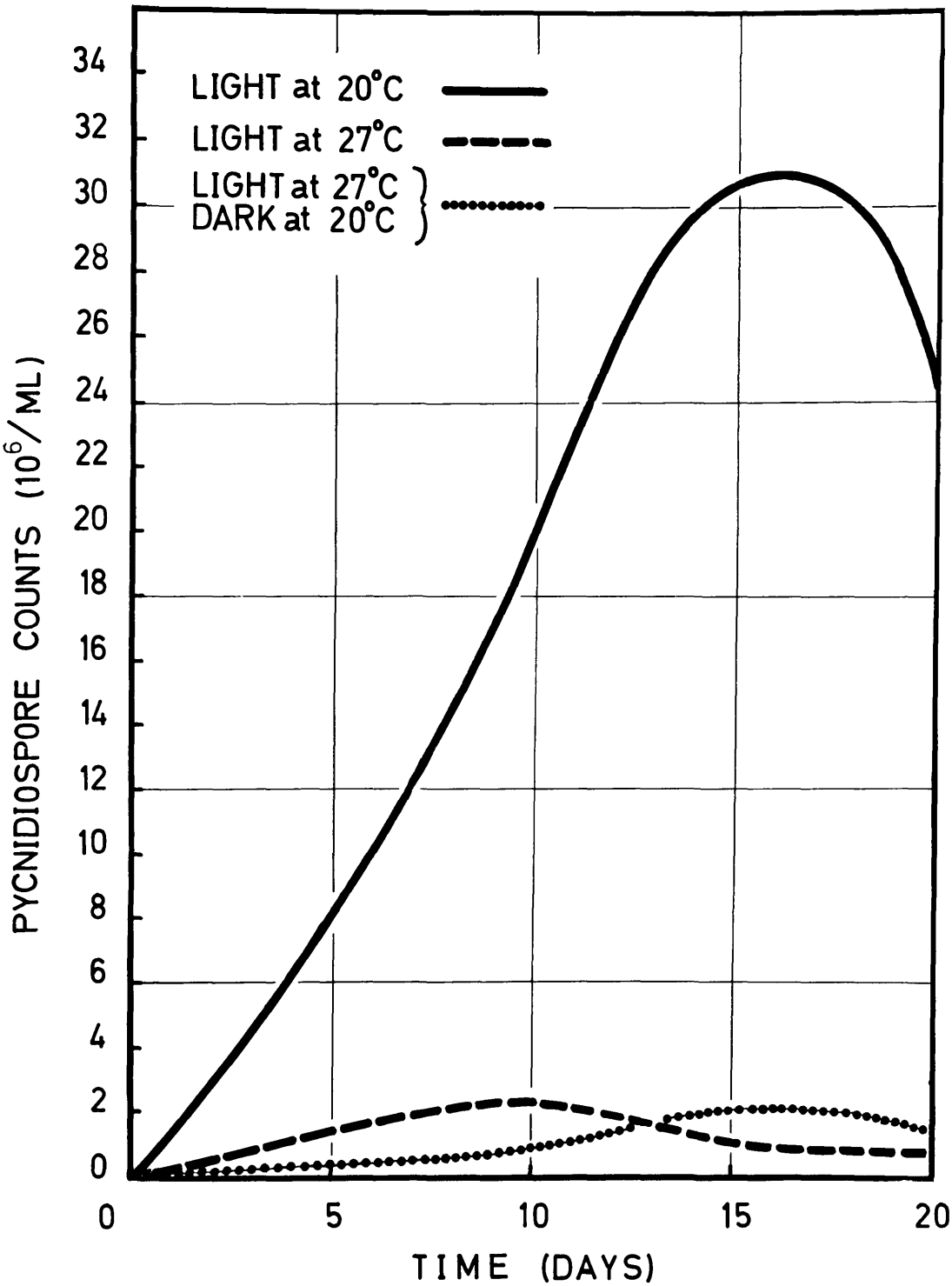


Table 18 - Average number of pycnidiospores produced by Guignardia citricarpa (a) after different periods of various light and temperature treatments

Light and Temperature treatments	Average pycnidiospore count (x 10^6 per ml) after:		
	10 Days	15 Days	20 Days
Light at 20°C	20.563*	32.125*	24.375*
Light at 27°C	2.400	1.025	0.975
Light at 27°C and dark at 20°C	0.625	1.963	1.700
LSD 0.05	3.880		

*Significantly different (P = 0.05)

Table 19 - Analysis of variance

Source	DF	SS	MS	F test	P= 0.05	P= 0.01
Light(L)	2	1,880,187	940,093.50	677.26**	3.40	5.61
Periods(P)	2	37,301	18,650.50	13.44**	3.40	5.61
L x P	4	77,487	19,371.75	13.96**	2.78	4.22
Replicates	3	3,776	1,258.66	0.91	3.01	4.72
Error	24	33,314	1,388.08			
Total	35	2,032,065				

Table 20 - Assessments of the number of spermatia produced by Guignardia citricarpa (a) exposed to different light and temperature treatments for different periods

Treatments	Treatment period											
	10 days				15 days				20 days			
	Replicates				Replicates				Replicates			
	1	2	3	4	1	2	3	4	1	2	3	4
Light 20°C	-	*	*	-	-	*	-	*	-	*	-	*
Light 27°C	**	*	*	***	**	***	**	*	**	*	**	**
Alternating Light 27°C and Dark 20°C	**	***	*	*	***	**	***	***	**	**	**	***

Spermatia per ml

- = 0 spermatia
* = max. of 30 x 10^6
** = max. of 100 x 10^6
*** = max. of 200 x 10^6

are described in the above experiment.

From Tables 18 and 19 and Figure 8, results show that continuous light at 20°C resulted in a significantly higher production of pycnidiospores than continuous light at 27°C and alternating light at 27°C/dark at 20°C. Secondly, pycnidiospore production was significantly higher after 15 days than after 10 and 20 days.

Results from Table 20 show that spermatial production was higher at 27° than at 20°C. This confirms results obtained in the previous experiment.

Growth in culture

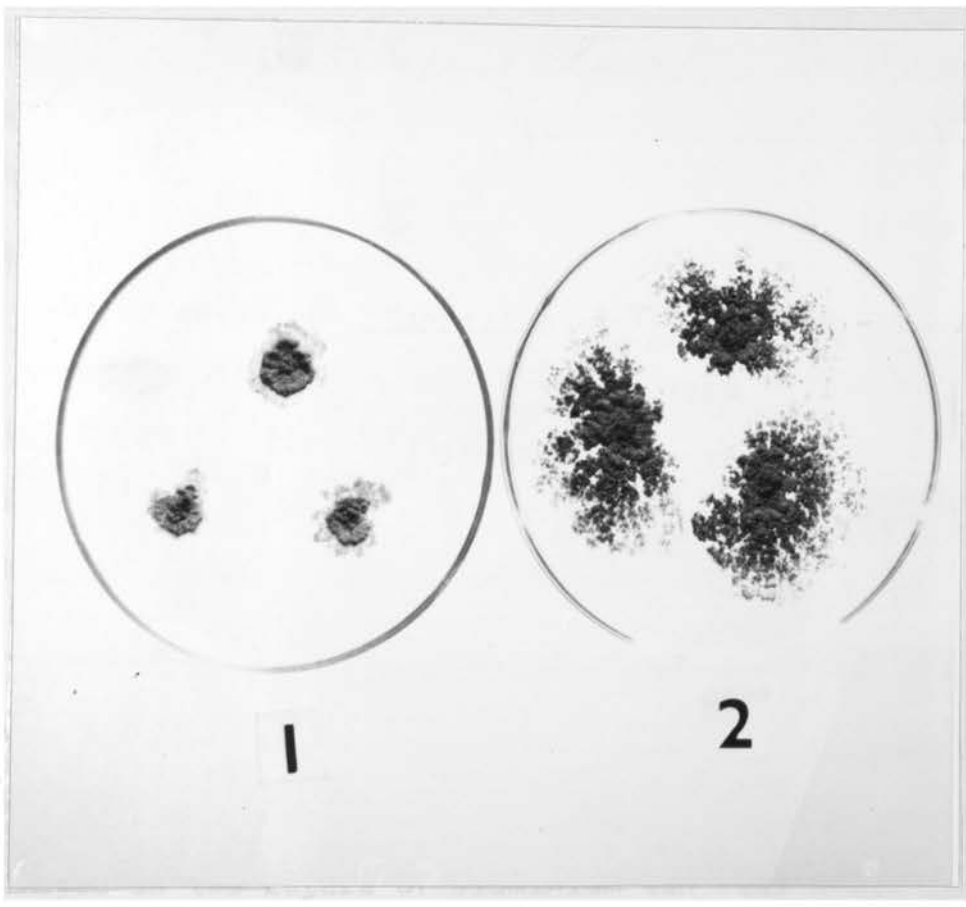
In previous experiments results showed that light markedly increased symptom expression of black spot on the fruits. The following experiments were conducted to determine the possible effect of light on the growth of the pathogen in culture.

In preliminary trials G. citricarpa (a) colonies on PDA plates, when exposed to artificial light, grew in a spreading fashion with fan-like extensions. In the dark, colonies were raised and compact in comparison (Figure 9). If colony diameters were used as a criterion of growth, then it would appear that light resulted in an increased growth of this fungus in culture. Cochrane (1958) however,

Figure 9 - Colonies of G. citricarpa (a) grown on PDA

1. Dark

2. Artificial light



mentioned the unsuitability of this method particularly where fungus colonies have an irregular outline.

In the following experiments G. citricarpa (a) was grown in liquid basal synthetic medium as described and exposed to light and dark conditions for periods ranging from 10 to 30 days.

In the first experiment the following treatments were arranged factorially viz. continuous light and continuous dark, and two treatment periods viz. 10 and 20 days. The temperature was maintained at 27°C in all the treatments.

The experiment was a 2 x 2 factorial with light treatments and periods as factors. The experimental design was a randomized block. Each treatment was replicated three times.

The fungus was grown in 125 ml flasks containing 50 ml of basal synthetic medium previously sterilized with BPL. In the dark treatment, flasks were completely wrapped in two layers of aluminium tin foil while in the light treatment, only the flask openings were covered with tin foil.

The flasks were randomly arranged on the bed of a controlled environment incubator-shaker with a light source mounted 46 cm above the flasks. The light intensity was measured at 10 Lux units, the temperature was set at

Table 21 - Average mycelium weights (mgm) of G. citricarpa (a) after different periods of various light treatments

Light treatments	Period		Average
	10 days	20 days	
Light	41.03	106.50*	73.76*
Dark	42.73	61.50	52.11
Averages	41.88	84.00*	

*Significantly different (P = 0.05)

Table 22 - Analysis of variance

Source	DF	SS	MS	F test	P= 0.05	P= 0.01
Light	1	1,437.85	1,437.85	15.06**	5.99	13.74
Periods	1	5,325.65	5,325.65	55.79**	5.99	13.74
L x P	1	1,608.49	1,608.49	16.85**	5.99	13.74
Replicates	2	148.83	74.42	< 1		
Error	6	572.71	95.45			
Total	11	9,093.53				

27°C and the shaker speed at 50 rpm. The mycelium inoculum was prepared by removing three globose colonies from an 11 day old shake culture and macerating it in 10 ml of sterile water with a MSE homogeniser for three minutes. After diluting to 50 ml with sterile water, 0.15 ml of the mycelial suspension was used as inoculum for each flask. Mycelium weights were taken after 10 and 20 days as described under Procedures.

Cotton wool stoppers were used initially but under the conditions of this experiment, (i.e. enclosed within tin foil) they were moist and later became contaminated with various fungi. Aluminium tin foil discs were used to replace the cotton stoppers and by holding the disc over the flask opening and tapping lightly, a tight fit was ensured.

Results from Tables 21 and 22 show that after 10 days there were no significant differences between light and dark treatments but after 20 days the mycelium weights in the light were significantly greater than those in the dark. Secondly, mycelium weights after 20 days were significantly greater than those after 10 days.

Colonies in the dark were a distinct yellow while those in the light were pink to beige coloured. Numbers of colonies were greater in the light than in the dark, although smaller in size than in the latter treatment. This was especially noticeable after 20 days.

Table 23 - Average mycelium weights (mgm) of G. citricarpa (a) after different periods of light treatments

Light treatments	Periods		Average
	15 days	30 days	
Light	209.55	267.72	238.63*
Dark	104.40	220.42	162.41
Average	156.98	244.07*	

*Significantly different (P = 0.05)

Table 24 - Analysis of variance

Source	DF	SS	MS	F test	P= 0.05	P= 0.01
Light	1	23,195.29	23,195.29	23.91**	5.12	10.56
Periods	1	30,223.82	30,223.82	31.16**	5.12	10.56
L x P	1	3,306.25	3,306.25	3.41		
Replicate	3	2,471.04	823.68	< 1		
Error	9	8,730.72	970.08			
Total	15	67,927.12				

In an attempt to accelerate the break-up of mycelium, indicated in the light treatment in the previous experiment, the shaker speed was increased from 50 to 65 rpm.

In this experiment the following treatments were arranged factorially viz. continuous light and continuous dark and two treatment periods viz. 15 and 30 days. The experimental design was in the form of a randomized block and treatments were replicated four times. Experimental details were the same as that described in the previous experiment.

From Tables 23 and 24 results show that mycelium weights in the light were significantly greater than those in the dark. After 15 days the growth in the light was nearly twice that in the dark. Secondly, the average mycelium weight after 30 days was significantly greater than that after 15 days.

In the next experiment the fungus was grown in two different media. The one medium was unexposed while the other was exposed to light for 16 days prior to inoculation.

The following treatments were used in a factorially arranged experiment:

(i) Continuous light and continuous dark

(ii) Two media viz. BSM unexposed and BSM exposed to light for 16 days prior to inoculation

(iii) Two treatment periods viz. 15 and 30 days

The experiment was designed as a randomized block with 10 replicates per treatment.

Flasks in the dark treatment were wrapped as far as the rim with aluminium tin foil and the openings were closed with dark-coloured cotton wool stoppers. Flasks in the light were treated similarly except that they were not enclosed with tin foil.

Results from Tables 25 and 26 show that after 15 days mycelium weights were significantly greater in continuous light compared with those in continuous dark while after 30 days differences between these treatments were not significant. Secondly, the average weights were significantly higher after 30 days compared with those of 15 days. Thirdly, the average mycelium weights in the medium exposed to light for 16 days prior to inoculation, were significantly greater than where the medium was inoculated immediately. Interaction effects between light and treatment periods were significant.

The average numbers of colonies per flask and colony diameters in the light after 15 days were 27.8 and 5 mm respectively, and that in the dark, 10.6 and 9 mm. After 30 days, besides the larger colonies, numerous small colonies, approximately 1 mm in diameter, were observed in both the light and dark treatments.

Table 25 - Average mycelium weights (mgm) of G. citricarpa (a) grown on two liquid media after two periods and different light treatments

Light treatments	Periods			
	15 days		30 days	
	Medium		Medium	
	Inoculated immediately	Inoculated after 16 days exposure to light	Inoculated immediately	Inoculated after 16 days exposure to light
Light	219.80*	267.64*	294.90	322.14
Dark	184.15	181.11	285.62	317.01
Averages	201.98	224.38	290.26	319.58
	213.18		304.92*	

*Significantly different (P = 0.05)

Table 26 - Analysis of variance

Source	DF	SS	MS	F test	P= 0.05	P= 0.01
Light(L)	1	23,351.80	23,351.80	16.15**	3.99	7.04
Medium(M)	1	13,349.00	13,349.00	9.24**	3.99	7.04
Periods(P)	1	168,436.30	168,436.30	116.53**	3.99	7.04
L x M	1	2,719.00	2,719.00	1.88		
L x P	1	14,473.70	14,473.70	10.01**	3.99	7.04
M x P	1	215.90	215.90	< 1		
L x M x P	1	3,817.98	3,817.98	2.64		
Replicates	9	18,768.90	2,085.43	1.44	2.02	2.70
Error	63	91,064.54	1,445.47			
Total	79	336,197.12				

Temperature studies on growth were conducted by Wager (1952) with isolates of G. citricarpa grown on solid media. He took colony measurements (diameters) and reported inconsistent results due to the irregular outline of the colonies.

In the next experiment the effect of temperature was determined on the growth of three isolates of G. citricarpa in liquid culture. The following treatments were used in a factorially arranged experiment:

(i) Three temperature levels viz. 22°, 27° and 32°C.

(ii) Three isolates viz. isolates 1 and 2 (G. citricarpa (a)) and isolate 3 (G. citricarpa (b))

(iii) Two treatment periods viz. 10 and 20 days.

The experimental design was a randomized block with three flasks per treatment. The experiment was repeated twice and each was regarded as a replicate.

Mycelium dry weight measurements were taken as described under Procedures. To reduce possible errors resulting from inherent variations in the incubators, in the second replicate, incubators were re-allocated at random. Flasks were shaken briefly by hand on alternate days.

Results from Tables 27 and 28 and Figure 10 show that significantly greater mycelium weights occurred at 27°C compared with those at 22° and 32°C. Differences between

Table 27 - Average mycelial weights (mgm) of three isolates of G. citricarpa in liquid culture after different periods of various temperature treatments

Temperature	Isolates						Average
	1		2		3		
	Period (days)		Period (days)		Period (days)		
	10	20	10	20	10	20	
22°C	56.15	133.00	48.50	82.80	96.65	215.80	105.53
27°C	76.55	138.80	70.85	121.45	241.25	434.45	180.56*
32°C	55.25	48.95	44.80	52.40	145.60	332.15	113.19
Average	84.78		70.13		244.32*		

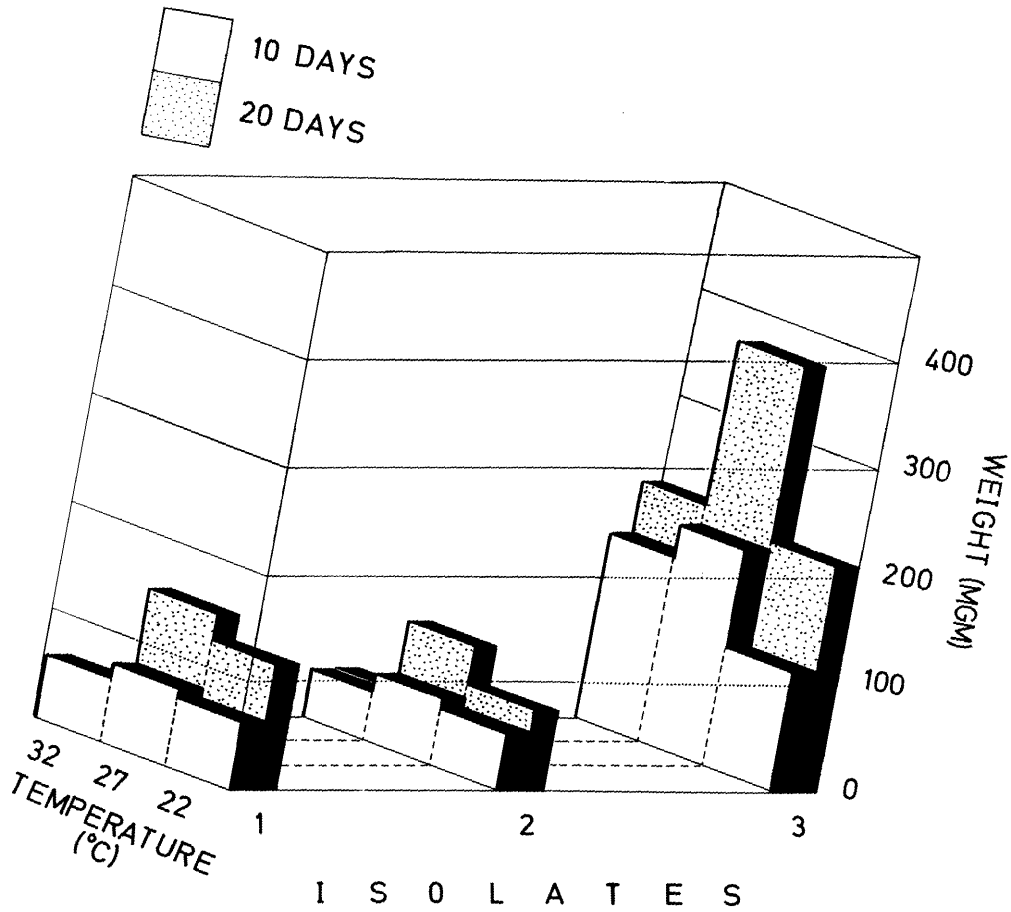
*Significantly different (P = 0.05)

Average mycelium weights after 20 days was 173.31* mgm which differed significantly from that after 10 days (i.e. 92.84 mgm)

Table 28 - Analysis of variance

Source	DF	SS	MS	F test	P= 0.05	P= 0.01
Temp.(T)	2	40,935.78	20,467.89	10.08**	3.59	6.11
Periods(P)	1	58,273.96	58,273.96	28.70**	4.45	8.40
Isolates(I)	2	224,021.37	112,010.69	55.18**	3.59	6.11
T x P	2	2,390.14	1,195.07	< 1		
T x I	4	36,612.20	9,153.05	4.51*	2.96	4.67
P x I	2	33,423.80	16,561.90	8.16**	3.59	6.11
T x P x I	4	5,851.25	1,462.81	< 1		
Replicates	1	4,816.36	4,816.36	2.37	4.45	8.40
Error	17	34,511.45	2,030.09			
Total	35	440,836.31				

Figure 10 - Average mycelial weights (mg) of three isolates of G. citricarpa in liquid culture after different periods and various temperature treatments



the last two treatments were non-significant. Secondly, results show that isolate 3 (G. citricarpa (b)) grew significantly better than either isolates 1 or 2 (G. citricarpa (a)). Thirdly, the average mycelium weights were significantly greater after the second period compared with those after the first period.

Limonene and fruit rind extracts

All citrus fruits contain oils of which 90 per cent are comprised of d-limonene. The oil is produced in oil glands embedded in the outer rind or flavedo. The possible antifungal activity, firstly of d-limonene ($C_{10}H_{16}$) and secondly of flavedo and albedo extracts (from the outer and inner areas of the rind respectively), were determined on G. citricarpa isolates in culture.

In the first experiment the following treatments were arranged factorially i.e. four concentrations of d-limonene viz. 0, 0.25, 0.50 and 1.00 per cent; two isolates, viz. isolate I (G. citricarpa (a)) and isolate 3 (G. citricarpa (b)) and two treatment periods viz. 5 and 10 days. The design was in the form of randomized blocks and treatments were replicated four times.

Various quantities of limonene were applied to macerated mycelium as described under Procedures. The number of colonies appearing on the plates after incubation

Figure 11 - Average colony counts of two isolates of G. citricarpa on PDA after different periods and various limonene treatments

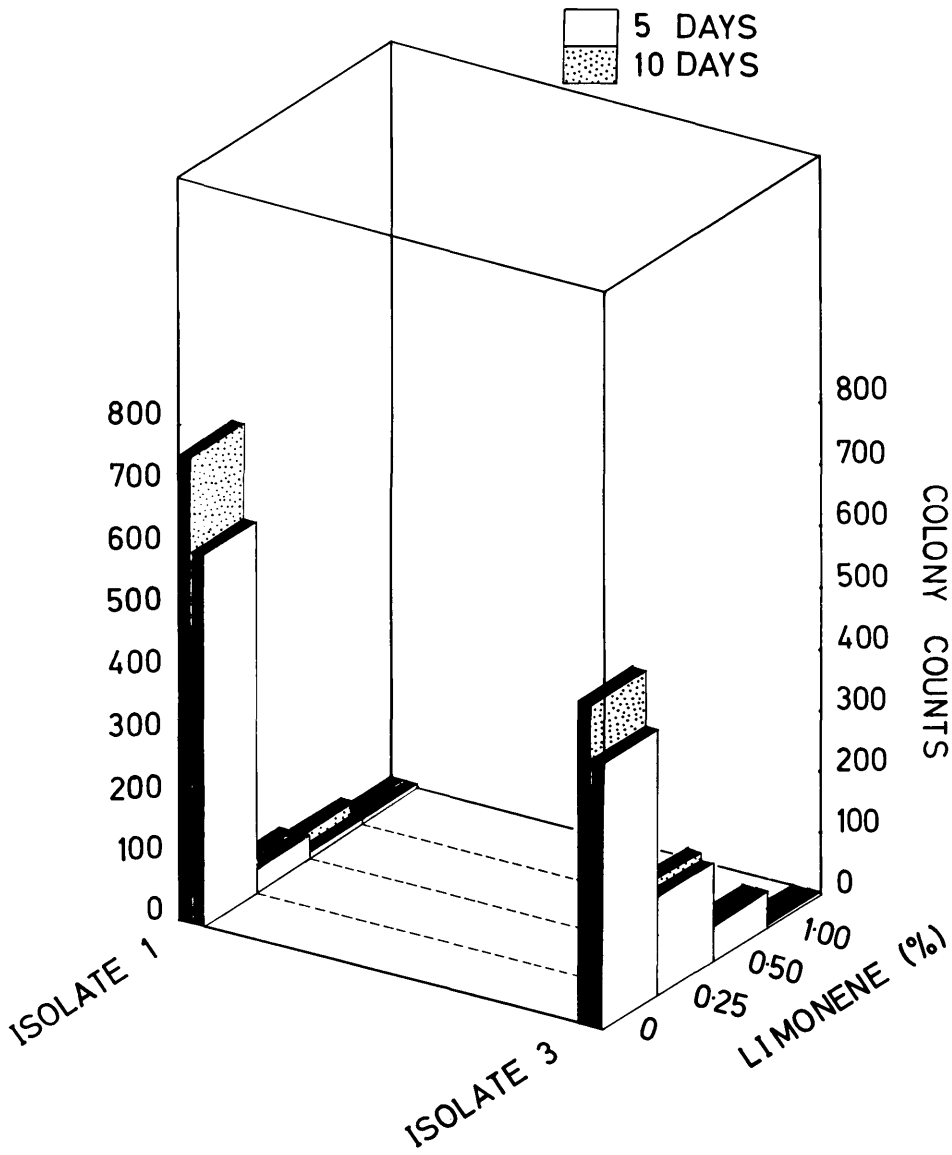


Table 29 - Average colony counts of G. citricarpa isolates 1 and 3 on PDA after two periods of various limonene treatments

Limonene concentration (%)	Isolates				Average
	1		3		
	Period (days)		Period (days)		
	5	10	5	10	
0	755.25	1051.00	706.50	875.80	847.13*
0.25	60.00	57.65	323.50	293.75	183.72*
0.50	18.75	47.50	105.25	89.76	65.31*
1.00	10.75	16.25	0	0	6.75
Average	252.18		299.32*		

*Significantly different (P = 0.05)

Average colony counts after 10 days was 303.93* which differed significantly from that after 5 days i.e. 247.50

Table 30 - Analysis of variance

Source	DF	SS	MS	F	P= 0.05	P= 0.01
Limonene (L)	3	7,224,111	2,408,037	4,894.38**	2.81	4.24
Isolates (I)	1	35,532	35,532	72.22**	4.05	7.21
Periods (P)	1	50,963	50,963	103.58**	4.05	7.21
L x I	3	281,562	93,854	190.72**	2.81	4.24
L x P	3	166,259	55,419	112.64**	2.81	4.24
I x P	1	10,404	10,404	21.15**	4.05	7.21
L x I x P	3	8,406	2,802	5.70**	2.81	4.24
Replicates	3	2,058	686	1.39		
Error	45	22,134	492			
Total	63	7,801,429				

at 25°C for 5 and 10 days, was counted and the results were analysed statistically.

Results from Figure 11 and Tables 29 and 30, show that the three limonene treatments (concentrations 0.25, 0.50 and 1.00 percent, respectively) differed significantly from the 0 per cent limonene treatment. Secondly, each of the limonene treatments differed significantly from each other. Thirdly, the average number of colonies per treatment after 10 days was significantly higher than that after 5 days. However, with isolate 3 (0.25 and 0.50 per cent limonene) and isolate 1 (0.25 per cent limonene) counts after 10 days were less than after 5 days. These differences were not significant.

From Table 25, results show that 0.25 and 0.50 percent limonene treatments resulted in a significantly greater reduction in the colony numbers in isolate 1 compared with isolate 3. However, the highest concentration, 1.00 per cent limonene, gave zero colony counts in isolate 3 which differed significantly from those of isolate 1.

The previous experiment was repeated except that the inoculum size was decreased (see under Discussion).

Results in Tables 31 and 32 show, as in the previous experiment, that the three limonene treatments significantly reduced colony counts compared with no limonene. The 0.25 per cent limonene was significantly less effective

Table 31 - Average colony counts of Guignardia citricarpa (a) and G. citricarpa (b), on PDA after two periods of various limonene treatments

Limonene (%)	Isolates				Average
	1		3		
	Period (days)		Period (days)		
	5	10	5	10	
0	449.00	437.25	162.50	179.50	307.06*
0.25	19.25	12.75	11.50	31.75	18.81*
0.50	6.25	6.50	13.50	15.00	10.31
1.00	3.25	3.00	7.25	6.75	5.06
Average	117.15*		53.47		

*Significantly different (P = 0.05)

Table 32 - Analysis of variance

Source	DF	SS	MS	F	P= 0.05	P= 0.01
Limonene	3	1,050,566.00	350,188.66	3,106.16**	2.81	4.24
Isolates(I)	1	64,897.56	64,897.56	575.64**	4.05	7.21
Periods(P)	1	100.00	100.00	< 1		
L x I	3	231,745.19	77,248.40	685.19**	2.81	4.24
L x P	3	120.25	40.08	< 1		
I x P	1	798.07	798.07	7.08*	4.05	7.21
L x I x P	3	745.68	248.56	2.20	2.81	4.24
Replicates	3	319.63	106.54	< 1		
Error	45	5,073.37	112.74			
Total	63	1,354,365.75				

than the 0.50 and 1.00 per cent limonene treatments. However, in this experiment differences between the last two treatments were not significant. A greater reduction in colony counts occurred with isolate 1 as compared with isolate 3 with 0.25 and 0.50 per cent limonene respectively. These results are similar to those in the previous experiment.

In the next two experiments, extracts from the flavedo, were compared with those from the albedo for their inhibitory action on G. citricarpa (a) in artificial culture.

The following treatments were used in a factorially arranged experiment:

(i) Flavedo and albedo extracts
(ii) North and south sides of the tree
(iii) Two treatment periods viz. 5 and 10 days

The experimental design was a randomized block with four replicates per treatment. The number of colonies appearing on the culture plates was counted and the data were analysed statistically (Table 34).

Methods of extraction from flavedo and albedo portions of Valencia oranges are described under Procedures. Fruits were randomly selected from the northern and southern aspects (five fruits per side) from five unsprayed Valencia trees during November. These fruits were dark-green and approximately 30 mm in diameter. After extraction and sterilization with BPL, the flavedo and albedo extracts

Table 33 - Average colony counts of G. citricarpa (a) after two treatment periods with flavedo and albedo extracts from fruits picked from the northern and southern sides of Valencia trees

Extracts	Position				Average
	North		South		
	Period (days)		Period (days)		
	5	10	5	10	
Flavedo	115.0	139.3	74.3	122.3	112.7
Albedo	275.0	409.0	268.3	365.3	329.4*
Average	234.56*		207.50		

*Significantly different (P = 0.05)
Average colony counts after 5 and 10 days were 272.3 and 360.3* respectively

Table 34 - Analysis of variance

Source	DF	SS	MS	F	P= 0.05	P= 0.01
Extracts(E)	1	375,627.78	375,627.78	379.77**	4.32	8.02
Position(Po)	1	5,859.03	5,859.03	5.92*	4.32	8.02
Periods(P)	1	45,980.28	45,980.28	46.49**	4.32	8.02
E x Po	1	26.26	26.26	< 1		
E x P	1	3,600.71	3,600.71	3.64	4.32	8.02
Po x P	1	88.77	88.77	< 1		
E x Po x P	1	10,844.39	10,844.39	10.96**	4.32	8.02
Replicates	3	611.07	203.69	< 1		
Error	21	20,770.68	989.08			
Total	31					

were applied to macerated mycelium as described under Procedures. A Control treatment of distilled water, was included but because positional effects could obviously not be obtained, the figures from this treatment were not included in the statistical analysis.

Results in Tables 33 and 34 show that flavedo extract resulted in a significantly greater reduction in the number of colonies of G. citricarpa compared with that of albedo extract. Secondly, flavedo extracts from fruits picked from the southern aspect of the trees resulted in significantly less colonies than that from the northern aspect.

In a second experiment the following treatments were used in a factorially arranged experiment:

(i) Flavedo and albedo extracts
(ii) North and south side of the tree
(iii) Two treatment periods viz. 5 and 10 days
(iv) Two sampling dates viz. December and January

The experimental design was in the form of randomized blocks and treatments were replicated four times. Colony counts were taken and the data, except for the Control figures, were analysed statistically (Table 36).

Results, which are given in Tables 35 and 36, show that the flavedo extracts resulted in significantly less colonies of G. citricarpa on the culture plates than that

Table 35 - Average number of colonies of G. citricarpa (a) on PDA plates after two periods, following treatment with flavedo and albedo extracts, from Valencia fruits picked at different times from two tree positions

Extracts	Periods (days)	December		January		Average
		Position		Position		
		North	South	North	South	
Flavedo	5	71.5	58.3	147.0	122.3	106.8
	10	77.7	69.5	160.8	147.5	
Albedo	5	189.7	248.3	131.8	149.0	183.7*
	10	250.5	205.5	144.0	151.0	
Control (distilled water)	5	213.8				201.4
	10	189.0				

Table 36 - Analysis of variance

Source	DF	SS	MS	F	P= 0.05	P= 0.01
Extracts(E)	1	94,633.14	94,633.14	50.02**	4.06	7.24
Position(Po)	1	118.27	118.27	< 1		
Time (Ti)	1	78.77	78.77	< 1		
Periods(P)	1	1,969.14	1,969.14	1.04	4.06	7.24
E x Po	1	2,364.39	2,364.39	1.25	4.06	7.24
E x Ti	1	95,712.89	95,712.89	50.60**	4.06	7.24
E x P	1	147.02	147.02	< 1		
Po x Ti	1	8.26	8.26	< 1		
Po x P	1	2,364.39	2,364.39	1.25	4.06	7.24
Ti x P	1	78.76	78.76	< 1		
E x Po x Ti	1	185.57	185.57	< 1		
E x Ti x P	1	159.37	159.37	< 1		
E x Po x P	1	4,241.24	4,241.24	2.24	4.06	7.24
Po x Ti x P	1	2,487.50	2,487.50	1.31	4.06	7.24
E x Po x P x Ti	1	1,881.53	1,881.53	< 1		
Replicates	3	2,063.05	687.68	< 1		
Error	45	85,127.20	1,891.72			
Total	63	293,620.49				

of albedo extracts, confirming results obtained in the preceding experiment. However, no significant differences were found between north and south positions nor between extracts from fruits in December compared with those in January.

The results from both experiments show a marked inhibitory effect of flavedo extracts which differed significantly in effectiveness from that of the albedo. As the flavedo possesses oil glands containing citrus oil (comprising 90 percent limonene) while the albedo does not, the evidence points strongly to limonene as the toxic principle.

It appears that extracts from fruits on the northern side of the tree are less toxic to G. citricarpa isolates than those from the southern side. The differences were significant in the first experiment (November fruit) but not significant in the second experiment, at a later stage of fruit development (see Discussion).

Osmotic pressure

Bain (1958) stated that the percentage moisture in the flavedo increased from approximately 70 [at an immature stage of citrus fruit development, to nearly 80 [at maturity. According to Lilly & Barnett (1951), changes in osmotic pressure of the medium may have a considerable effect on the rate and amount of growth in fungi.

The next experiment was conducted to determine whether osmotic pressure changes occur in the rind of Valencia oranges during fruit development up to maturity (July/August) i.e. the period of maximum symptom development of citrus black spot disease. As Seville oranges appear resistant to this disease, this cultivar was included as a comparison.

The following treatments were arranged factorially i.e. two cultivars viz. Valencia and Seville; two positions viz. north and south sides of the tree and four sampling dates viz. February, May, June and July. The experimental design was in the form of a randomized block and treatments were replicated five times.

An Osmometer, which measures the freezing point depression was used to determine the osmotic pressure of flavedo extracts as described under Procedures. Osmotic pressures were expressed in terms of milliosmols and the data were analysed statistically (Table 39).

From Tables 37, 38 and 39 results show that average osmotic pressures of flavedo extracts were significantly greater in July than in June, which in turn differed significantly from that in February, but not in May. Secondly, the average osmotic pressure for Valencia fruits was significantly greater than that for Seville. Positional differences also occurred where average osmotic pressure (OP) readings from the north side were significantly greater than

Table 37 - Average osmotic pressures in milliosmols, of flavedo extracts from fruits of different citrus cultivars, picked at different periods and from various positions on the tree

Time	Cultivar				Average (in millios-mols)
	Valencia		Seville		
	Position		Position		
	North	South	North	South	
February	643.2	605.6	752.8	751.8	688.4
May	860.2	862.4	768.8	673.8	791.3
June	971.2	950.8	692.2	660.2	818.6
July	1009.6	934.6	724.8	671.6	835.2*
Averages	871.0	838.3	734.6	689.4	
	854.7*		712.0		

*Significantly different (P = 0.05)

Average osmotic pressure for north side was 802.8* which differed significantly from the south i.e. 763.8

Table 38 - Average osmotic pressure, in milliosmols, of flavedo extracts from fruits of different cultivars, picked at different periods

Time	Cultivar	
	Valencia	Seville
February	624.4	752.3*
May	861.3*	721.3
June	961.0*	676.2
July	972.1*	698.2
	LSD 0.05 = 61.25	

Table 39 - Analysis of variance

Source	DF	SS	MS	F	P= 0.05	P= 0.01
Time(T)	3	260,281	86,760	32.28**	2.76	4.13
Cultivar(C)	1	407,266	407,266	151.51**	4.00	7.08
Position(Po)	1	30,420	30,420	11.32**	4.00	7.08
T x Po	3	6,183	2,061	< 1		
T x C	3	553,187	184,396	68.60**	2.76	4.13
C x Po	1	794	794	< 1		
T x C x Po	3	13,453	4,484	1.67	2.76	4.13
Replicates	4	62,748	15,687	5.83**	2.52	3.65
Error	60	161,267	2,688			
Total	79	1,495,599				

that from the south.

Results from Tables 38 and 39 show that the interaction effect, Time (T) x Cultivar (C), was significant. Valencia flavedo resulted in significantly greater OP readings in July than June, in June than May and May than February respectively. However, Seville resulted in the highest OP in February, which differed significantly from that in June.

Field experiments

Evaluation of basic nature of fruit blemish

Blemish marks render fruit unattractive and therefore unsuitable for export. In recent years a continuous blemish on the side of the fruit resulted in serious export losses. Citrus thrips was suspected as being the main cause of this blemish.

In the first experiment three factors, namely wind, thrips Scirtothrips aurantii and mites Panonychus citri were investigated as possible causes of blemishes on the sides of citrus fruits.

There were seven treatments, details of which are given in Table 40. The experiment was laid out as a randomized block design with four replications of single-tree plots in a three year old midseason orchard at Nelspruit.

Trees were divided into four sections, north, south, east and west respectively, and results from 50 fruits in each section were recorded separately.

The materials and methods used are described under Procedures. In certain treatments, trees were protected from wind by twelve foot high hessian screens. Trees in the other treatments were exposed to the wind which was particularly severe just prior to summer storms when the tender fruits were subjected to a severe buffeting against the mid-ribs of leaves and the dry twigs and branches. Thrip infestations were high in those treatments not receiving insecticidal sprays (see under Procedures).

The following forms of blemish were distinguished on fruits at harvest: thrip injury with the characteristic blemish ring around the stem-end; continuous blemish and scratches and scabby lesions on the sides of fruits. The latter two blemishes were classed into grades 0 to 4 according to the extent of the markings on the fruit (Table 3, Procedures).

Results from Tables 40 to 43 inclusive, show that percentages of fruits with scratches and scabby lesions were significantly higher in wind-exposed treatments 1, 3 and 6 compared with other treatments where trees were protected from wind. Secondly, the percentage fruits with thrip injury was highest in the treatments where thrips were not controlled (treatments 1, 4 and 7) however, both treatments

Table 40 - Percentages of Pineapple midseason oranges at harvest showing various blemishes, after exposure to wind, thrips Scirtothrips aurantii and mites, Panonychus citri

Treatments			Percentage fruits with blemish		
Number	Factors				
	Wind (W), Mites (M) and Thrips(T)				
	Protection offered	Exposed to	Blemish ring at stem-end	Continuous blemish (a)	Scratches & Scabby Lesions (a)
1	0	W,T,M	22.0	15.4	56.3*
2	W,T,M	0	9.0	2.1	24.0
3	T,M	W	11.0	6.1	54.0*
4	W,M	T	31.0*	3.3	32.9
5	W,T	M	12.0	3.7	33.3
6(b)	T,M	W	7.0	10.3	50.7*
7(c)	W,M	T	31.0*	5.0	14.0

*Significantly different (P = 0.05)

(a) moderate to severe blemish (grades 2, 3 and 4)

(b) same as treatment 3, except trees received an early parathion spray only

(c) same as treatment 4 except trees received a late parathion spray only

Thrip infestations were controlled in (b) but not in (c), in the above Table

Table 41 - Analysis of variance (Thrip ring)

Source	DF	SS	MS	F
Treatments	6	63,240.49	10,540.08	5.61**
Replicates	3	14,698.44	4,899.48	2.61
Error a	18	33,846.65	1,880.37	
Position	3	981.57	327.19	
T x P	18	7,614.02	423.00	
Error b	63	98,725.88	1,567.08	
Total	111	148,252.54		

Table 42 - Analysis of variance (Continuous blemish)

Source	DF	SS	MS	F
Treatments	6	5,703.45	950.58	
Replicates	3	8,523.59	2,841.20	4.29*
Error a	18	11,913.92	661.90	
Position	3	978.12	326.04	1.92
T x P	18	3,613.18	200.73	
Error b	63	10,680.04	169.52	
Total	111	41,412.30		

Table 43 - Analysis of variance (Scratch marks and irregular scarring)

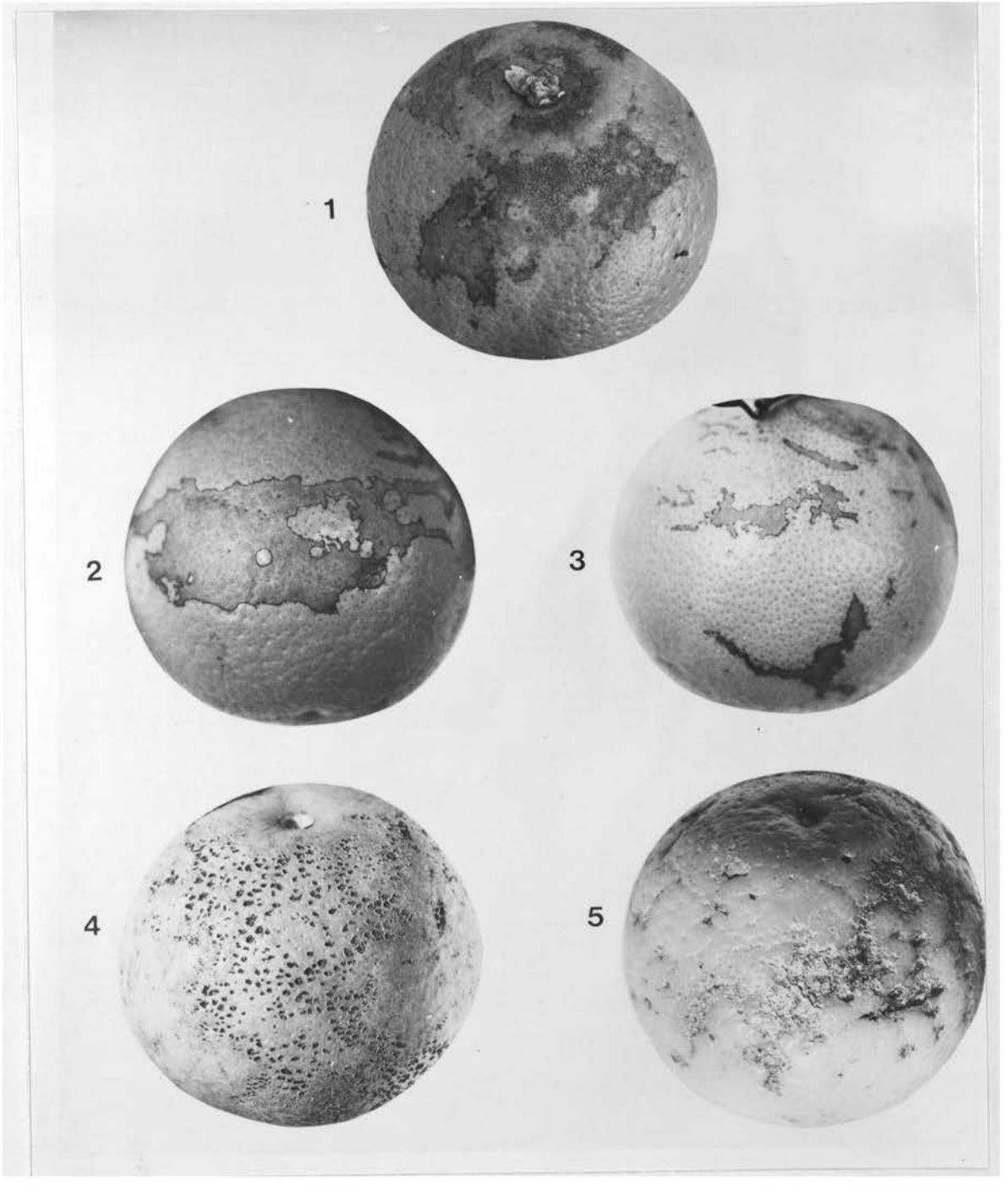
Source	DF	SS	MS	F
Treatments	6	48,858.20	8,143.03	6.23**
Replicates	3	5,174.79	1,724.93	
Error a	18	23,526.50	1,307.02	
Position	3	598.67	199.56	
T x P	18	9,903.55	550.20	
Error b	63	37,789.85	599.84	
Total	111	125,851.56		

Figure 12 - Various blemishes on midseason oranges at harvest

1. Thrips ring at the stem-end resulting from infestations of Scirtothrips aurantii at an early stage of fruit development

2 & 3. Continuous and broken, smooth-surface blemish as a result of wind injury inflicted during September and early October respectively

4 & 5. Scratch marks and scabby lesions resulting from wind injury during late October and November



4 and 7 were significantly higher than the other treatments. Thirdly, there were no significant differences between treatments with regard to the continuous blemish on the side of the fruit.

Results from this experiment show a definite correlation, firstly between wind and scratches and scabby lesions and secondly between thrip infestations and ring blemish around the stem-end of citrus fruits. There was no positive relationship between wind and continuous blemish, although the highest counts of fruits showing this blemish were recorded in the three treatments exposed to wind. There was no correlation between mite infestations and fruit blemish in this experiment.

In the above experiment, of the three agencies investigated, wind appeared as the major factor causing blemishes on the side of citrus fruits. In the next experiment, the effect of wind, induced at various stages of fruit development, was determined in relation to the form of fruit blemish. The trees, which were protected from wind by vertical screens, were shaken for five minute periods to simulate severe wind turbulence frequently encountered just prior to a summer storm. The young trees were approximately eight ft in height and were shaken as uniformly as possible (see under Procedures).

The experiment was a 2 x 4 factorial with cultivars and time of injury respectively, as factors. Experimental

design was in the form of randomized block. As screens had to be constructed around each tree, it was not practical to use more than 16 trees or one tree per treatment. At harvest, trees were divided into four equal plots according to the method used by Rich (1952), and approximately 30 fruits from each ¼-tree plot were examined and assessed for blemish. The data, which were analysed statistically, appears in Tables 45 and 46.

Results from Tables 44 to 46 inclusive, show that the percentage fruits of both cultivars with continuous side blemish, were significantly higher when treated in September/early October than at a later period. Secondly, treatment of fruits during late October and November respectively, resulted in significantly higher percentages of fruits with scratch markings than that in the previous treatment.

Injury to pea-sized fruits in September/early October, resulted in the immediate appearance of dark-green bruise marks which became silvery-grey after a few weeks. The edge of the blemish was regular in outline and the surface at the same level as the surrounding healthy tissue. These blemishes became more noticeable as the fruits coloured at maturity and appeared as golden-brown, smooth, continuous areas which in severe cases covered most of the fruit surface (Figure 12, 2 and 3).

Table 44 - Percentage midseason oranges at harvest with moderate to severe continuous blemish and scratch marks after trees were shaken in summer at different periods to simulate wind-storm conditions

Simulated wind conditions in:	Percentage fruits with side blemish			
	Continuous blemish		Scratch markings	
	Cultivar		Cultivar	
	Hamlin	Pineapple	Hamlin	Pineapple
0 (Control)	0	1.2	4.0	2.3
September	46.0*	54.8*	0	2.6
October	0	1.0	63.4*	41.6*
November	0	0.5	48.5*	34.2

*Significantly different (P = 0.05)

Table 45 - Analysis of variance (Continuous blemish)

Source	DF	SS	MS	F
Wind (W)	3	130,980.40	43,660.13	355.71**
Position	3	430.16	143.39	
Error a	9	1,104.65	122.74	
Cultivar(C)	1	1,130.50	1,130.50	11.55**
W x C	3	842.46	280.82	2.87
Error b	12	1,174.41	97.87	
Total	31	135,662.58		

Table 46 - Analysis of variance (Scratch markings)

Source	DF	SS	MS	F
Wind (W)	3	121,531.92	40,510.64	59.50**
Position	3	698.56	232.85	
Error a	9	6,127.17	680.80	
Cultivar(C)	1	833.34	833.34	4.17
W x C	3	4,395.98	1,465.33	7.33**
Error b	12	2,397.62	199.80	
Total	31			

Fruit injury during late October and November resulted in raised, rough lesions, usually with irregular edges (Figure 12, 4 and 5).

Mechanical injury

In the previous experiment fruits were injured by shaking the trees to simulate wind conditions. In the next experiment fruits were individually damaged in order to obtain a greater uniformity of blemish. The object of the experiment was to compare injury at an early stage compared with that at a later stage of fruit development.

There were eight treatments comprising two cultivars viz. Valencia and Navel oranges and four times of injury viz. October, November, December and January.

The experiment was a 2 x 4 factorial with cultivars and time of injury, respectively, as factors. The experimental design was a randomized block. Each treatment consisted of 50 fruits viz. 10 fruits from each of 5 trees per cultivar. Since the four times of injury were represented on each individual fruit, each fruit was regarded as a replicate.

Fruits which were relatively free of blemish were chosen from an orchard at Nelspruit. On the equator of each fruit, four positions were ringed in weather-proof ink

Figure 13 - A. Surface view of and B. vertical section through, fruit blemishes resulting from mechanical injury inflicted during early October, November, December and January (1 to 4 respectively) Small square cuts made in fruits E. at an early stage, October and L. at a late stage in December

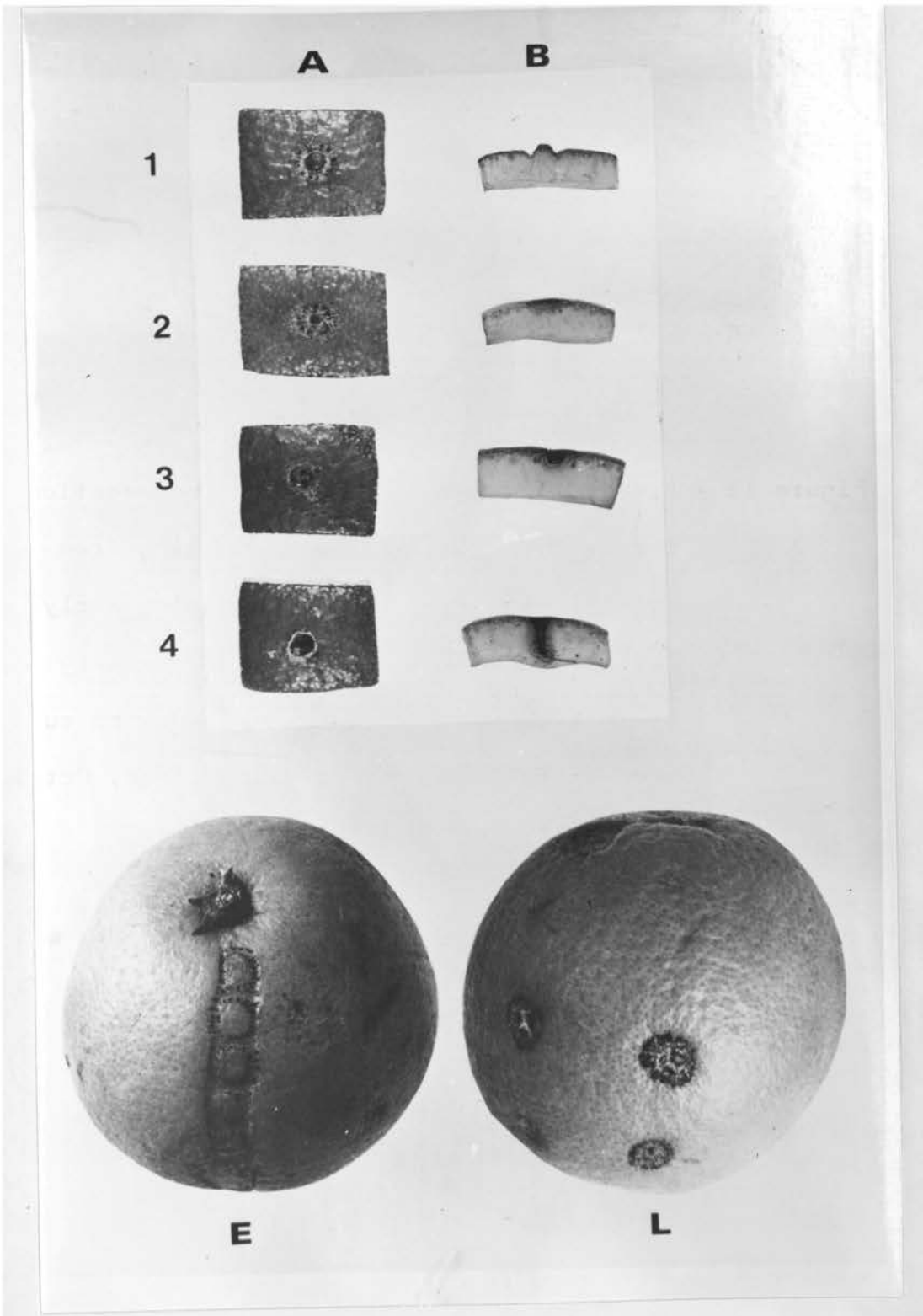


Table 47 - Average blemish rankings after mechanical injury at various stages of development on fruits of two cultivars prior to maturity

Time of injury	Average blemish ranking on:	
	Cultivar	
	Valencia	Navel
1. October	1.06*	1.10*
2. November	2.11	2.09
3. December	3.06	3.02
4. January	3.77	3.79

*Significantly different (P = 0.05)

Table 48 - Analysis of variance

Cultivar	χ^2_r	(χ^2 P = 0.01)
Valencia	124.56**	11.35
Navel	121.88**	11.35

and each position was allotted a treatment number from 1 to 4. A small metal cylinder, with a cutting depth adjusted to one mm was used to cut a ring (one mm in diameter) in the centre of each position on fruits at the dates given in Table 47. As injured fruit dropped readily results were taken three months after injury.

Fruit injuries were classed into the following grades: 1, tissue at centre alive; 2, 3 and 4, tissue at centre dead, with the formation of shallow, moderate and deep depressions respectively (Figure 13). Friedman's two-way analysis of variance was used to rank blemishes according to the severity of blemish as described under Procedures.

Results from Tables 47 and 48 for both cultivars, show that average rankings for blemish were significantly less in early October than at a later period. In the former treatment the central area of the lesion remained alive and the blemish was smooth-surfaced. Injury inflicted in November, December and January resulted in the appearance of a dead central area with a raised, scabby blemish. It would appear that injury in October resulted in the formation of normal repair tissue, while later injury to the fruits probably involved an additional factor in the rind and the subsequent development of a severe form of fruit blemish.

Phytotoxic effects of citrus rind extracts

In order to gain further information regarding the toxic principle in the rind, extracts obtained from immature fruits were used to treat older fruits and vice versa.

In this experiment the following treatments were used in a factorially arranged experiment:

(i) Flavedo extracts from fruits of three sizes viz. 15, 25 and 35 mm diameter.

(ii) Test fruits of three diameters viz. 15, 25 and 35 mm diameter.

(iii) Two treatment periods viz. 3 and 5 weeks.

The experimental design was in the form of a randomized block. As three different extracts were applied to each fruit, the fruits were regarded as replicates. Replicates ranged from 17 to 24 per treatment.

Friedman's two-way analysis of variance was used to rank blemishes according to the severity of blemish where 3> 2> 1. An average of 2.5 was taken if two blemishes were the same.

The cold press method, described under Procedures, was used to make rind extracts from fruits of various sizes (Table 49). Fruits to be treated, were selected in the same size range as above and on the equator of each, positions were ringed in weatherproof ink and each position was

Table 49 - Rank totals for severity of fruit blemish after various periods of treatment with different rind extracts from fruits at various stages of development

Diameters of fruits (mm) used for rind extracts.	Fruit diameter (mm) of treated fruits					
	15		25		35	
	Period (weeks)		Period (weeks)		Period (weeks)	
	3	5	3	5	3	5
15	29.0*	28.5*	22.5+	32.0*	31.5*	24.0*
25	55.0	52.0	33.5	49.0	55.0	41.5
35	54.0	57.5	46.0	63.0	57.5	48.5
LSD 0.05 FR	13.82	15.89	13.66	16.23	16.23	14.45

*Significantly different from both treatments, (25 and 35 mm)

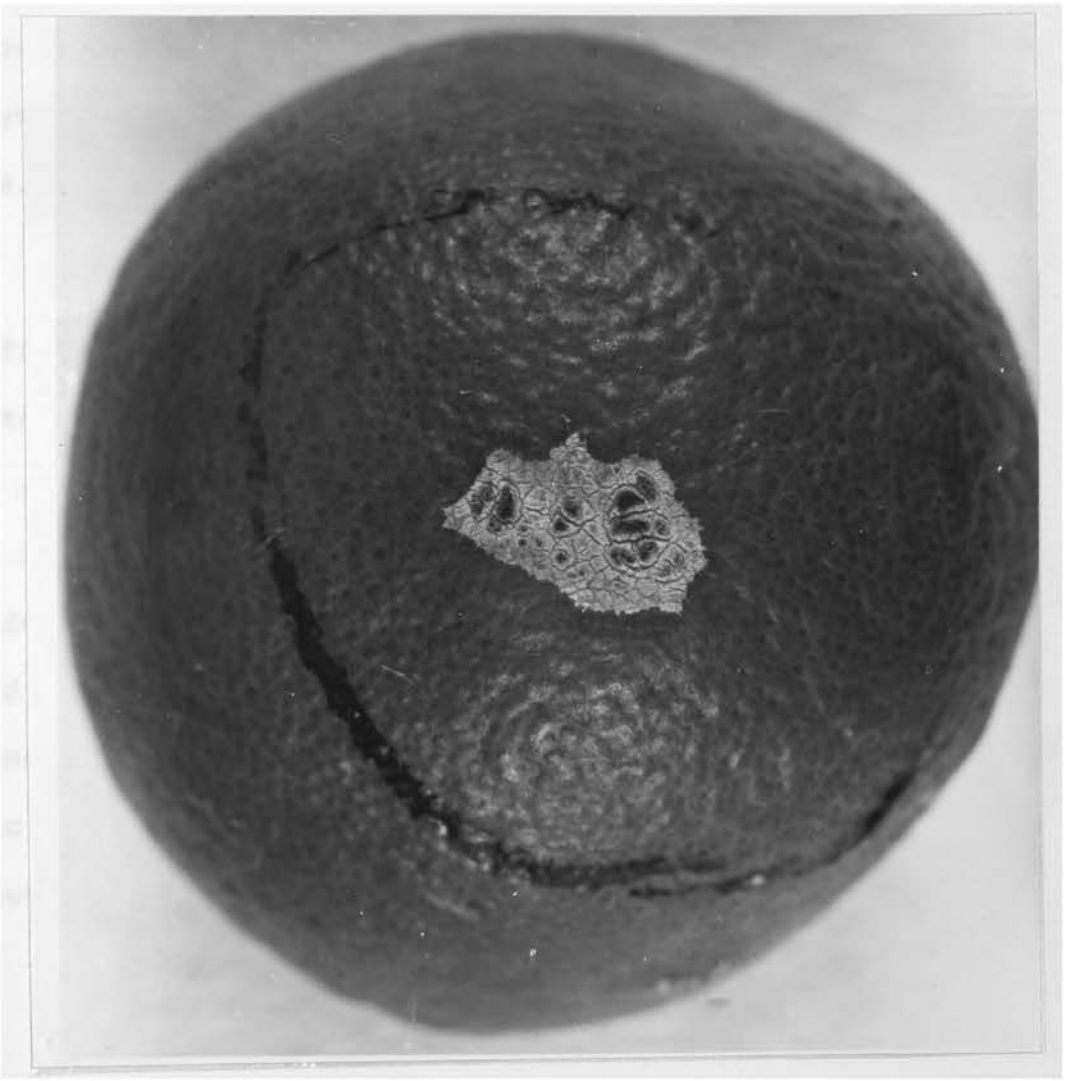
+Significantly different from treatment 35 mm only

Table 50 - Friedman's two-way analysis of variance

Statistic	Fruit diameters (mm)					
	15 mm		25 mm		35 mm	
	Period (weeks)		Period (weeks)		Period (weeks)	
	3	5	3	5	3	5
χ^2_r	18.81*	20.57*	16.25*	20.03*	17.10*	16.76*
$\chi^2_{(0.001)}$	13.82	13.82	13.82	13.82	13.82	13.82

*Rank sums for treatments, highly significantly different

Figure 14 - Response of a green, immature Valencia orange to applications of small quantities of flavedo extract.



allotted a treatment number from 1 to 3. With a micro-pipette 1/4 ml of extract was applied to the centre of each marked area. Care was taken not to damage the tissue mechanically. Any blemishes which formed could only have resulted from the toxic action of the extract. Immediately after treatment, all fruits were protected from wind by enclosing them in open-ended paper bags and insecticide sprays were applied to control thrips and red mite infestations.

Results in Tables 49 and 50 show that after three and five weeks, peel extracts from small, 15 mm diameter fruits, resulted in significantly less blemish than that from the larger fruits, 25 and 35 mm diameter, respectively when applied to test fruits of various diameters. Close examination revealed that extracts from 15 mm fruits (pea-sized) were virtually innocuous to the fruit rind while extracts from fruits of 25 and 35 mm diameter, were directly toxic and gave rise to raised, rough, scabby fruit blemish (Figure 14).

Copper blemish

Experiments were conducted to determine the stage when citrus fruits are most susceptible to fungicidal spray damage.

Figure 15 - An existing blemish accentuated by raised, rough, blackened outline due to copper

Table 51 - The application of copper and Mancozeb foliar sprays applied at different times and in various combinations to young midseason and Valencia trees.

Treatments	Times of application				
	October	November		December	
	1st Week	1st Week	3rd Week	2nd Week	4th Week
1 Control	-	-	-	-	-
2	-	M	M	M	M
3	Cu	M	M	M	M
4	-	Cu	M	M	M
5	-	M	Cu	M	M
6	-	M	M	Cu	M
7	-	M	M	M	Cu
8 Standard +	-	Cu	Cu	Cu	Cu
9	-	Cu + I	Cu + I	Cu + I	Cu + I
10	-	Cu (0.6)	Cu (0.6)	Cu (0.6)	Cu (0.6)
11	-	Cu + S	Cu + S	Cu + S	Cu + S

Cu = Cuprous oxide (50 percent metallic copper) at 0.91 kg/455 l

Cu (0.6) = Cuprous oxide at 0.68 kg/455 l

Cu + I = Cuprous oxide at 0.91 kg/455 l + Insecticide (parathion)

Cu + S = Cuprous oxide at 0.91 kg/455 l + Safener (slaked lime) at 0.45 kg/455 l

\- = no sprays were applied

M = Mancozeb (80 percent active), zinc complexed manganese ethylene bisdithiocarbamate, at 0.68 kg/455 l water

\+ = Treatment 8, four sprays of copper fungicide regarded as standard treatment for black spot control i.e. at the time the experiment was conducted (further details under Procedures)

Table 52 - Average percentage copper-blemished fruits and percentages non-exportable fruits, of Pineapple and Hamlin midseason cultivars after different spray applications

Treatments	Percentage fruits					
	Pineapple			Hamlin		
	Stipple	Dark blemish	non-ex-portable	Stipple	Dark blemish	non-ex-portable
1.+	0*	0*	2.6*	0*	0*	1.0*
2.	0*	1.5*	12.9*	0*	1.1*	12.7*
3.	0.5*	1.1*	13.7*	1.1*	5.7*	10.1*
4.	0.4*	0.5*	17.4*	0*	0*	3.4*
5.	0*	6.1*	4.7*	0*	4.2*	3.0*
6.	15.9*	32.4	30.1	33.0	34.1	18.2*
7.	42.5	36.4	45.6	49.2	34.4	30.6
8.	41.4	46.4	50.1	37.4	49.7	38.6
9.	30.5	44.6	45.7	39.2	38.9	23.6
10.	57.1	40.2	52.8	55.3	47.3	30.1
11.	0*	0*	4.4*	0*	0*	1.0*

*Significantly different from treatment 8 (P = 0.05)
+Refer Table 51

Table 53 - Analysis of variance (% fruits with Stipple)

Source	DF	SS	MS	F
Treatments	10	158889.90	15888.99	19.83**
Blocks	1	5636.46	5636.46	7.03*
Error a	10	8013.29	801.33	
Cultivars	1	353.98	353.98	4.18
T x C	10	2600.47	260.05	3.07*
Error b	11	931.30	84.66	
Total	43	176425.40		

Table 54 - Analysis of variance (% Fruits with darkened blemish)

Source	DF	SS	MS	F
Treatments	10	162042.82	16204.28	12.59**
Blocks	1	13199.92	13199.92	10.25*
Error a	10	12872.92	1287.29	
Cultivars	1	8.82	8.82	
T x C	10	1218.78	121.88	
Error b	11	7010.43	637.31	
Total	43	196353.69		

Table 55 - Analysis of variance (% Non-exportable fruit)

Source	DF	SS	MS	F
Treatments	10	6279.84	627.98	11.27**
Blocks	1	442.28	442.28	7.94*
Error a	10	557.06	55.71	
Cultivars	1	509.32	509.32	15.67**
T x C	10	269.03	26.90	
Error b	11	357.46	32.50	
Total	43	8414.99		

*Significantly different (P = 0.05)

In the first experiment (Nelspruit site) there were 22 treatments comprising 11 different times of copper fungicide applications, and two cultivars i.e. Pineapple and Hamlin midseason oranges. Details of the various spray treatments are given in Table 51. The experiment was a 2 x 11 factorial with cultivars and timing of copper fungicide sprays as factors, respectively. The experimental design was a randomized block with single-tree plots and four replicates per treatment. A motorised power-sprayer, adjusted to a pressure of 27.5 bars (viz. 400 lb/in^2) was used for the application of all foliar sprays.

After results were taken at harvest the data was analysed statistically (Tables 53 to 55). In order to stabilise variance the transformation $\Phi = \text{arc sin}\sqrt{p^2 + \frac{1}{2}}$ was applied to the data.

The results showed two forms of copper injury on the fruits at harvest. Firstly, a black stippling occurred on the fruits, which were classed into the following grades:

0 = no stipple

1 = faint, localized stipple

2 = faint, general stipple

3 = heavy, distinct stipple

Secondly, a darkening of existing blemish due to the effect of copper (Figure 15). Fruits were classed into various grades as follows:

0 = None

1 = < 25

2 = 25 to 50

3 = 50 to 75

4 = 75 to 100*

*Percentage of blemish area darkened by copper.

Fruits were graded into two categories, exportable and non-exportable on the basis of blemish marks viz. stipple and darkened markings, according to the regulations set down by the S.A. Co-operative Citrus Exchange (Procedures).

Results from Tables 52 to 55 show that treatments 1, 2, 3, 4, 5 and 11 resulted in significantly less percentage fruits with stipple, darkened blemish and non-exportable fruit than treatment 8 (standard treatment). The results were the same for both cultivars except for treatment 6 where differences occurred between certain categories. Copper markings were greatly reduced following an early application i.e. September and October compared with a copper spray applied later i.e. November and December, respectively. Secondly, where copper applications were increased from one to four, copper-blemish was severe, but could be significantly reduced in intensity by the addition of slaked lime to the fungicide spray (Treatment 11). Thirdly, neither adding parathion to copper sprays nor decreasing copper concentrations from 0.91 to 0.68 kg/455 l, had any significant effect on blemish compared with that of the standard treatment. Treatment 2,

receiving four sprays with Mancozeb showed no stippling on the fruit and virtually no darkening of blemish. The small percentage of darkened blemish, comparable with that of the control (unsprayed) treatment, may be due to the effect of sooty mould Capnodium salicinum Mont, or other factors.

The experiment was repeated the same year in a three year old Valencia orchard near Nelspruit. Treatments are described under Table 51 except that in treatment 11, sodium carbonate was used as the safener with copper instead of slaked lime.

Results from Tables 56 to 58 inclusive, show that treatments 2, 3, 4 and 6 resulted in significantly less darkening of blemish compared with treatment 8 (standard), while treatments 5 and 7, also receiving only one copper application, did not differ significantly from the standard treatment. Secondly, results in the category non-exportable fruits are contradictory as treatments 3 and 4 resulted in a reduced darkening of blemish compared with the standard treatment, yet no significant differences were recorded between these treatments and that of the standard treatment with regard to percentage non-exportable fruits. The opposite results were obtained with treatment 7, where percentage fruits showing darkening of blemish was high, a low figure was obtained for percentage non-exportable fruit. In this experiment percentage fruit stippling was not evaluated and may have been the reason for increased rejection

Table 56 - Average percentage copper-blemished fruits and percentages non-exportable fruits of Valencia oranges, after different spray applications

Treatments	Percentage fruits	
	Darkening of blemish	non-exportable
1.*	-	-
2.	1.9*	16.4*
3.	9.5*	27.0
4.	12.0*	27.5
5.	29.2	30.1
6.	9.5*	11.1*
7.	20.0	8.0*
8.	52.5	36.7
9.	66.2	41.2
10.	43.9	38.1
11.	14.4	21.6

*Refer Table 51

*Significantly different from treatment 8 (P = 0.05)

Table 57 - Analysis of variance (% Non-exportable fruit)

Source	DF	SS	MS	F
Treatments	9	2285.35	253.93	3.23*
Blocks	2	221.00	110.50	1.41NS
Error	18	1414.46	78.58	
Total	29	3920.81		

Table 58 - Analysis of variance (% Fruits with darkened blemish)

Source	DF	SS	MS	F
Treatments	9	182617.29	20290.81	5.63**
Blocks	2	8141.17	4070.59	
Error	18	64910.17	3606.12	
Total	29	255668.63		

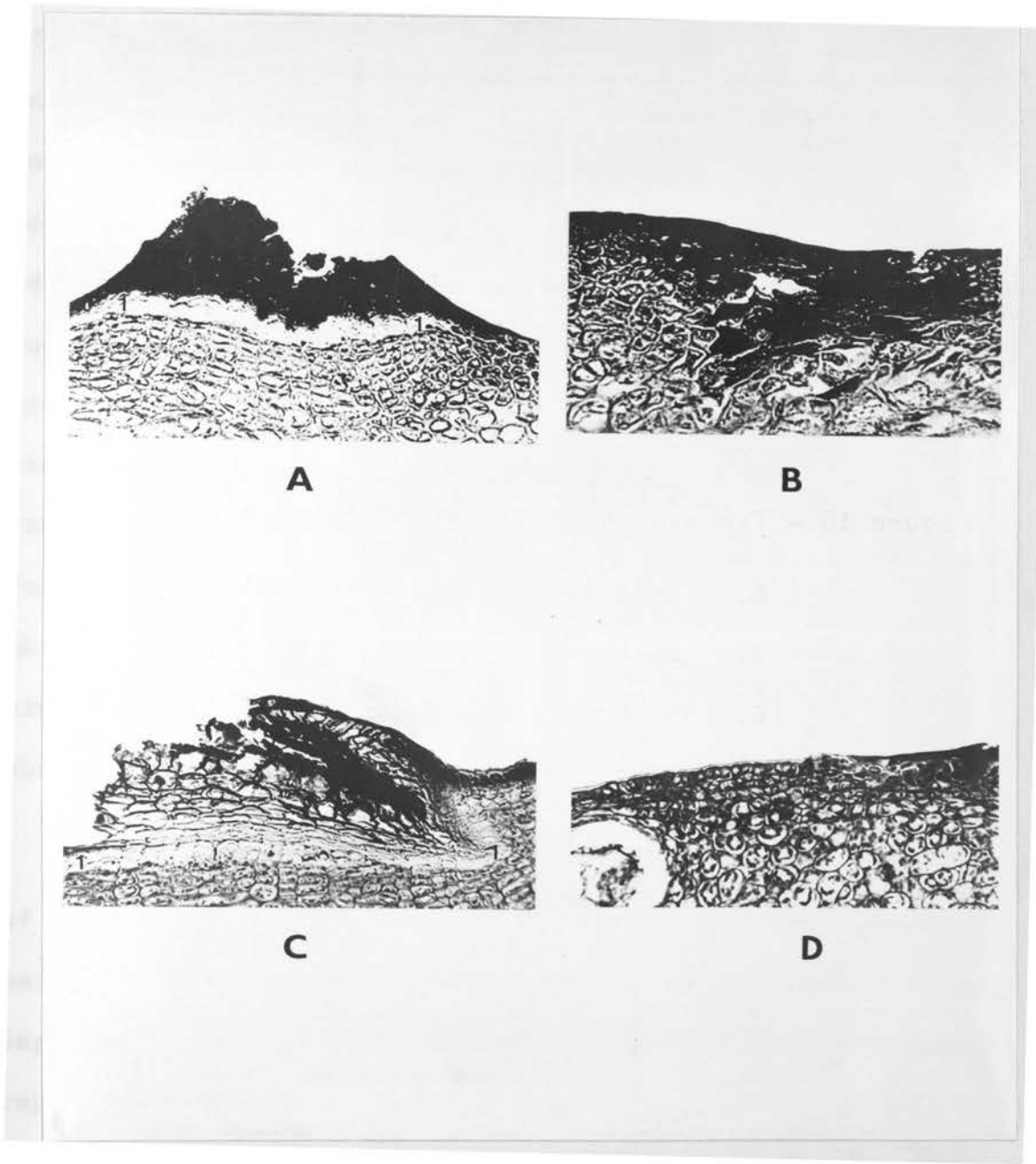
Figure 16 - Transverse sections of orange peel (x 200)

A. Stipple mark due to copper

B. Darkened copper blemish

C. Scab mark resulting from November wind injury

D. Continuous blemish caused by September wind injury



of fruit for export in treatments 3 and 4.

Indications from the previous experiment are that early copper applications possibly result in less darkening of fruit scars than later sprays in November and December. However, results were contradictory in the next experiment. Secondly, one copper application generally resulted in less darkening of blemish than four copper sprays. Thirdly, the addition of parathion to copper, or reducing the rate of copper fungicide from 0.91 to 0.68 kg/455 l in both experiments had no noticeable effect on blemish compared with the standard treatment. Both safeners i.e. slaked lime and sodium carbonate, effectively reduced copper blemish, the former appeared more effective than the latter. Conflicting results in these experiments may have been due to random wind-scarring on fruits since wind was not controlled in the above experiments.

In transverse section (Figure 16) the various types of blemish, which have been discussed, appear to differ mainly in the depth of cork penetration into the flavedo. September wind resulted in the formation of an even-surfaced repair tissue (D) at epidermal level while November wind gave rise to rough, raised, corky tissue (C) with the formation of a definite phellogen layer (1 - 1). Secondly, stipple marks (A) appeared as a black homogeneous mass separated from the healthy tissue by layers of phellogen (1 - 1). However, the effect of copper on an existing blemish (B),

resulted in darkly stained tissue of the flavedo without the formation of a phellogen layer.

DISCUSSION

After infection during Spring and Summer the fungus remains latent in the peel of the fruit, and depending on cultivar and prevailing environmental conditions, the latent period may extend from two to as long as ten months (Mc Onie, 1967). This may lead to serious practical problems since fruits for export which appear free of black spot disease at harvest, may develop severe symptoms of this disease during transit from packhouses to the ports prior to shipment overseas. These experiments were aimed at determining the effects of various environmental and physiological factors both on the development of symptoms on the fruit and on the pathogen in artificial culture.

Light and temperature effects

Fruit

Various workers reported that the highest incidence of this disease occurred on fruits on the sunny or warmer side of the tree i.e. north-western aspect in the Southern Hemisphere. In addition, higher lesion counts were obtained on fruits on upper compared with the lower branches of unsprayed trees and secondly, more lesions were observed on exposed compared with shaded areas of the fruit peel (Kiely, 1948;

Wager, 1952; Calavan, 1960, and Kotzé, 1963). However, it is uncertain whether these were temperature or light effects, or a combination of both factors. Kiely (1949) stated that peak disease development in the orchard was at 30°C. Kotzé (1963) reported that the period of increased temperature in late Winter ccincided with increased development of lesions on fruits in the orchard. Wager (1952) obtained highest lesion counts at temperatures from 27° to 30°C on harvested fruits in the laboratory. However, no mention was made regarding the effect of light in his experiments.

It was hypothesised that both light and higher temperatures result in increased symptom development on the fruit. In experiments where latently-infected fruits were subjected to artificial light and dark conditions in the laboratory, results in Tables 5, 6 and 7 show that continuous light resulted in significantly more fruit symptoms than alternating light/dark, which in turn was significantly higher than the dark treatment. Raising the temperature from 20° to 27°C resulted in a significant increase in fruit symptoms. The average number of fruit symptoms was significantly higher after 15 days compared to 10 days. From Table 6 it can be seen that interaction effects between light and temperature were highly significant. Continuous light at 20°C and continuous dark at 27°C, resulted in significantly less fruit symptoms than continuous light at 27°C. These results support the hypothesis that both light and higher temperatures result in increased symptom development on the fruit.

Results in Table 6 show that replicates were significantly different. Each replicate consisted of fruit picked from the same trees but at different periods viz. July, August and September. Fruits picked in September gave significantly more lesions than fruits in August, which in turn differed significantly from that in July. This is consistent with reports by Kiely (1948) and Kotzé (1963), who observed that increased symptom development at a later stage of picking was related to increased temperature. In these experiments, in order to reduce treatment variations due to differences between replicates, a $\sqrt{x+1}$ transformation was applied to the data. Tukey's test of additivity (Table 7), showed that this transformation had been effective in stabilising variance.

Possibly at the low light intensities of approximately 10 Lux units in these experiments, light had a quantitative effect since continuous light differed significantly from the alternating light/dark treatments (Table 5). At the time of picking at the start of the experiment, fruits showed few or no disease symptoms notwithstanding the fact that they had previously been exposed to sunlight for several months while on the tree. It is possible that a physiological change occurred in the peel immediately after picking, which in the presence of light, was conducive to the development of the pathogen and subsequent symptom development. Fruit respiration may play a role in this respect and evidence presented by various workers is given. According to Seberry et al

(1967) and Kotzé (1963) various oils and wax compounds, applied to picked fruits, resulted in a considerable reduction of the post-harvest development of black spot. This was ascribed to the reduction in the rate of fruit respiration resulting from these treatments. From this it appears that increased fruit respiration is directly correlated with symptom development on the fruit. Sinclair (1961) mentioned that workers in Australia reported an increase in respiration rate immediately after harvest although this was not confirmed by Vines, Grierson and Edwards (1968) who reported that a climacteric rise does not occur in citrus. Sinclair (1961) mentioned that light and increased temperature appeared to increase respiration rate in the fruit. It is possible that these factors, which apparently result in increased respiration of the fruit, may be indirectly associated with increased symptom development.

Mc Onie (1967) showed that the fungus remained latent as a small knot of mycelium between the cuticle and the epidermis. It was postulated that the fungus, in this sub-cuticular position, may be affected directly by light. In laboratory experiments continuous light and continuous dark were applied to opposite halves of latently-infected fruits. The temperature was kept at 27°C and results were taken after 15 days. Results given in Tables 8 and 9 show that a significant increase in numbers of fruit lesions occurred on the light compared with the dark side. These results indicate that a translocatable principle is probably not involved and

that light may have a direct effect on the pathogen within the peel tissue.

There are two possible practical applications of the effect of light and temperature on symptom development. Firstly, export fruits could possibly be enclosed in dark-coloured wrappers in order to reduce light intensity, thereby replacing the transparent tissue wrappers. Secondly, pack-houses could, by subjecting fruits to continuous artificial light at 27°C, determine within a 14-day period, the potential black spot on a farmers crop. This test could preferably be applied approximately two weeks before harvest by selecting five symptomless fruits from the north-western aspect of each tree from 20 percent of the trees in the orchard. Percentage non-exportable fruits i.e. with more than three black spot lesions per fruit, could be calculated. Packhouses could thus guard against including latently-infected fruits with healthy fruits, thereby decreasing the chance of rejection and the cost of repacking of fruits in an entire consignment at the ports, due to black spot development on fruits during transit.

Flavedo discs

It was observed by Kiely (1948) that pycnidia developing on dead leaves on the orchard floor occurred mainly on that side exposed to the sun's radiation. It was

postulated therefore, that light and higher temperature may induce sporulation of G. citricarpa on pieces of flavedo tissue in culture. Fruit lesions did not develop but instead the fungus grew over the piece of peel and within a week had formed a black crust in which pycnidia were embedded. Pycnidiospore counts were made after the crust was macerated and left overnight in sterile water. As only zero counts of pycnidispores were obtained in the dark, this treatment was regarded as being significantly different from continuous light and alternating light/dark treatments, which did not differ significantly from each other (see Tables 10 and 11). Secondly, significantly more pycnidiospores were produced at 27° than at 20°C. Thirdly, significantly greater numbers of spores were recorded after 10 compared with 7 days. Results from sporulation assessments, which took mainly the crust development into account (as shown in Figure 2 and Table 12), did not coincide with results obtained with spore counts. In fact, in certain treatments there appeared to be a higher percentage of crust development on the flavedo discs after 7 days than that after 10 days. This method does not appear as effective as the quantitative method where the numbers of pycnidiospores were counted. The results obtained from spore counts show that conditions most conducive to pycnidiospore production on pieces of flavedo in culture viz. artificial light at 27°C, apparently coincides with those conditions resulting in maximum post-harvest lesion development on entire fruits.

Artificial medium

Mycelial growth

Lesion development is due to the active growth of the pathogen, and pycnidia formation as a result of the inhibition of growth (Kiely, 1948). As lesion development on fruits was increased by both light and higher temperatures, it was postulated that these conditions might be conducive to the growth of G. citricarpa in artificial media. Previously Wager (1952) conducted temperature studies on the growth of this pathogen on solid artificial medium and reported inconsistent results due to the irregular outline of the colonies.

In these experiments the effects of three temperatures viz. 22°, 27° and 32°C were determined on the growth of two pathogenic and one non-pathogenic isolate of G. citricarpa in artificial liquid basal synthetic medium. Results from Tables 27, 28 and Figure 10, show that mycelial growth was significantly better at 27°C compared with that at 22° and 32°C. Differences between the latter two treatments were not significant. Secondly, the average mycelial weights were significantly greater after 20 compared with 10 days. These results support the hypothesis that the optimal temperature for growth of the pathogen coincides approximately with those temperatures most conducive to fruit symptom development. Results from Table 27 show that the non-pathogen (Isolate 3) grew significantly better than the

pathogenic isolates 1 and 2 in liquid medium, which confirms results previously obtained by Frean (1964). It is interesting to note that according to Garret (1963), pathogenic isolates which show a high degree of specialization, appear to be slower growing in culture compared with those fungi which are less specialized. This could be true for G. citricarpa where the rate of growth of the pathogen is reduced compared with that of the non-pathogen.

From Figure 10, it is clearly seen that the response to temperature treatments was the same for both pathogenic and non-pathogenic isolates. This leads one to suspect that other factors besides temperature are possibly responsible for differences in pathogenicity between these isolates. Possibly these are genetical differences when it is seen that Luttrell (1948) separated out three forms or physiologic races of Guignardia bidwellii on the basis of pathogenicity. In the same way, G. citricarpa can be separated into two forms on the basis of pathogenicity.

The effect of artificial light on the growth of G. citricarpa was investigated. Previously Schüepp (1961), found that light had no direct effect on either growth or sporulation of this pathogen in culture. However, these results are questionable since he exposed plate cultures to the sunlight but did not control either temperature or humidity factors. The writer conducted preliminary experiments with the fungus grown on PDA and noted that in

continuous artificial light, colonies grew in a spreading fashion compared with a compact growth in the dark (Figure 9). In the next experiment the pathogen was grown in liquid basal synthetic medium and the flasks were placed in an incubator-shaker where the temperature was set at 27°C and the light intensity at 10 Lux units. Results from Tables 21 to 26 show that average mycelial weights in the light were significantly greater than in the dark. As well, numbers of colonies were higher in the light compared with the dark treatment, although colonies were smaller in size in the former treatment. Mycelial growth in the light was significantly greater than that in the dark after 20 days as compared to that after 10 days (Tables 21 and 22). In a second experiment (Tables 23 and 24) a significant difference between the light and dark treatments were found after 15 days, but not after 30 days. Three times as many colonies were counted in the light compared with the dark after 15 days while after 30 days the mycelium in both treatments appeared to have broken up to form approximately the same number of small globose colonies. There was a direct correlation between increased numbers of colonies and higher mycelial weights in the light compared with that in the dark.

Various workers showed that the effect of light was exerted through the medium. It was previously reported by Yusef and Allam (1967) with species of Pleurotus and various other fungi tested, that light appeared to

favour the break-down of one or more substances in the synthetic medium beneficial for mycelial growth. Weinhold and Hendrix (1963) also studied the effect of light on culture media and found that exposure of potato dextrose broth to light intensities ranging from 25 to 100 Lux units made the medium inhibitory to Rhizoctonia solani Kühn and to various other fungi tested. It was postulated that a stimulatory effect of artificial light on growth of G. citricarpa was exerted through the medium. In this experiment half the medium was unexposed while the other was exposed to light for 16 days prior to inoculation. Results from Tables 25 and 26 show that mycelial weights in both media were significantly greater in the light compared to the dark after 15 days while after 30 days differences between light and dark treatments were not significant. Secondly, the average mycelial weights in the medium previously exposed to light for 16 days was significantly greater than that in unexposed medium. Possibly light resulted in a reaction in the medium stimulatory to the growth of the fungus. It is also possible that the pathogen, situated subcuticularly in the fruit, may be stimulated to develop through the effect of light on the cells in the outer layers of the flavedo.

Certain technical problems relating to these experiments need further clarification. Firstly, the buffer system used could not include an organic compound as this could either serve as a carbon source or have an inhibitory effect e.g. Frean (1964) found that citric acid reduced the

growth of this pathogen compared with that of the control. With regard to phosphate buffers used in these experiments, the ratio of KH_2PO_4 to K_2HPO_4 was designed to give a final pH of 6.0, according to Rabie and Steyn (1964). However, after sterilization with BPL, the final pH dropped to 5.8. Nevertheless the pH of the medium even after 30 days of mycelial growth, never dropped to below 4.9. Frean (1964) found that pH only became inhibitory to the growth of this fungus below a value of 4.2. It appears from this evidence that the buffer system used in these experiments was effective. Secondly, flasks were enclosed initially with cotton wool stoppers but under the conditions in this experiment (i.e. enclosed within tin foil in the dark treatment) the cotton wool was slightly moist and became contaminated with various fungi. In the first two light experiments, aluminium tin foil discs were used as stoppers and by tapping them lightly while being held over the opening of the flask, a tight fit was ensured. In the third experiment, dark-coloured cotton wool stoppers were used and the flasks, except for the openings, were covered in two layers of aluminium tin foil.

Sporulation

Kiely (1948) observed that pycnidia of this fungus occurred in greater numbers on that side of the leaf exposed to the sun's radiations. It was postulated therefore,

that certain light and temperature conditions result in increased sporulation of the pathogen in culture. In the first experiment the fungus was cultured on PDA slants and subjected to different light and temperature treatments for various periods. Results in Tables 13 and 14 show that continuous light was significantly better than alternating light/dark which was significantly superior to that of continuous dark. Secondly, pycnidiospore production was significantly greater at 20° compared to that at 27°C. Thirdly, the optimal sporulation period was 15 days where average numbers of pycnidiospores differed significantly from that after 10 and 20 days. Results from Table 14 show that interaction effects between light and temperature were significant. Continuous light at 20°C and alternating light/dark at 20°C, both proved significantly superior to other treatment combinations.

The writer previously showed that a reduced mycelial growth of the pathogen occurred at 22°C and 32°C compared with that at 27°C. In these sporulation experiments, besides the effect of light on increasing sporulation, lower temperatures viz. 20°C resulted in a significant increase in pycnidiospore production compared with that at 27°C. This supports the statement made by Cochrane (1958) to the effect that sporulation in fungi is possibly not so much due to a stimulation effect but rather results from the inhibition of mycelial growth.

It is interesting to note that temperature effects on sporulation of the pathogen are not the same in artificial medium as in the host tissue. The fungus grown on pieces of rind in culture, sporulated better in the light at 27°C than at 20°C. Secondly, light at 27°C increased symptom development significantly, and also the subsequent pycnidial production within the lesions, compared with light at 20°C. It may be assumed that it is possibly not a temperature inhibition of growth but some other factor limiting for growth of the fungus in the host tissue which induces sporulation of the pathogen.

In a second experiment the effect of different artificial media was determined on the sporulation of this fungus in culture. Results in Tables 15 and 16 show that pycnidiospore production was significantly greater on potato dextrose agar compared to basal synthetic agar. Possibly certain constituents in the former natural medium were conducive to sporulation in comparison with those of the synthetic medium. Results from Table 15 show that continuous light was significantly better than alternating light/dark, which was significantly superior to the dark treatment. Secondly, maximum spore production was recorded after 15 days, where the average count differed significantly from that after 10 and 20 days. These results coincide with those of the previous experiment. The reduction of the number of pycnidiospores after 20 days compared with 15 days may be due possibly to spores

becoming embedded within the gelatinous matrix of the pycnidium at this stage, to such an extent that only relatively few were recovered from the pycnidia.

Results from Table 17 show that spermatial production appeared to increase when the fungus was grown on PDA compared with BSA and at 27°C compared to 20°C. As well, light appeared to increase production of spermatia compared to the dark but only at 27°C and not at 20°C. These results differ from those of pycnidiospore production viz. temperature inhibition of growth is apparently not a prerequisite for increased spermatial production. According to Kiely (1948), spermatia, which are thought to have a sexual function, always precede perithecial formation on dead leaves. This suggests that the spermatia are produced at a specific stage in the life-cycle of the fungus. Results from the writer's experiments indicate that certain physiological conditions in the host may be important in this respect as light, temperature and nutritional factors (Table 17) had a marked effect on spermatial production.

It was postulated that light and temperature conditions approximating those occurring in a packhouse at harvest would result in increased sporulation of the fungus in artificial medium. Results from Tables 18 and 19 show that alternating light at 27°C/dark at 20°C and secondly, continuous light at 27°C were significantly inferior to continuous light at 20°C. These results do not

support the hypothesis and indicate rather that higher temperatures during the day and lower night temperatures are relatively ineffective in inducing sporulation compared with lower temperatures during the day. Results in Table 18 show that highest pycnidiospore production was recorded after 15 days, which differed significantly from that after 10 and 20 days. Spermatial production (Table 20) was higher at 27°C than at 20°C. These results confirm those obtained in previous experiments.

Limonene and peel extracts

All citrus fruits possess oil of which 90 percent is comprised of d-limonene. Murdoch and Allen (1960) reported that orange peel oil and d-limonene, at concentrations from 0.02 to 0.1 percent, were toxic to the Zygosaccharomyces sp. They suspected that d-limonene was the main antimicrobial component as this was present in high concentrations in both treatments. Kiely (1948) mentioned that he found it difficult to obtain isolations from hard spot lesions and attributed this to the fact that the mycelium was killed in the lesion. It is possible that oil cells were ruptured in the vicinity of the lesion and the citrus oil thus liberated was toxic to the fungus. Sinclair (1961) reported that less citrus oil occurred at the stem-end compared with the stylar-end while Calavan (1960) observed that the black spot lesions appeared to be more numerous nearer the stem-end of the fruit.

It is uncertain whether there is a correlation between decreased limonene concentration and increased lesion development at the stem-end or whether this part of the fruit, being more exposed, is thus more subject to infection.

From the evidence presented, it was postulated that citrus oil (d-limonene) is toxic at low concentrations to isolates of G. citricarpa in artificial culture. The fungus was treated with various limonene concentrations just prior to plating out on PDA. This experiment was repeated twice. In the second experiment the inoculum was reduced in order to minimize the danger of interference between developing colonies on the plates through competition (Parkinson & Waid, 1960). Results in Tables 29 to 32 show that limonene, even at concentrations as low as 0.25 percent, resulted in a significant reduction in the number of colonies compared with that of the untreated Control. The 1 percent limonene was significantly superior to 0.5 percent limonene, which in turn was significantly better than the 0.25 percent limonene treatment. The average number of colonies counted after 10 days was significantly higher than that after 5 days. These results support the hypothesis that d-limonene at concentrations as low as 0.25 percent is toxic to the pathogen in culture. It is interesting to note that both the pathogenic and non-pathogenic isolates reacted similarly to the limonene treatments. Mc Onie (1964) stated that both isolates were readily obtained from citrus peel but only the pathogen could be isolated

from black spot lesions. In order to explain this difference it appears that other factors besides limonene may influence the development of the fungus in the peel.

As d-limonene is the main constituent of citrus oils which occurs in oil glands in the flavedo, it was postulated that flavedo extracts have an inhibitory effect on the pathogen in culture compared with albedo extracts. In these experiments the extracts were chemically sterilized with BPL and a Control treatment of distilled water, treated similarly with BPL, was included as a comparison. Sterilization by filtration was tried initially and found to be unsatisfactory as the lighter fractions or terpenes remained on the filter pads. Results given in Tables 33 to 36 show that flavedo extracts had a significantly greater inhibitory effect on the fungus than albedo extracts. These results lend support to the above hypothesis.

Kiely (1948) and Kotzé (1963) reported that the disease was more severe on fruits on the side more exposed to sunlight viz. north, than on the south side of the tree. It was postulated that this was due to reduced antifungal activity in the peel of fruits on the north compared with the south side of the tree. According to Braverman (1949) the keeping quality of citrus oils is affected by light, oxidation and temperature. Light resulted in a large increase in the non-volatile residues. He mentioned that workers in Israel reported that increased amounts of non-

volatile residues occurred in the oil as the season advanced and this was attributed to the action of the UV rays of the sun. Results from Tables 33 and 34 show that fruit extracts from the north side resulted in significantly less inhibition of the pathogen in culture compared with that from the south side. Results in the next experiment, Tables 35 and 36, show the same tendency in the treatments with flavedo extracts, however, treatment differences were not significant. Variations in these results occurred primarily in the albedo treatments. A certain amount of difficulty was experienced in separating the albedo from the flavedo parts of the fruits. Particularly in the case of larger fruits with prominent oil glands i.e. December and January, in the second experiment, a certain amount of oil may have been included in the albedo extracts from some fruits and not from others which would explain the variations obtained. However, these results support the hypothesis that the anti-fungal activity of limonene is reduced in fruits from the northern or sunny side compared with the southern side possibly as a result of limonene break-down due to prolonged exposure to radiation from the sun.

Osmotic pressure

Bain (1958) reported that the percentage moisture in Valencia fruits increased progressively from an immature stage through to maturity. This coincides with the period

of increased symptom development on the fruit. It was postulated that osmotic pressure changes occurring in the flavedo during this period may influence the development of the pathogen and hence symptom development on the fruit. According to Lilly and Barnett (1951) changes in osmotic pressure may have a considerable effect on the growth of fungi in artificial medium. In this experiment flavedo extracts were made from fruits picked from the north and south sides of Valencia and Seville trees. According to Mc Onie (1964), Seville oranges appear resistant to this disease. This cultivar was included in this experiment as a comparison with Valencias. Results from Tables 37, 38 and 39 show that osmotic pressure readings of Valencia flavedo extracts in July were significantly higher than that in June, which in turn was significantly greater than those in May and February. Seville flavedo extracts gave just the opposite readings, where osmotic pressure values in February were significantly higher than those in May, June and July. The results with Valencia fruits support the above hypothesis that osmotic pressure changes in the flavedo may influence the development of the pathogen and hence symptom development of the fruit. Secondly, results from Table 37 show that the average osmotic pressure readings of fruit extracts from the north side were significantly greater than that from the south side. There appears to be a correlation between higher disease incidence on the north side of the tree with increased osmotic pressure values of the flavedo

of the fruit. It may be assumed possibly, that increased osmotic pressure in the flavedo tissue directly or indirectly stimulates the growth of the pathogen and subsequent development in the fruit.

Fruit blemish

Citrus fruits are characterized by their tough outer skin and many oil glands. Even with this tough rind the fruits are peculiarly susceptible to surface injury. The resulting blemish is often out of all proportion to the initial injury. Blemishes may result in a considerable reduction in external quality and therefore export of citrus fruits. The position is worsened by copper, applied until recently for black spot control, which accentuates, by darkening, existing fruit blemishes.

In recent years, side blemish on the fruit has increased and because thrip infestations also occurred, it was suspected that a relationship might exist between the two. Bedford (1943) previously mentioned that an identical blemish on the side of the fruit was caused by thrip and by early wind. He did not however, specify which of the two agents was the most important in causing side blemish to fruits. It was postulated that wind is the major cause of side blemishes on citrus fruits. Wind, thrips, Scirtothrips aurantii and mites, Panonychus citri were investigated

as possible causes of fruit blemish. Results from Tables 40 to 43 show a definite correlation between wind and criss-cross scratch marks on the fruit. Although differences were not significant, the highest numbers of fruits with continuous side blemish were recorded in the three wind treatments. In a second experiment trees were shaken at different periods to simulate wind-storm conditions in the orchard. Results from Tables 44 to 46 show that numbers of fruits with continuous blemish were significantly higher when fruits were treated in September/early October than in later months. Histological studies (Figure 16) showed continuous blemish (D) as a greyish superficial area extending into the epidermal cell layers of the fruit. The blemish was even-surfaced and on the same level as the surrounding healthy tissue. Treatment of fruits during late October and November respectively, resulted in significantly higher percentages of fruits with criss-cross scratch markings. In transverse section (Figure 16, C) the scratch markings appeared as raised areas of corky cells separated from the healthy tissue by a phellogen layer (1 - 1). This latter type of blemish was far more severe than continuous blemish and evoked a definite response from the fruit tissue as evidenced by the formation of a phellogen layer.

These results support the hypothesis that wind is the main factor causing side blemish on the fruit and furthermore, that two distinct types of wind blemish resulted when fruits were subjected to severe wind conditions at an

early compared to a later stage of fruit development. Independant work by Dodson (1966) on one aspect of this problem, coincided with investigations by the writer. He made observations which were in agreement with the results obtained by the writer.

Injury inflicted by wind to pea-sized fruits in September resulted in the immediate appearance of dark-green bruises where the tender fruit had been rubbed against the leaf midribs, twigs and branches. Within four weeks a continuous, smooth-surfaced, translucent or light-green to silver-grey blemish appeared which varied in size from a pin-head to that covering more than half of the fruit surface. The blemish, which occurred on the side of fruits, was smooth-surfaced, continuous and usually with a regular outline. Fruit injury in early October however, resulted in broken blemish areas with irregular outlines. As fruits reached maturity these blemishes became noticeable as yellow or golden-brown, smooth-surfaced, shiny areas which in severe cases covered most of the fruit surface. When the fruit reached the size of a golf ball, approximately in November, the peel became harder and wind damage at this stage gave rise to rough, corky scars on the fruit.

It is interesting to note that Tal and Monselise (1965) referred to a blemish appearing on the sides of Shamouti oranges and grapefruit as "spreading spot". This they ascribed to increased pressure in the endocarp and the

formation of small splits in the rind which led to the formation of fruit blemish. They mentioned that fruits were protected from external injury on the tree by enclosing them in paper bags after fruit set. According to the writer this may have been too late as the small fruits could easily have been bruised by wind without the injury being discernible at that stage. Possibly what they referred to as "spreading spot" may in fact have been wind blemish on the fruit.

In field experiments fruits were injured mechanically at different stages of development by cutting circles in the rind with a small cylinder. Results from Tables 47 and 48 show that with fruits treated in early October, the central area of the injury remained alive and the resulting blemish was smooth-surfaced (Figure 13). Treating fruits similarly in November, December and January resulted in dead tissue at the centre of the injury and a raised, scabby blemish. When blemishes were ranked according to the severity of injury using Friedman's two-way analysis of variance, blemishes resulting from the October treatment were significantly less severe than those in November, December and January.

According to Hall (1930) citrus oils are directly toxic to the fruit. He mentioned that the harmful element in the oil was probably limonene which acts by direct toxic action on the sound tissue of the peel. The writer

confirmed this by applying commercial d-limonene in small quantities to unblemished fruits. Within 24 hours the tissue of the flavedo had collapsed resulting in a sunken blemish lesion. Fawcett (1916) showed that lemon oil, acting for only eight seconds on the rind, was sufficient to cause fruit injury.

The formation of the oil glands at different stages of fruit development was shown in histological studies by Bain (1958). Oil glands in pea-sized fruit were shown to consist of numbers of central gland cells. At a later stage of fruit development oil glands enlarged further and the central gland cells were replaced by drops of oil. The correlation between the production of citrus oil and fruit size was also reported by Bartholomew & Sinclair (1946) who found that up to the time that Valencia fruits were mature, the yield of oil was directly correlated with surface area of the fruit. It was hypothesised that the smooth-surface, continuous side blemish originates from normal repair tissue which results from injury to small, pea-sized fruit in September/early October while unsightly, scabby, raised blemish is due to the toxic effect of citrus oil following injury at a later stage of fruit development. In this experiment, flavedo extracts from fruits of various sizes were applied to fruits of a similar size range on the same trees. Results from Tables 49 and 50 show that extracts from 15 mm fruits (pea-sized) were virtually innocuous to the fruit rind while extracts from larger fruits

of 25 and 35 mm diameters were directly toxic and resulted in raised, rough, scabby fruit blemish. These results support the hypothesis by showing that differences between "early" and "late" blemish may be ascribed to the added phytotoxic effect of citrus oil produced in fruits at a slightly later stage of development.

Copper markings

In Florida, where copper was applied for melanose (Phomopsis citri (Faure)) control, excessive blackening and corking of existing blemish was reported by Knorr et al (1957) and Pratt (1958). These authors stated that the later the copper sprays were applied in the season the greater the intensification of rind blemish. However, experiments conducted by Mc Onie and Smith (1964) on the timing of copper sprays in citrus orchards in the Transvaal, gave completely conflicting results. This they attributed to the fact that wind, which is a pre-disposing factor for copper blemish, was not controlled in their experiments.

In these experiments the treatments consisted of different times and concentrations of copper fungicide applications. In the first experiment with midseason oranges, two forms of copper blemish appeared on fruits in certain treatments viz. a black stippling or "flyspeck" occurred predominantly at the stylar-end where drops of

spray deposit had accumulated on the fruit and secondly, a darkening of existing blemish and a raised, black ridge around the border of the blemish, Figure 15. Results from Tables 51 to 55 show that copper markings were significantly less with early applications i.e. September and October compared to those applied later i.e. November and December. Secondly, where applications were increased from one to four sprays, copper blemish was severe. Blemishes were significantly reduced with the addition of a safener (slaked lime) to the copper sprays. Mancozeb did not result in any fruit stippling nor in noticeable darkening of blemish compared to that of the Control (unsprayed) treatment. The percentage fruits regarded as non-exportable in the standard treatment (treatment 8) was approximately 50 percent and 40 percent respectively with the Pineapple and Hamlin cultivars. It appears that fruits from Pineapple cultivar are more susceptible to copper blemish than that from Hamlin. Secondly, results show that when Mancozeb replaced copper (Treatment 1) or when single applications of copper were made during October, November (Treatments 1 to 5) or when slaked lime was added to the copper sprays, a significant increase in percentage export fruit was recorded compared ~~to~~ with that of the standard treatment.

The experiment was repeated in a young Valencia orchard in the same season as that of the previous experiment. Results from Tables 56, 57 and 58 were contradictory eg. treatments 3 and 4 showed significantly less darkening

of blemish than the standard copper treatment yet the former treatments did not differ significantly from the standard with regard to the percentage non-exportable fruits. As well, in Treatment 7 where the percentage fruits showing darkening of blemish was high, a low figure was obtained for percentage non-exportable fruit. In this experiment percentage fruit stippling was not evaluated and may have been the reason for increased rejection of fruit for export in treatments 3 and 4. The addition of parathion to copper or reducing the rate of copper from 0.91 to 0.68 kg/455 l (i.e. 2 to 1½ lb/100 gals) in both experiments, had no noticeable effect on blemish compared to the standard treatment. Safeners, slaked lime and sodium carbonate effectively reduced copper blemishes on the fruit in comparison with the standard treatment. The conflicting results in these experiments may have been due to the effect of random wind scarring on fruits since wind was not controlled in these experiments. Mc Onie and Smith (1964) reported similar difficulties in their experiments.

Histological studies revealed (Figure 16) stipple marks (A) as a black homogeneous mass separated from the healthy tissue by a definite phellogen layer (l - l). This indicates that stippling is the result of direct injury of copper to fruit tissue. In transverse section, darkened blemish due to the effect of copper appeared as darkly stained tissue in the flavedo (B) without the formation of a phellogen layer. This appears to support the

assumption held previously, that copper applied to an existing blemish is not directly phytotoxic but darkens the blemish thus making the fruit appear unsightly.

SUMMARY

1. Notwithstanding the application of preventative measures against black spot on citrus caused by Guignardia citricarpa Kiely losses frequently result due to the post-harvest development of the symptoms on export fruit in transit from the packhouses to ports prior to shipment overseas.

2. As little was known regarding the physiology of the fungus, i.e. from the onset of infection until the symptoms appear on the fruit, research was aimed at determining effects of environmental and physiological factors both on the fungus and on symptom development. The following aspects were investigated:

a. Light and temperature effects on:

(i) fruit symptom development,

(ii) sporulation of the fungus on peel discs,

(iii) growth and sporulation of G. citricarpa on artificial media.

b. Effect of limonene and fruit rind extracts on G. citricarpa in culture.

c. Osmotic pressure of peel extracts at various stages of fruit development.

3. Artificial light at an intensity of 10 Lux units resulted in a significant increase in the post-harvest

development of symptoms on latently infected fruit compared with fruit kept in the dark.

4. Raising the temperature from 20°C to 27°C resulted in a significant increase in the number of fruit symptoms.

5. Continuous light at 27°C resulted in a significant increase in the number of fruit symptoms after 10 and 15 days in comparison with either continuous light at 20° or continuous dark at 27°C.

6. Alternating light/dark (12 hourly) was not as effective as continuous light in inducing fruit symptom development.

7. Significantly higher numbers of fruit lesions were recorded after 15 days than after 10 days.

8. A significant increase in symptom development occurred on that half of the fruit exposed to the light compared with the opposite half kept in the dark.

9. On pieces of latently-infected flavedo discs in culture, the number of pycnidiospores produced was significantly higher in continuous light compared with continuous dark treatment and secondly were significantly more at 27°C than at 20°C.

10. On artificial media the pathogenic isolate of G. citricarpa sporulated significantly better in continuous light compared with alternating light/dark

treatment. Lowest pycnidiospore counts were recorded in the continuous dark treatment which differed significantly from both light treatments.

11. Pycnidiospore counts were significantly higher in all light treatments at 20°C compared with 27°C on artificial media.

12. On artificial culture media continuous light at 20°C and alternating light/dark at 20°C increased pycnidiospore production significantly compared with a continuous dark at 20°C.

13. The optimal period for sporulation in culture was 15 days where the average number of spores differed significantly from that after 10 and 20 days.

14. Production of both pycnidiospores and spermatia were significantly higher on potato dextrose agar compared with that on a basal synthetic agar.

15. Production of spermatia, unlike pycnidiospore production, was higher at 27°C compared with 20°C.

16. The effects of light and temperatures approximating conditions in the packhouse were determined on the sporulation of this pathogen in culture. These conditions viz. light at 27°C alternating 12 hourly with dark at 20°C, and also a control treatment of continuous light at 27°C resulted in a significant

reduction in pycnidiospore production compared with continuous light at 20°C.

17. Significantly greater mycelial weights of the pathogenic and non-pathogenic isolates occurred in liquid basal synthetic medium at 27°C compared with that at 22°C and 32°C. This supports the hypothesis that optimal growth temperature of the fungus coincides with that temperature most conducive to symptom development on the fruit.

18. Colonies of the pathogenic isolate on PDA grew in a spreading fashion when exposed to continuous artificial light as compared with colonies in the dark which were raised, compact and of smaller diameter.

19. Significantly greater mycelial weights of the fungus were recorded in liquid basal synthetic medium under continuous artificial light conditions compared with the dark treatment. There appeared to be a direct correlation between increased mycelial weights and an increase in the number of colonies in the light treatment.

20. All citrus fruits possess oil of which 90 per cent is d-limonene. Various quantities of commercial d-limonene were applied to macerated mycelium before being plated out on PDA. The number of colonies appearing on the plates after incubation

at 25°C for 5 and 10 days were counted. Limonene concentrations as low as 0.25 per cent resulted in a significant reduction in the number of colonies appearing on the plates. Both the pathogenic and non-pathogenic isolates reacted similarly to treatment with limonene.

21. Citrus oil (d-limonene) occurs in oil glands embedded in the flavedo. It was postulated that flavedo extracts have an inhibitory effect on the fungus in culture in comparison with that of the albedo extracts. Results showed that a significant reduction in numbers of colonies occurred on culture plates treated with flavedo extracts compared with that of albedo extracts.

22. Extracts from the fruit on the north side of the trees resulted in a greater reduction in the number of colonies compared with extracts from fruits on the south side.

23. Osmotic pressures of Valencia flavedo extracts increased significantly from February (immature stage) to July (i.e. at fruit maturity). Seville, which appear resistant to this disease, did not show this increase in osmotic pressure. Average osmotic pressure readings of flavedo extracts from the north side were significantly higher than those from the south side. Symptom development on fruits was

previously reported to be more severe on the north compared with the south side.

24. Copper sprays applied for black spot control were previously reported to darken existing fruit blemishes. As the exact cause of different side blemishes on the fruit was unknown and since these blemishes make the fruit more prone to copper blemish, an investigation into the possible causes of side blemish was warranted.

25. Thrips Scirtothrips aurantii Faure, red mite Panonychus citri McG and wind, were investigated as possible causes of blemish. Results showed that wind was the major cause of side-scarring on the fruit resulting in a continuous, smooth-surfaced blemish when fruits were injured just after fruit set (viz. pea-sized) compared with scratch markings when injury occurred at a later stage of fruit development.

26. Different types of fruit scarring due to mechanical injury at various stages of development were investigated. These results confirmed results obtained in experiments on various forms of wind injury at different stages of fruit development.

27. The difference in the type of blemish was shown to be due to a toxic factor in citrus peel extracts occurring mainly at a later stage of fruit development compared with pea-sized fruits (15 mm) which did not contain this toxic factor.

28. A darkening of existing blemish and secondly, the formation of stipple marks on the fruit are both attributed to copper injury. In histological studies the former appeared merely as darkly stained areas in the blemish area. Stipple marks however, appeared to be separated from the uninjured cells by a phellogen layer which suggests that this was a direct form of copper injury.

29. Experiments on the timing of copper sprays in relation to copper markings on the fruits were conducted but results were inconclusive. This may have been due to random wind-scarring on fruits as wind was not controlled in these experiments.

OPSOMMING

1. Ten spyte van die toepassing van maatreëls om swartvlek op sitrus, veroorsaak deur Guignardia citricarpa Kiely, te voorkom, kom daar dikwels verliese voor tydens die vervoer vanaf die pakhuise na die hawens as gevolg van die ontwikkeling van siektesimptome op die uitvoervrugte voor hul verskeping.

2. Omdat daar van die fisiologie van die swam, veral vanaf die begin van infeksie tot die verskyning van simptome op die vrugte, weinig bekend was, is die navorsing toegespits op die bepaling van die invloed van omgewings- en fisiologiese faktore sowel op die swam as op die ontwikkeling van simptome. Die volgende aspekte is ondersoek:

a. Die invloed van lig en temperatuur op:

(i) die ontwikkeling van simptome op die vrugte,

(ii) die sporulasie van die swam op skilskyfies,

(iii) die groei en sporulasie van Guignardia citricarpa op kunsmatige media.

b. Die invloed van limoneen en skilekstrakte op G. citricarpa in vitro.

c. Bepaling van osmotiese druk van skilekstrakte op verskillende stadia van vrugontwikkeling.

3. Blootstelling aan kunsmatige lig teen 'n sterkte van 10 Lux eenhede het gelei tot 'n betekenisvolle toename

in die na-oesontwikkeling van simptome op latent-besmette vrugte in vergelyking met vrugte wat in die donker gehou is.

4. 'n Toename in temperatuur van 20° tot 27°C het gelei tot 'n betekenisvolle toename van vrugsimptome.

5. Aanhoudende lig by 27°C het 'n betekenisvolle toename in simptoomontwikkeling in vergelyking met òf aanhoudende lig by 20°C òf aanhoudende donker by 27°C veroorsaak.

6. Afwisselende tydperke van twaalf uur lig en twaalf uur donker was nie so gunstig vir die vorming van simptome op die vrugte as aanhoudende blootstelling aan lig.

7. Betekenisvol hoër aantalle vrugletsels het ontwikkel na 15 dae as na 10 dae.

8. Op die helfte van vrugte wat aan lig blootgestel was, is 'n betekenisvol groter aantal letsels waargeneem as op die teenoorgestelde helfte, wat in die donker gehou is.

9. Op stukkies latent-besmette flavedo in kultuur was die aantal piknidiospore wat gevorm is betekenisvol hoër in aanhoudende lig as in aanhoudende donker, en was tweedens betekenisvol hoër by 27° as by 20°C.

10. Op kunsmatige media het die patogene isolaat van G. citricarpa in aanhoudende lig betekenisvol beter gesporuleer as wanneer tydperke van blootstelling aan

lig met donker periodes afgewissel is. Die laagste piknidiospoortellings is waargeneem na aanhoudende blootstelling aan donker, en het betekenisvol verskil van die twee behandelings, waarby die swam aan lig blootgestel was.

11. Wanneer die swam op kunsmatige media gekweek is, was piknidiospoortellings in al die ligbehandelings betekenisvol hoër by 20°C as by 27°C.

12. Op kunsmatige media is meer piknidiospore gevorm na blootstelling aan aanhoudende lig by 20°C en na afwissellende blootstelling aan lig en donker ook by 20°C as na aanhoudende blootstelling aan donker by 20°C.

13. Die optimale tyd vir sporulasie in kultuur was 15 dae; op dié stadium het die gemiddelde aantal spore betekenisvol verskil van die aantalle na 10 en 20 dae.

14. Die produksie van beide piknidiospore en spermatia was betekenisvol hoër op aartappel-dekstrose-agar (ADA) as op 'n basale sintetiese agar (BSA).

15. Die produksie van spermatia was in teenstelling met dié van piknidiospore hoër by 27° as by 20°C.

16. Navorsing is gedoen met betrekking tot die invloed van lig en temperatuur, soos dit voorkom onder pakhuistoestande met betrekking tot die vorming van spore in

vitro. Onder nagebootste pakhuistoestande naamlik, blootstelling aan lig by 27°C, vir 12 uur afgewissel met donker by 20°C, is betekenisvol minder piknidiospore gevorm as by 'n Kontrole behandeling naamlik aanhoudende lig by 20°C.

17. Die miseliumgewigte van sowel die patogene as die niepatogene isolate op die vloeibare basale sintetiese medium was by 27°C groter as by 22°C en 32°C. Dit bevestig die hipotese dat die optimal temperatuur vir die groei van die swam dieselfde is as dié wat optimaal is vir simptoomontwikkeling op die vrug.

18. Kolonies van die patogene isolaat op ADA het in aanhoudende lig op 'n spreidende wyse gegroei in teenstelling met die verhewe, kompakte kolonie tiepe wat in die donker ontwikkel het.

19. In aanhoudende lig is groter miselium opbrengste in die vloeibare sintetiese medium verkry as in die donker. 'n Direkte korrelasie is gevind tussen die toename van miselium-gewig en die toename in die aantal kolonies.

20. Alle sitrusvrugte bevat olie waarvan 90 persent uit d-limoneen bestaan. Verskillende hoeveelhede van kommersiële d-limoneen is by gemassereerde miselium gevoeg, voordat dit uitgeplaat is op ADA. Die aantal kolonies wat by 25°C na 5 en 10 dae ontwikkel het, is getel. Limoneen-konsentrasies so laag as 0.25

persent het 'n betekenisvolle vermindering in die aantal kolonies wat ontwikkel het teweeggebring. Beide die patogene en nie-patogene isolate het op dieselfde wyse op behandeling met limoneen gereageer.

21. Aangesien sitrusolie (d-limoneen) in spesiale olie-kliere in die flavedo voorkom, is veronderstel dat flavedo-ekstrakte 'n sterker inhiberende invloed op die swam in kultuur sal hê as albedo-ekstrakte. Resultate het getoon dat betekenisvol minder kolonies in kultuur ontwikkel het, waar flavedo-ekstrakte by-gevoeg is, as waar albedo-ekstrakte bygevoeg is.

22. Byvoeging van ekstrakte van vrugte afkomstig van die noordekant van bome het gelei tot 'n groter vermindering van die aantal kolonies as byvoeging van ekstrakte van vrugte afkomstig van die suidekant.

23. Die osmotiese druk van Valensia flavedo-ekstrakte neem vanaf Februarie (onvolwasse stadium) tot Julie (dit wil sê by rypheid) betekenisvol toe. Seville, wat blykbaar bestand is teen hierdie siekte, het nie hierdie toename in osmotiese druk getoon nie. Die gemiddelde osmotiese druk van ekstrakte van vrugte afkomstig van die noordekant was betekenisvol hoër as dié van vrugte van die suidekant. Simptoomont-wikkeling op vrugte is volgens vroeëre verslae erger aan die noordekant as aan die suidekant.

24. Dit is voorheen gerapporteer dat koperbespuitings vir swartvlekbeheer 'n neiging het om die reedsbestaande letsels donker te kleur. Aangesien die oorsaak van die verskillende letsels op die kant van die vrug onbekend was en aangesien hierdie merke die vrug meer onderhewig maak aan koperbeskadiging, is 'n ondersoek op moontlike oorsake van letsels as wenslik beskou.

25. Blaaspootjie, Scirtothrips aurantii Faure, rooimyt Panonychus citri McG en wind, is ondersoek as moontlike oorsake van hierdie letsels. Daar is gevind dat wind die hoofoorsaak van hierdie letsels was. Aaneenlopende, gladde letsels het ontstaan wanneer die vrugte net na vrugsetting naamlik ertjiegroote, beseer is en krapmerke in teenstelling ontwikkel as gevolg van latere beserings.

26. Verskillende tipes vrugletsels wat op verskillende stadia van ontwikkeling deur meganiese beskadiging veroorsaak is, is ondersoek. Hierdie resultate het resultate bevestig wat in eksperimente met varierende vorms van windbeskadiging op verskillende stadia van vrugontwikkeling verkry is.

27. Die verskil in die tipe van letsel is aangetoon om te wyte te wees aan 'n toksiese faktor aanwesig in die ekstrak van sitrusskil wat hoofsaaklik in latere stadia van vrugontwikkeling voorkom in vergelyking met kleiner vrugte (15 mm diameter) wat nie die toksiese

faktor bevat nie.

28. Die donkerder kleur van bestaande letsels en die vorming van stippelvlekke op die vrug word albei toegeskryf aan koperbeskadiging. Die eersgenoemde merke het in histologiese studies slegs as donkergekleurde areas voorgekom. Dit lyk of die stippelvlekke van die onbeskadigde selle geskei is deur 'n fellogeenlaag wat mag beteken dat dit 'n direkte vorm van koperbeskadiging is.

29. Proewe is gedoen om die verband vas te stel tussen die tye waarop met koper bespuit is en die mate van vrugbeskadiging. Die resultate was egter onoortuigend. Dit mag die gevolg gewees het van toevallige wind beskadiging van die vrugte aangesien geen poging aangewend is om wind beskadiging uit te skakel nie.

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