CHAPTER 2

DEVELOPMENT AND VIABILITY OF BLACK SPOT LESIONS ON CITRUS FRUIT

ABSTRACT

Citrus black spot (CBS) lesion types occurring commonly on fruit were indexed into four groups, viz. lesion type A (hard spots or shot-hole spots), B (false melanose, speckled blotch or inky spots), C (freckle spots) and D (virulent, spreading or galloping spots). This indexing accommodated the inclusion of atypical CBS lesions occurring on fruit into one of these classes. Freckle spot was the first symptom to develop on harvested naturally-infected symptomless Valencia fruit after 5 - 7 days of incubation at 27 °C, high humidity and 24 h fluorescent lighting. Pycnidia formed in these lesions contained viable conidia after 9 – 13 days of incubation. Mycelium in the margins of viable hard spot lesions survived packhouse processing while conidia present in the lesions were rendered sterile by processing and also rapidly lost viability on unprocessed fruit under optimal storage conditions. CBS symptoms could not be induced in healthy mature intact Valencia leaves by artificial inoculation with *P. citricarpa* conidia. Conidia of *Phyllosticta citricarpa* germinated abundantly in citrus juice extracts (Valencia orange, Eureka lemon and Texas Star Ruby grapefruit) while germination in distilled water, 0.5 g l^{-1} sucrose and citrus peel washings was negligible.

INTRODUCTION

Guignardia species, including their *Phyllosticta* and *Leptodothiorella* anamorphic states, comprise an economically important group of about 40 plant pathogenic species (Hawksworth & David, 1989). Among them, *Guignardia citricarpa* Kiely (anamorph: *Phyllosticta citricarpa* (McAlp.) Van der Aa), the causal agent of citrus black spot

(CBS), is a pathogen of major concern to citrus-growing in South Africa (Doidge, 1919; Wager, 1949; Kotzé, 1963; McOnie, 1964; Brodrick, 1969).

CBS is characterised by lesions which develop on maturing fruit of lemon, sweet orange, grapefruit and tangerine citrus varieties (McOnie, 1964). Various types of lesions can be distinguished during development of the disease, e.g. speckled blotch, freckle spot, hard spot and virulent spot (Kiely, 1948; Calavan, 1960; Kotzé,1963). There is, however, no general consensus regarding this terminology. For instance, hard spot is also known as shot-hole or type "A" spot, virulent spot as spreading or type "D" spot, freckle spot as type "B" spot and speckled blotch as false melanose or type "C" lesions (Kiely, 1948; Brodrick, 1969; Garrán, 1996). Furthermore, CBS lesions often cannot be classified into a specific category (S. Kamburov, personal communication). Also, although the *G. citricarpa* teleomorph does not occur in any of the lesion types on citrus fruit (McOnie, 1964; Kotzè, 1981), it is not clear to what extent each type contributes towards the production of viable conidial inoculum. A need thus exists for reassessing the lesion classification scheme, particularly with regard to viability of the various spots.

The purpose of this study is to propose a modified classification system for CBS fruit symptomology, present evidence on pycnidial development in various lesion types, to elucidate the effect of packhouse processing on conidial and mycelial viability in these lesions, investigate the susceptibility of citrus leaves to conidial infection and, to additionally, describe a medium for assessing the viability of *P.citricarpa* conidia.

MATERIALS AND METHODS

Lesion development on citrus peel. Valencia fruit exposed to a high inoculum of *G. citricarpa* ascospores in the orchard, but not showing visible CBS symptoms, were harvested in the 1997 season at Malelane Estates. The fruit did not receive any postharvest treatment except for surface-sanitising in 70 % ethanol for 5 minutes. Fifty fruit were placed in each of three growth chambers (Conviron) with 24 h exposure to full-

strength fluorescent light at 27 °C under high humidity. Lesion development was monitored and samples were prepared for electron microscopy at different developmental stages of the disease. Citrus peel blocks (ca. 5 mm^3) containing the dissected lesions were fixed for 24 h in 6 % glutaraldehyde. The samples were then washed three times for 15 min in 0.7 M phosphate buffer, dehydrated for 15 min in ascending percentages (50 %, 70 %, 90 %, 100 %) absolute ethanol, and dried in a critical-point dryer (Hitachi HCP-2). After goldplating in an Eiko IB3 ion coater, specimens were viewed in a Hitachi 400 scanning electron microscope.

Viability of different types of hard spot CBS lesions. Valencia oranges exhibiting typical hard spot CBS symptoms were collected in July 1997 at Malelane Estates and either left untreated or treated at the Crocodile Valley packhouse near Nelspruit. The packhouse process comprised a high-pressure descaler water spray (20 – 25 kPa), hot water bath (42 °C for 2 minutes), brushing with imazalil sulphate (1000 ppm Fungazil 75 % SP, Janssen Pharmaceutica) and waxing.

Hard spot lesions on the treated and untreated fruit were categorised into four classes, viz (a) red margin hard spots (RMH), (b) brown margin hard spots (BMH), (c) dark brown margin hard spots (DBMH) and (d) black margin hard spots (BLMH) (see Figs 9-12). Three lesions of a particular hard spot lesion category were selected per fruit and demarcated with a permanent marker. Twenty treated and 20 untreated fruit of each hard spot category were incubated at 27 °C under high humidity and continuous fluorescent lighting.

Five treated and untreated fruit from each hard spot category were removed from the incubator after 5, 14 and 21 days respectively. Each of the three selected hard spot lesions on each fruit was covered with a drop of spore germination medium (Chapter 3). When 50% or more of the pycnidia (3 to 200) present in the lesion had released their contents into the droplet, the conidia were harvested with a sterile 0.5 mm diameter glass capillary tube and streaked onto a single Petri dish containing the medium (described in Chapter 3). Concommitantly, a citrus peel block (ca. 3 mm^3) was dissected aseptically

from the lesion margin of each selected hard spot and plated onto medium in a separate Petri dish. Petri dishes were incubated for 10 days at 22 °C under high humidity and 24 h fluorescent lighting, and inspected visually for *P. citricarpa* colonies.

Evaluation of media for sustaining germination of *P. citricarpa* conidia. Germinability of conidia of *P. citricarpa* isolate from Natal (PPRI 5350) was determined as described in Chapter 3 in sterile distilled water (SDW) supplemented with the following substances (rates are per litre of SDW): (a) 15 g blended mature Valencia orange peel (flavedo and albedo), (b) 0.5 g sucrose, (c) 5 g citric acid (d) 20 ml mature Valencia orange juice, (e) 20 ml Eureka lemon juice, (f) 20 ml Star Ruby grapefruit juice, and (g) citrus peel washings from three mature Valencia oranges agitated for 8 hours in a litre SDW on a slow-speed rotary shaker. The various suspensions and solutions were filtered through Whatman no1 paper and autoclaved for 20 min at 120 °C. Unamended SDW served as control. Conidial concentration was 1 x 10^7 ml^{-1}, three replicates were used, and germination was recorded after 48 h.

Categorising of CBS symptoms. Symptoms on citrus fruit from various localities in South Africa were photographed to obtain a representative photo-gallery of the different symptoms occurring on fruit. Variations on the commonly-described symptoms were noted and the lesion types were categorised into main groups.

***P. citricarpa* leaf inoculation.** Ten 5-year-old greenhouse-reared Valencia orange on Swingle citrumelo citus trees were pruned, removing all existing leaves, and allowed to flush. Mature CBS-free leaves were selected in the new flush, washed with SDW, and inoculated with a conidial suspension of *P. citricarpa* isolate from Natal (PPRI 5350). Inoculation was accomplished by sticking a 15-mm diameter rubber ring with Vaseline® to the abaxial or adaxial surface of respectively five leaves on each tree, and depositing 500 µl of a 1 x 10^7 ml^{-1} conidial suspension of the *P. citricarpa* isolate in each of the rings. Control leaves received 500 µl SDW per rubber ring adaxially on five leaves per tree. After inoculation, each leaf was covered for one week with a small plastic bag to maintain a humid environment.

Inoculated leaves were harvested and inspected for CBS symptoms after four weeks. Isolations were made from the inoculated leaf areas on potato-dextrose agar (Merck, Biolab), and plant tissue sections were processed and observed under the electron microscope as described earlier.

RESULTS

Lesion development on citrus peel. The first lesions on mature CBS-infected Valencia fruit appeared as freckle spots after 5 – 7 days incubation at 27 °C with high humidity and constant fluorescent lighting. Development of these small, red, sunken dots on the citrus rind was restricted, with no notable tissue colonisation beyond the infection loci (Fig. 1). Small numbers of conidia were present in some of the pycnidia after 9 – 13 days (Figs. 2 & 3). At 20 days, lesions that developed under optimal conditions each contained more than one centrally-positioned pycnidium (Fig. 4). However, these pycnidia were at different stages of maturity, indicating that they would not release their conidia synchronously. In mature pycnidia present at 20 days, myriads of conidia were closely packed in a gelatinous mass (Figs. 5 & 6). The gelatinous substance and cap-like appendages on each conidium (Fig. 7) facilitated attachment of the conidia to the substrate.

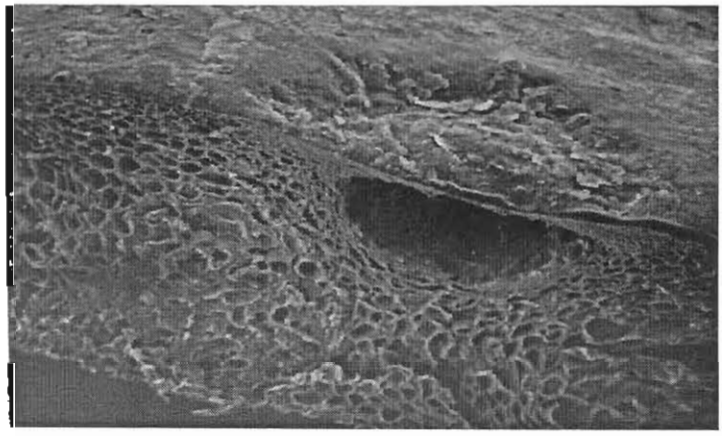

Figure 1: Section through a freckle spot lesion caused by *Phyllosticta citricarpa* on a Valencia orange after 7 days under optimal conditions for disease development.

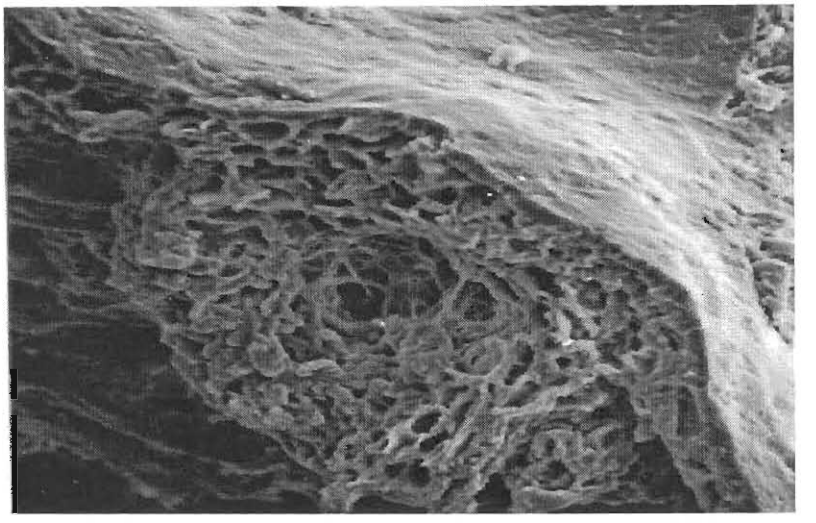

Figure 2: Primordial pycnidia of *Phyllosticta citricarpa* developing in a freckle spot lesion on Valencia citrus peel after 9 days under optimal conditions for disease development.

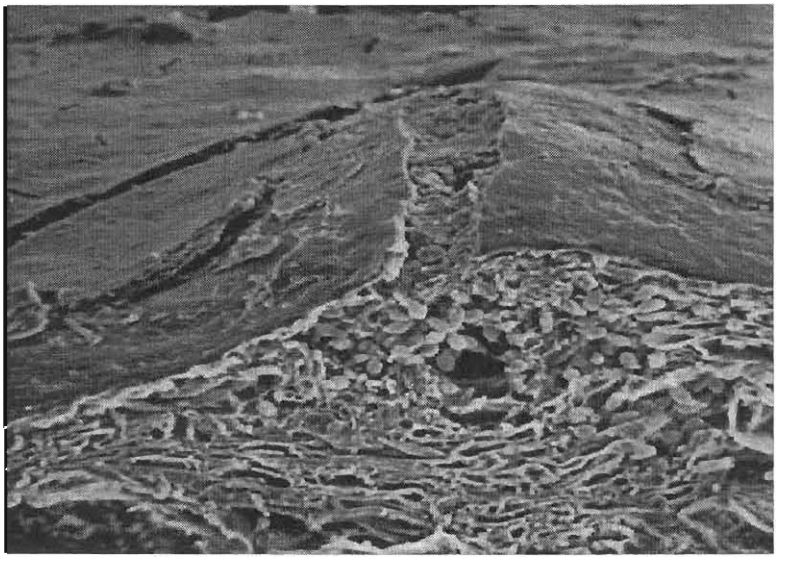

Figure 3: Conidia of *Phyllosticta citricarpa* in the first pycnidium formed in a freckle spot lesion after 13 days under optimal conditions for disease development.

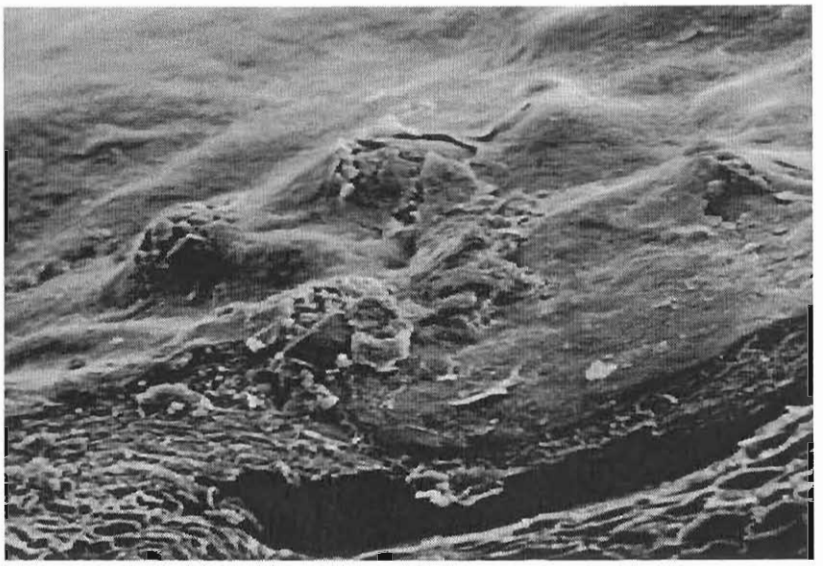

Figure 4: Pycnidia of *Phyllosticta citricarpa* at different stages of maturity after 20 days under optimal conditions for disease development.

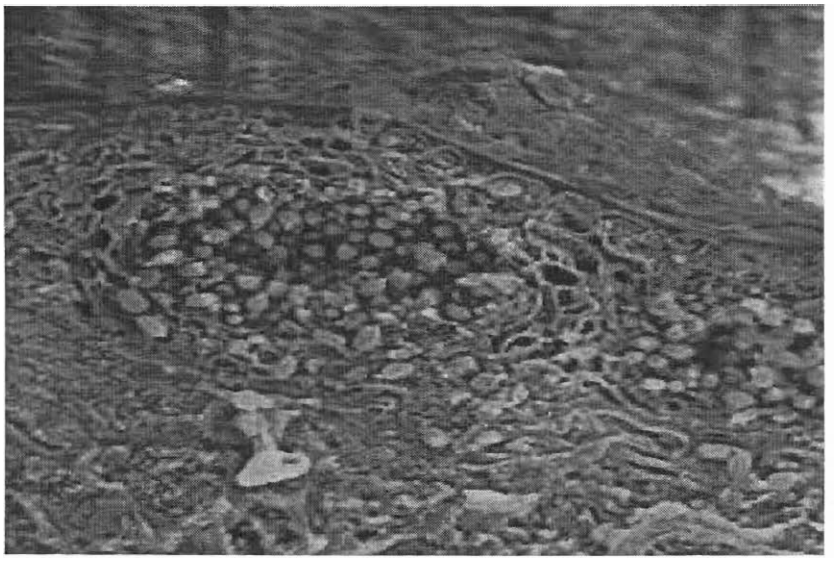

Figure 5: Fully-developed pycnidium of *Phyllosticta citricarpa* filled with mature viable conidia in a freckle spot lesion after 20 days under optimal conditions for disease development.

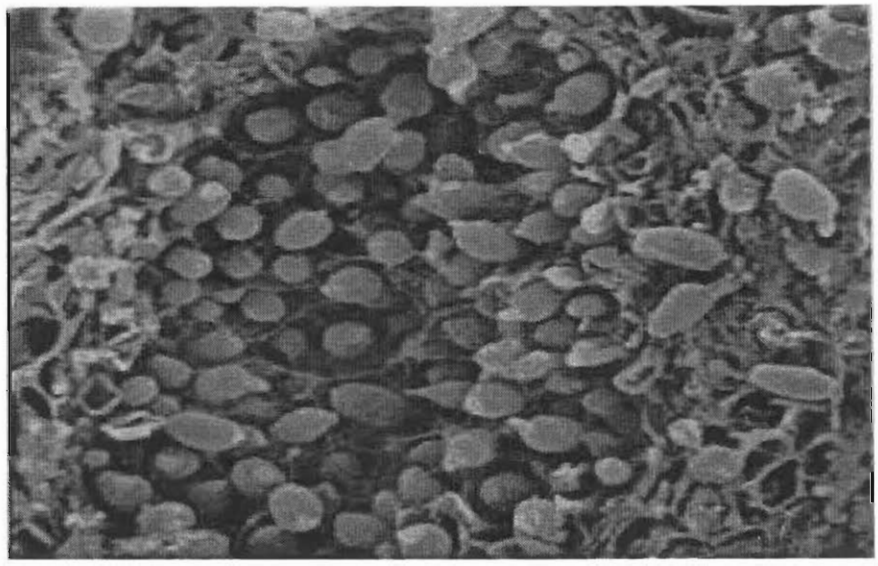

Figure 6: Enlargement of Fig. 5 showing numerous viable conidia of *Phyllosticta citricarpa* imbedded in a gelatinous mass ready for dispersal.

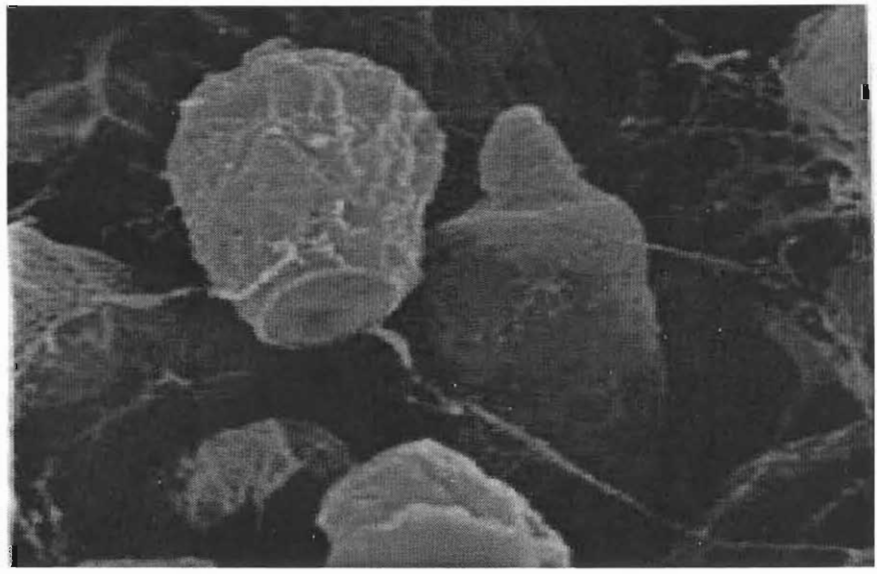

Figure 7: Viable conidia of *Phyllosticta citricarpa* present in a pycnidium on fruit incubated for 20 days. Note the gelatinous cap-like appendage on each conidium which is used for attachment.

Viability of different types of hard spot CBS lesions. At day 5, only RMH-type hard spot lesions on unprocessed fruit yielded an appreciable percentage of viable *P. citricarpa* conidia (Table 1). However, the incidence of viable conidia progressively declined to zero at day 21. Viability of conidia in BMH lesions on unprocessed fruit was about 73% less than in RMH lesions at day 5 and also of a transient nature. Very few conidia from DBMH lesions germinated when plated out, and none at all from BLMH lesions. Packhouse processing drastically reduced conidial viability, with RMH lesions being the only type yielding some viable conidia after processing, albeit only temporarily.

Table 1: Viability of *Phyllosticta citricarpa* conidia in different categories of CBS hard spot lesions on Valencia orange fruit subjected or not subjected to packhouse treatments.

Hard spot Category[x]	%Viable conidia on untreated fruit[y]			%Viable conidia on treated fruit[y]		
	5d	14d	21d	5d	14d	21d
RMH	43.3 a	8.3 b	0c	1.4 c	0 c	0 c
BMH	10.6 a	1.2 b	0 b	0 b	0 b	0 b
DBMH	0.3 a	0 a	0 a	0 a	0 a	0 a
BLMH	0 a	0 a	0 a	0 a	0 a	0 a

[x] RMH = red margin hard spots; BMH = brown margin hard spots; DBMH = dark brown margin hard spots; BLMH = black margin hard spots.

[y] Means in a row (based on 15 replicates) followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

A similar tendency was evident with mycelial viability, although packhouse processing had a less pronounced effect on survival (Table 2). BLMH lesions nevertheless contained no viable mycelium, whether fruit were treated or not.

Table 2: Viability of *Phyllosticta citricarpa* mycelium in different categories of CBS hard spot lesions on Valencia orange fruit subjected or not subjected to packhouse processing.

Hard spot category[x]	% Viable mycelium on untreated fruit[y]			% Viable on treated fruit[y]		
	5d	14d	21d	5d	14d	21d
RMH	98.2 a	90.4 a	87.2 a	67.2 b	54.6 b	58.3 b
BMH	83.3 a	74.8 b	63.2 b	17.3 c	11.3 cd	8.7 d
DBMH	8.6 a	5.9 a	4.3 a	1.2 b	0.4 b	1.2 b
BLMH	0 a	0 a	0 a	0 a	0 a	0 a

[x] RMH = red margin hard spots; BMH = brown margin hard spots; DBMH = dark brown margin hard spots; BLMH = black margin hard spots.

[y] Means in a row (15 replicates) followed by the same letter do not differ significantly (P= 0.05) according to Duncan's multiple range test.

Evaluation of media for sustaining germination of *P. citricarpa* conidia. Best germination of *P. citricarpa* conidia occurred in SDW supplemented with Eureka lemon or with Valencia orange juice, although Star Ruby grapefruit juice also supported good germination (Table 3). Stimulation of germination was evident in the citric acid solution, but considerable variation existed between replicates. Compared to the juice extracts, germination in the Valencia orange peel suspension was low, but nevertheless higher than in Valencia peel washings, sucrose solution, and SDW.

Table 3: Germination rate of *Phyllosticta citricarpa* conidia in sterile distilled water (SDW) supplemented with various substances.

Substance (per litre SDW)	PH	% Germination[x]
Sterile distilled water	7.25	0.2 c
15 g mature Valencia orange peel	4.62	27.8 d
0.5 g sucrose	6.92	0.3 c
5 g citric acid	4.00[y]	33.4 bd
20ml mature Valencia orange juice	4.34	63.9 a
20 ml mature Eureka lemon juice	3.84	69.2 a
20 ml mature Star Ruby grapefruit juice	3.93	44.0 b
Valencia peel washings	6.52	0.8 c

[x] Means in a column (based on 3 replicates) do not differ significantly (P = 0.05) according to Duncan's multiple range test.

[y] pH adjusted with 1 N NaOH.

Categorising of CBS symptoms. Four main types of CBS lesions could be distinguished, viz. Type A (hard spot or shot-hole spot) (Figs. 8-12), Type B (false melanose or speckled blotch) (Figs. 13 & 15), Type C (freckle spot) (Figs. 15-17), and Type D (virulent spot, spreading spot or galloping spot) (Figs. 18 & 19).

Type A symptoms (Fig. 8) are commonly present and may have a green halo on mature fruit or a yellow halo on green fruit. The symptom can be distinguished from other lesion types by a parchment-like, grey-white to light-brown depressed centre in which pycnidia may or may not be present. The rind of these lesions is slightly raised and vary in colour from light red to pitch black. Variations within lesion type A include type A1, (RMH) with a red margin (Fig. 9), type A2 (BMH) with a brown margin (Fig. 10), type A3 (DBMH) with a black margin and no halo (Fig. 11), and type A4 (BLMH) with a black margin (Fig. 12).

Type B lesions (Fig. 13) are an early-season expression of infection by the CBS pathogen and appear within months after fruit has reached the resistant stage towards infection. These lesions are small, circular, slightly raised, dark brown to inky black, surrounded by tiny black specks, and do not produce pycnidia. With time they may develop into type A lesions, whereas type C lesions can appear amongs them on mature fruit. Type B_1 lesions (mud-cake blotches) (Fig. 14) differ from classical false melanose or speckled blotch in being larger in diameter and having an abrasive texture. The centres of these lesions are cracked, giving them a mud-cake like appearance. They also do not contain pycnidia.

Type C lesions (Fig. 15) are red to dark red, sunken, and usually appear on mature fruit after the colour has changed from green to orange. Individual spots sometimes coalesce into type D lesion (Fig. 16). One or several (Fig. 17) pycnidia may be present in the lesions, but the grey-white craters characteristic of type A lesions are absent.

Type D symptoms (Fig. 18) mainly develop late in the season on fully mature fruit, even after harvesting (Fig. 19). The necrotic, sunken lesions are brown to brick red at the periphery, irregular, confluent and spread rapidly. Numerous black pycnidia may be present in collapsed tissue in the centre of old type D lesions. However, pycnidia and conidia are absent in newly-formed virulent spots.

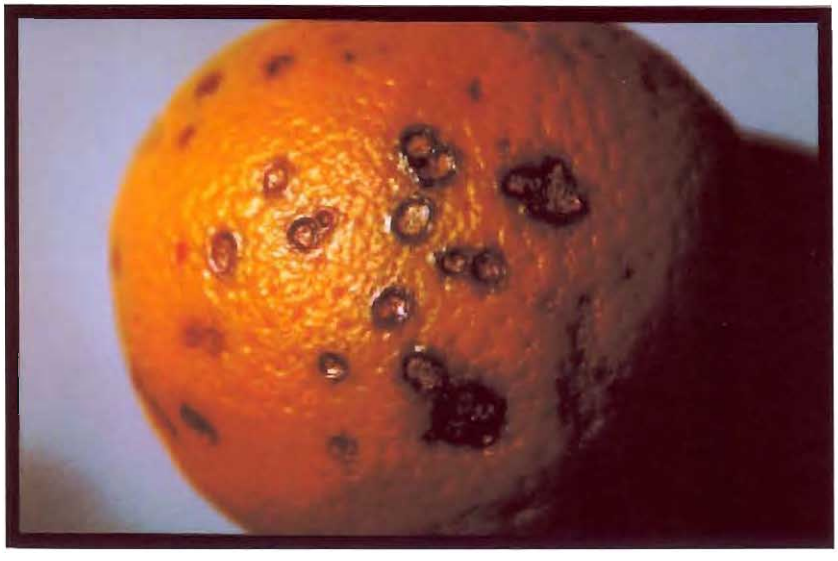

Figure 8: Citrus black spot lesion type A , hard spot or shot-hole spot.

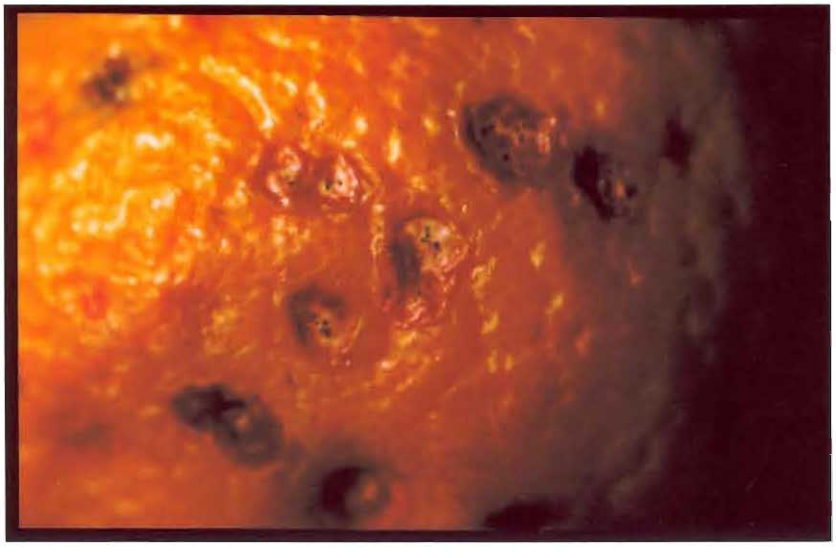

Figure 9: Citrus black spot lesion type A1, red margin hard spot (RMH).

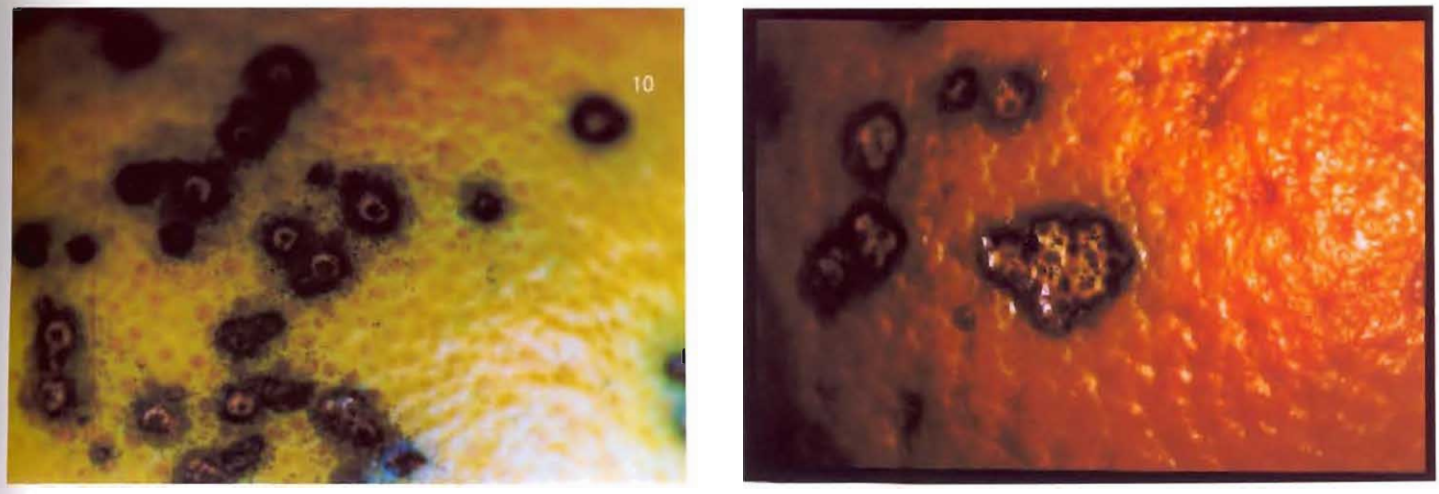

Figure 10: Citrus black spot lesion type A2, brown margin hard spot (BMH).

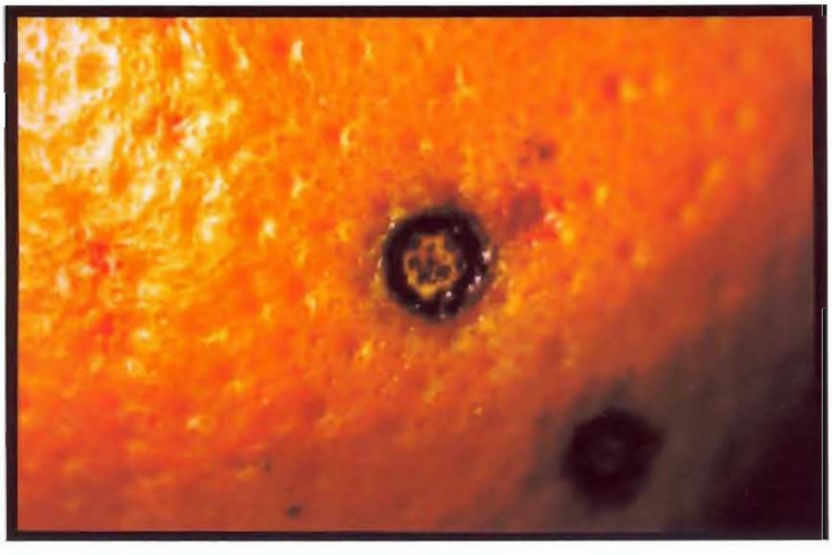

Figure 11: Citrus black spot lesion type A3, dark brown margin hard spot with no halo present (DBMH).

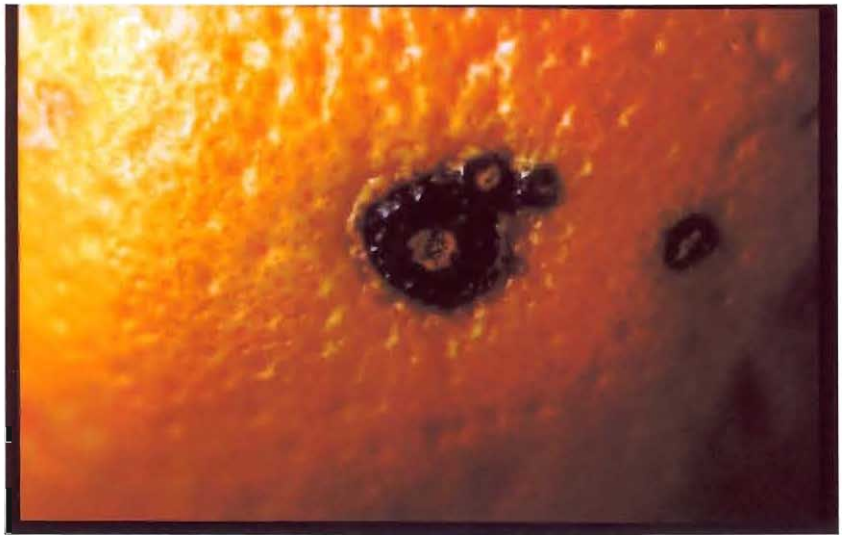

Figure 12: Citrus black spot lesion type A4, black margin hard spot (BLMH).

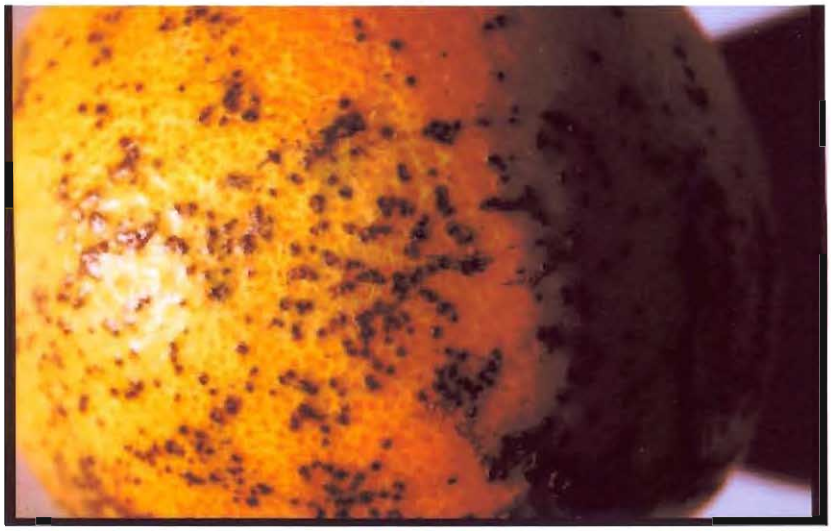

Figure 13: Citrus black spot lesion type B, false melanose or speckled blotch

Figure 14: Citrus black spot lesion type B2, mud-cake blotches.

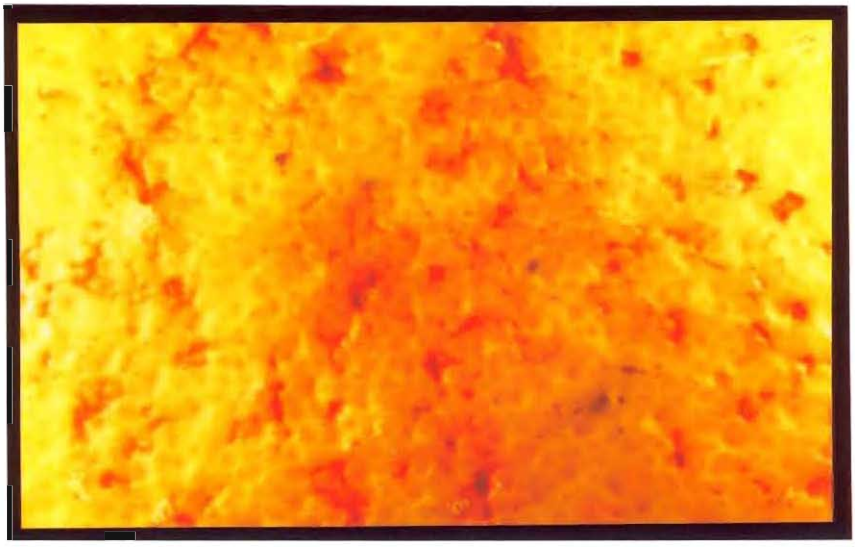

Figure 15: Citrus black spot lesion type C, freckle spot.

Figure 16: Coalescing type C freckle spots forming type D virulent spots.

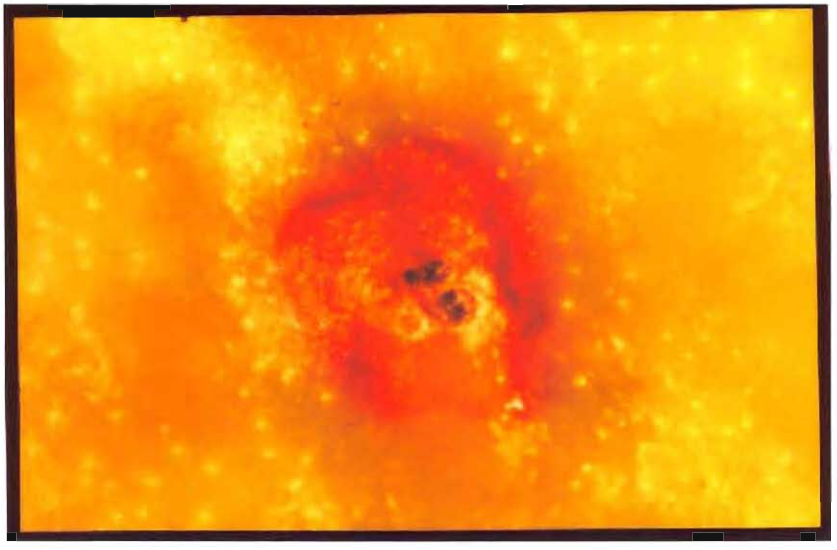

Figure 17: Citrus black spot lesion type C freckle spot with several pycnidia in the centre.

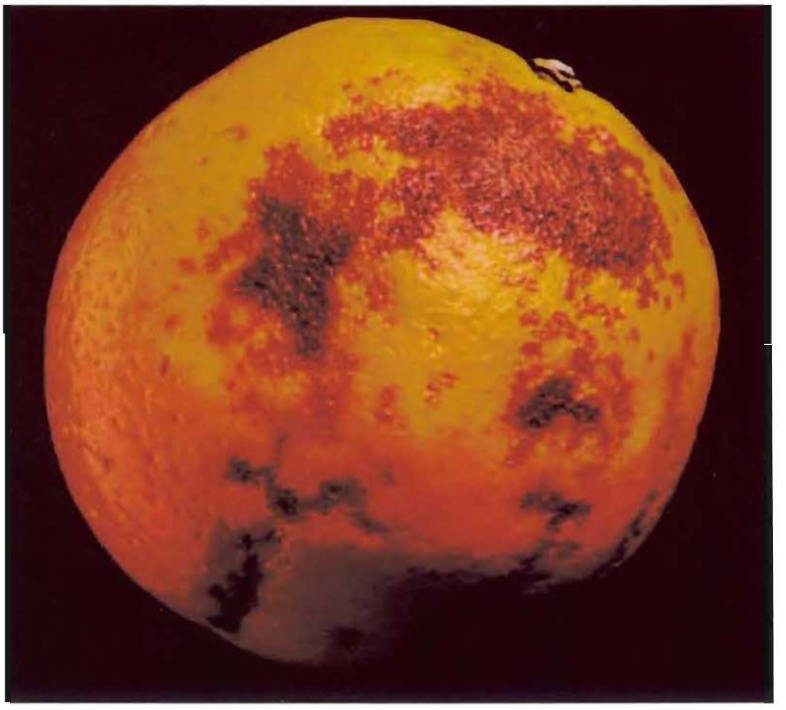

Figure 18: Citrus black spot lesion type D, virulent, spreading or galloping spot.

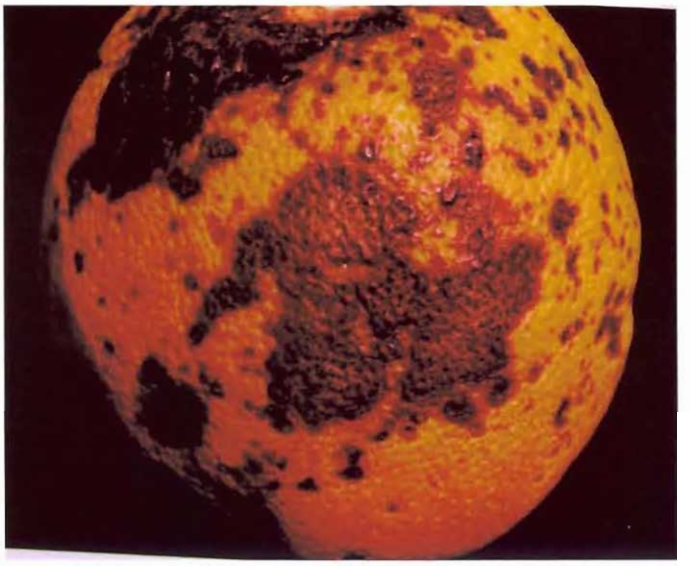

Figure 19: Virulent spot which developed after post harvesting.

***P.citricarpa* leaf inoculations.** *P. citricarpa* could not be re-isolated from any of the artificially inoculated mature Valencia leaves (Table 4). *C. gloeosporoides*, on the other hand, developed from about 40% of the inoculated and uninoculated leaf discs plated out. Electron microscopy indicated poor adhesion of *P. citricarpa* to the intact natural surface of leaves, but good adherence was observed in wounds and cracks (Fig. 20), which typically contained mucous clusters of conidia (Fig. 21). However, no conidial germination was evident after 4 weeks

Table 4: Isolation frequency of *Phyllosticta citricarpa* and *Colletotrichum gloeosporioides* from intact Valencia citrus leaves artificially inoculated or not inoculated with a conidial suspension of the CBS pathogen.

Tree no	Inoculation	% *P. citricarpa*[x]	% *C. gloeosporioides*[x]
1	Abaxial	0 a	34.2 b
	Adaxial	0 a	59.3 a
	Control	0 a	54.5 a
2	Abaxial	0 a	13.2 c
	Adaxial	0 a	66.8 a
	Control	0 a	43.2 b
3	Abaxial	0 a	25.3 b
	Adaxial	0 a	51.4 a
	Control	0 a	57.6 a
4	Abaxial	0 a	38.6 a
	Adaxial	0 a	10.3 b
	Control	0 a	12.5 b
5	Abaxial	0 a	66.6 a
	Adaxial	0 a	74.4 a
	Control	0 a	23.5 b
6	Abaxial	0 a	16.0 c
	Adaxial	0 a	62.4 a
	Control	0 a	42.7 b
7	Abaxial	0 a	8.5 b
	Adaxial	0 a	21.1 a
	Control	0 a	24.5 a
8	Abaxial	0 a	63.4 a
	Adaxial	0 a	62.7 a
	Control	0 a	50.5 b
9	Abaxial	0 a	39.5 a
	Adaxial	0 a	46.2 a
	Control	0 a	18.5 b
10	Abaxial	0 a	27.3 b
	Adaxial	0 a	56.4 a
	Control	0 a	58.3 a
Mean	Abaxial	0 a	33.3 a
	Adaxial	0 a	44.4 a
	Control	0 a	42.5 a

[x] Mean of 15 isolations from 5 leaves on each of 10 trees; values in columns within trees followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

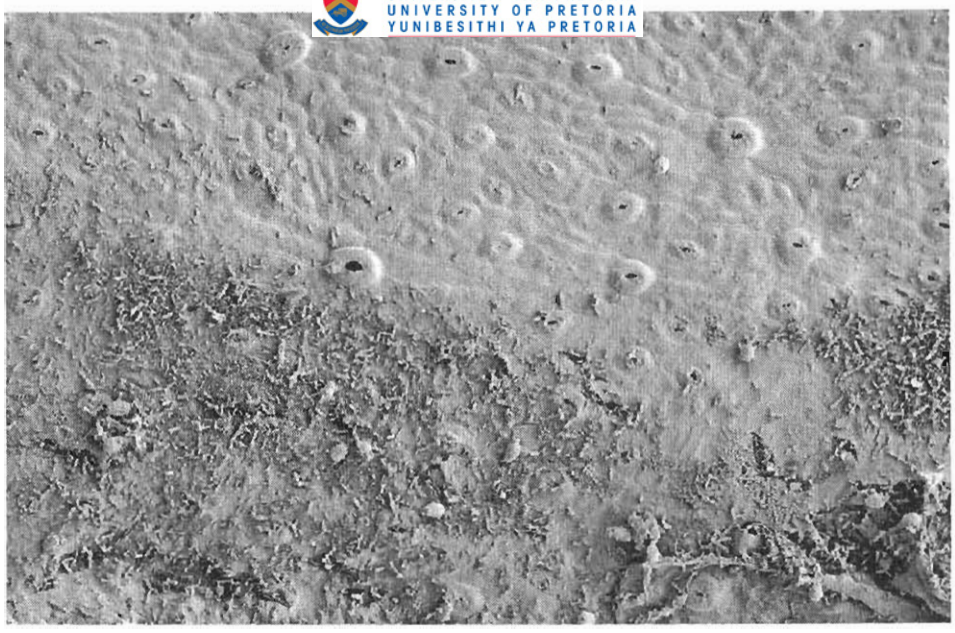

Figure 20: Poor adhesion of *Phyllosticta citricarpa* conidia to the intact natural leaf surface of abaxially-inoculated Valencia leaves (top of picture), and good adhesion in wounds and cracks

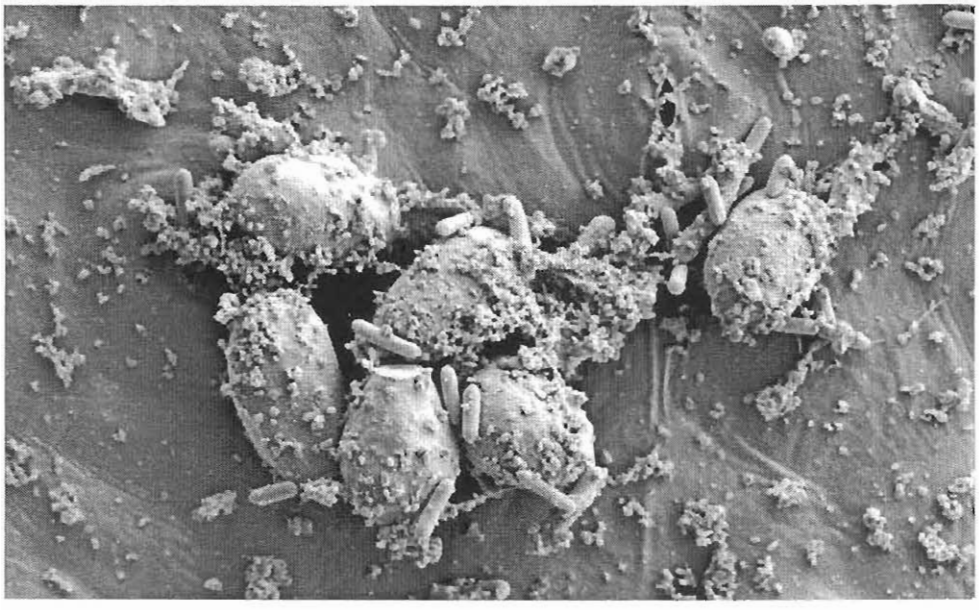

Figure 21: *Phyllosticta citricarpa* conidia in a wound on a Valencia leaf.

DISCUSSION

Brodrick & Rabie (1970) showed that light and temperature promote CBS symptom development. In the present investigation, CBS lesions developed within 5 – 7 days on symptomless, naturally-infected Valencia oranges at a storage temperature of 27 °C and 24 h fluorescent lighting. Lesions appearing first, were freckle spots and upon further incubation they developed into virulent spots. Pycnidia were produced after 9 – 13 days of incubation under optimal conditions. Viable conidia could be discerned in these pycnidia the moment they changed colour from whitish translucent to brownish black, i.e. from day 13 onwards. No conidia were present in primordial pycnidia sampled after 10 days.

Brodrick & Rabie (1970) observed significantly slower CBS lesion development at 20 °C than at 27 °C, whereas Wager (1948) found that lesion development at 4.5 °C was delayed to such an extent that practically no further increase occurred. The recommended export temperature for citrus varies between 10 °C and 11 °C (Venter & Cook, 1998). It is thus clear that under export conditions, lesion development and conidium production would be delayed. Furthermore, latently-infected fruit do not have the potential to produce new viable *P. citricarpa* conidia within the time allowed for marketing.

Valencia oranges receiving no postharvest treatment contained higher percentages of viable mycelium and conidia in the different hard spot lesions than fruit subjected to standard packhouse treatments. The combination of packhouse treatments and incubation time reduced the conidial inoculum present on fruit to zero. This is in accordance with findings in Chapter 4. It is clear from the higher percentage mycelium surviving the packhouse treatments that a brush-on application of imazalil sulphate is less effective than an imazalil, 2,4-D and guazatine dip treatment. Findings by Kiely (1948) and Darnell-Smith (1918) were confirmed that negligible *P. citricarpa* conidial germination occurs in distilled water. Germination in citrus peel extracts was not comparable to

germination in juice extracts. This might have been the result of toxicity of citrus peel extracts to *P. citricarpa* (Brodrick, 1971).

Grouping of the four basic CBS symptoms present on citrus fruit into lesion types A, B, C and D not only consolidated the various existing classification systems, but facilitated allocation of atypical CBS symptoms to a specific type (Table 5). The only lesion type capable of producing a significant amount of viable conidia was Type A1, red margin hard spot. Conidial inoculum produced by freckle and virulent spots can be regarded as insignificant compared to viable hard spots. Type A, "hard spots" are conspicuous blemishes on harvested fruit and are actively culled in the packhouse. It is furthermore unlikely for Type A lesions to develop in transit (Wager, 1948) and the chances of new viable conidia reaching CBS-free recipient countries, therefore remote. Lesion Type B holds no threat to importing countries. It is an early season expression of CBS containing no pycnidia or conidia and is also actively culled in the packhouse. Thus, the only viable CBS propagules potentially capable of reaching their end-destination via citrus fruit are viable mycelium in the margin of Type C and D lesions. Only under extreme conditions will it be possible to spread through viable mycelium on fruit to intact citrus leaves. This infection phase, together with a summer-rainfall climate, are essential for the continuation of the CBS disease cycle which will ultimately lead to a CBS epidemic (Kotzé, 1981).

Table 5: Comparison of proposed and existing citrus black spot lesion descriptions.

Proposed CBS lesion classification on fruit	Existing CBS lesion classification on fruit	Reference	Description of lesion type	Viable conidia and mycelium after packhouse processing
Type A	Hard spot (synonym: shot-hole spot)	Darnell-Smith, 1918; Kiely,1948; Calavan,1960; Kotzé, 1963, 1981; Brodrick, 1969; Garrán, 1996	▪ Circular crater-like lesions often surrounded by a green or yellow halo. ▪ Rims of the crater are slightly raised with a red to pitch black colour. ▪ Parchment-like centres are grey-white to light brown and may or may not contain numerous pycnidia.	Viable mycelium with the ability to produce viable conidia.
Type B	Speckled blotch (synonyms: false melanose; inky spot)	Kotzé, 1963; McOnie, 1965; Brodrick, 1969; Garrán, 1996.	▪ An early season expression of CBS. ▪ Lesions are small, circular and slightly raised. ▪ Lesions are dark brown to inky black, surrounded by tiny black specks and have an abrasive texture.	Viable mycelium. No conidia are found in this lesion type.
Type C	Freckle spot	Kiely,1948; Wager, 1948; Calavan,1960; Kotzé, 1963, 1981; Brodrick, 1969	▪ Small red to brick red sunken, irregular to circular lesions, which develop on mature fruit after the colour has changed from green to orange. ▪ No grey-white crater center is present. ▪ Pycnidia can develop in the lesions but never in the same quantities as in Type A lesions.	Viable mycelium in the margins of lesions.
Type D	Virulent spot (synonyms: spreading or galloping spot)	Darnell-Smith, 1918; Wager, 1948 Kiely,1948; Calavan,1960; Kotzé, 1963, 1981; Brodrick, 1969; Garrán, 1996	▪ Develop late in the season on fruit which is fully matured. ▪ Lesions are sunken and brown to brick red at the periphery, irregular, confluent and spread rapidly. ▪ Pycnidia may develop in the centers of these lesions upon incubation.	Viable mycelium. This lesion type frequently develops in transit and thus can contain viable conidia

Reisolation of *P. citricarpa* from artificially-inoculated intact mature Valencia leaves was unsuccessful and Koch's postulates could not be satisfied. Wager (1953) also failed to establish infection in intact citrus leaves by spraying a *P. citricarpa* conidial suspension onto healthy trees during the few months following petal drop. He proposed that spells of wet weather are necessary for the conidia to germinate and penetrate young fruit or leaves. The reluctance of *P. citricarpa* conidia to adhere to natural leaf surfaces observed

here corroborates the findings in Chapter 4 regarding conidial adhesion to the natural waxy layer present on fruit. However, more detailed investigations are necessary to determine if the age and state of citrus leaves could determine susceptibility towards infection by *P. citricarpa* conidia infection.

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