CHAPTER 7

Leaf litter management as a non-chemical means of reducing citrus black spot

7.1 Abstract

A four year study was carried out in a commercial Valencia orange orchard to assess the effect of leaf litter removal and mulching on citrus black spot inoculum and disease development on fruit. All leaves on the orchard floor were manually removed and burned between August and early October each year. Eight rows of 18 to 20 trees each were used for the treatments and received no chemical spray for citrus black spot control. The soil surface in four of the eight rows was mulched with a layer of wheat straw in October, whereas leaves were removed once again from the non-mulched area in the other four rows, a month later. Twelve Valencia orange trees in an adjacent orchard used as control, received no chemical spray for citrus black spot control or any leaf litter removal or confinement. No ascospores of *G. citricarpa* could be trapped with a volumetric spore trap in the orchard receiving leaf litter treatments during October to February for the 2001/2002, 2002/2003 and 2003/2004 seasons. Evaluation of fruit at harvest, indicated a mean citrus black spot severity index over four years of 0.4, 1.5 and 12.2 in the mulched, non-mulched and control rows, respectively. Except for the 2001/2002 season, there were no significant differences of citrus black spot infected fruit between trees where leaves were removed or removed and mulched with wheat straw. Results showed that leaf litter management by leaf removal and mulching allowed a reduction of up to 97% in citrus black spot development compared to control.

7.2 Introduction

South Africa (SA) is the second largest exporter of fresh citrus fruit in the world after Spain, although citrus growing in SA is a relative small industry compared to other countries (FAO, 2010b). During 2008, SA produced 2.2 million metric tonnes of fruit (FAO, 2010a) of which 64% were exported (FAO, 2010b). *Guignardia citricarpa* Kiely, the causal agent of citrus black spot (CBS), is an important quarantine organism that has resulted in sanitary and phytosanitary trade barriers for countries with CBS exporting to especially the European Union and United States of America (USA) (European Union, 1998). Furthermore, control of CBS contributes significantly to the production cost of citrus, contributing to the economic importance of CBS.

Control of CBS to a large extent relies on preventative fungicide sprays applied up to six times during the period of fruit susceptibility, from October to January (Kotzé, 1981;

Schutte *et al.*, 1997, 2003; Miles *et al.*, 2004). Since intensive fungicide spray programmes are expensive and have resulted in development of resistance in *G. citricarpa* to benomyl (Herbert & Grech, 1985), alternative non-chemical control measures are needed. Furthermore, environmental and human health concerns have led to increasing restrictions on the use of chemical fungicides (Janisiewicz & Korsten, 2002). This shift has resulted in greater emphasis in agriculture on adopting alternative approaches and use of integrated control measures.

Sanitation practices can contribute to disease control and resistance management in an integrated approach. Orchard sanitation whereby infected late hanging fruit are removed before the new crop sets and pruning of dead and possible infected twigs are widely practiced within SA (Kotzé, 1981). These sanitation practices effectively remove or reduce pycnidiospore inoculum in the trees, but no sanitation practices are currently employed to reduce inoculum from leaf litter, especially airborne ascospores that are considered the main inoculum source of CBS (Kotzé, 1981).

Pseudothecia of the pathogen develop on dead infected leaves on the orchard floor within 40 to 180 days after leaf drop, depending on the temperature and frequency of wetting (Kotzé, 1981). Once mature, ascospores are discharged mainly during spells of rain (Kotzé, 1963). Ascospore production, maturation and release are seasonal with most spores captured during October to February in summer rainfall regions (Swart & Kotzé, 2007). The most critical period for infection occurs at fruit set and can persist for four to five months (Kotzé, 1981). Reduction or removal of CBS inoculum from the orchard floor should significantly reduce infection of fruit. Therefore infected leaf litter must be removed before and during this critical infection period, to reduce the available CBS inoculum.

Mulching is an ancient technique and has many advantages such as reduction in water use due to limited evaporation, improved water infiltration, increased soil fertility, structure, porosity and aeration, reduced fertilizer use, less temperature fluctuations of soil, control of soil-borne diseases and reduced weed growth (Casale *et al.*, 1995; Wolstenholme *et al.*, 1996; Faber *et al.*, 2003). The net result is improved root growth and reduction in physiological stress, resulting in better fruit set, larger fruit and higher yields (Casale *et al.*, 1995; Wolstenholme *et al.*, 1996; Faber *et al.*, 2003). Disadvantages of mulching include that suitable material is either not available or too expensive, can create a fire hazard in dry winter months, may house insect pests and is labour intensive. Under certain conditions it can be toxic to plants, releasing toxic amount of ammonia upon degradation (Casale *et al.,* 1995; Wolstenholme *et al.*, 1996; Faber *et al.*, 2003).

Mulching has been used to control fungal foliar and fruit diseases by reducing the release of airborne ascospores from infected leaf or fruit litter. Mulching significantly reduced the release of ascospores of *Guignardia bidwellii* (Ellis) Viala & Ravaz from over wintering mummified grape berries (Becker & Pearson, 1993). A significant reduction in *Venturia inaequalis* (Cooke) G. Winter inoculum and scab symptoms on apple was achieved with various leaf litter management strategies, including mulching (Sutton *et al*., 2000; Vincent *et al*., 2004; Holb *et al*., 2006; Gomez *et al*., 2007). In citrus, mulching with grass resulted in a reduction of CBS at harvest although mulching on its own was not as effective as fungicidal sprays or mulching combined with sprays (Schutte & Kotzé, 1997).

Since mulching without fungicidal sprays did not reduce CBS to acceptable levels in a two-year study (Schutte & Kotzé, 1997), the effectiveness of leaf litter sanitation over a longer period needed to be investigated. Therefore, the aim of this study was to evaluate the effect of leaf litter removal and mulching with wheat straw in a commercial citrus orchard on CBS incidence over four seasons.

7.3 Materials and methods

The experiment site comprised an orchard near Burgersfort, Mpumalanga planted with Valencia Orange on Rough Lemon rootstock. All the leaves on the orchard floor from the entire estate were manually removed and burned between August and early October each year for three years before commencement of the study and for the duration of the study. Trees used during the 2001/2002 season were 31 years old. Four adjoining rows of 16 trees each were selected in each of two adjacent orchard blocks, 1.36 and 1.03 ha in size, respectively. All trees in the two sets of four rows of trees including those in an additional border row on each side received no chemical spray for CBS during 2001 to 2002. Late October 2001 the entire orchard floor in four rows in one block was mulched with a layer of wheat straw, whereas leaves were again removed from the non-mulched area in the other four rows in the adjacent block. The wheat used for mulching was cultivated on a nearby field on the same estate. The wheat straw was spread under the trees selected for mulching in such a way that the entire orchard floor was covered with a layer *ca*. 20 cm thick. All the trees in the two blocks used during the 2001/2002 season were removed and burned during 2003 due to generally poor tree condition and yield.

Valencia Orange on Rough Lemon rootstock trees used during the 2002/2003, 2003/2004 and 2004/2005 seasons were 34 years old at commencement of treatment. During 2002 to 2005 a total of eight adjacent rows of 20 trees each were used in a 5.56 ha block. All trees, including those in an additional border row on each side of the eight rows of trees used for the study, received no chemical spray for CBS during 2002 to 2005. Late October the surface under the trees in four of the eight rows, was mulched with a layer of wheat straw, whereas leaves were again removed from the non-mulched area in the other four rows. Twelve Valencia Orange on Rough Lemon rootstock trees in an adjacent orchard served as control during 2001 to 2005. Control trees received no chemical treatment for CBS or leaf removal and mulching for the duration of the study and were less than 250 m from the treatment trees.

Trees were evaluated in July the following year shortly before harvest. Forty-eight evenly-distributed fruit on each tree were assessed for CBS severity. Fruit were randomly selected to include 12 fruit per wind direction. From each wind direction, four fruit were from the top (top 33% of trees), the middle (middle 33% of tree) and the bottom (bottom 33% of tree) of the tree. From the four fruit per wind direction and horizontal position, two fruit were on the outside of the tree (peripheral) and two fruit on the internal side of the tree. Fruit were assessed according to a rating scale of 0 to 3, where 0 = clean; 1 = 1-5 spots per fruit; 2 = 6-50 spots per fruit; 3 = > 50 spots per fruit (Fig. 7.1). A severity index was calculated for each tree by means of the following formula, adapted from De Wet (1987):

$$\text{CBS-index} = 100 \times (0n_0 + 0.25n_1 + 0.5\, n_2 + 0.75n_3) / n_{\text{total}}$$

Where n represents the total number of infected fruit in each of the categories.

A Quest volumetric spore trap (Interlock Systems, Pretoria) was operated in the same block used for mulching treatments during October to February for 2001/2002, 2002/2003 and 2003/2004 seasons, but not for the 2004/2005 season. The spore trap was placed on a platform to ensure that the orifice is about 1 m from the soil surface (Fig. 7.2). The eight-day rotating disk was sprayed with a thin layer of petroleum jelly (Interlock Systems) to capture spores and replaced every seven days with a new petroleum-coated disk. Disks were stained with Trypan blue and the whole capturing surface was systematically examined at 100X and 400x magnification using a compound light microscope.

Data were analysed separately using the statistical program, SAS 9.2. One-way analysis of variance (ANOVA) was used to test for differences between values. The Student's *t*-least significant difference (LSD) was calculated at the 5% level of significance to compare means of significant effects.

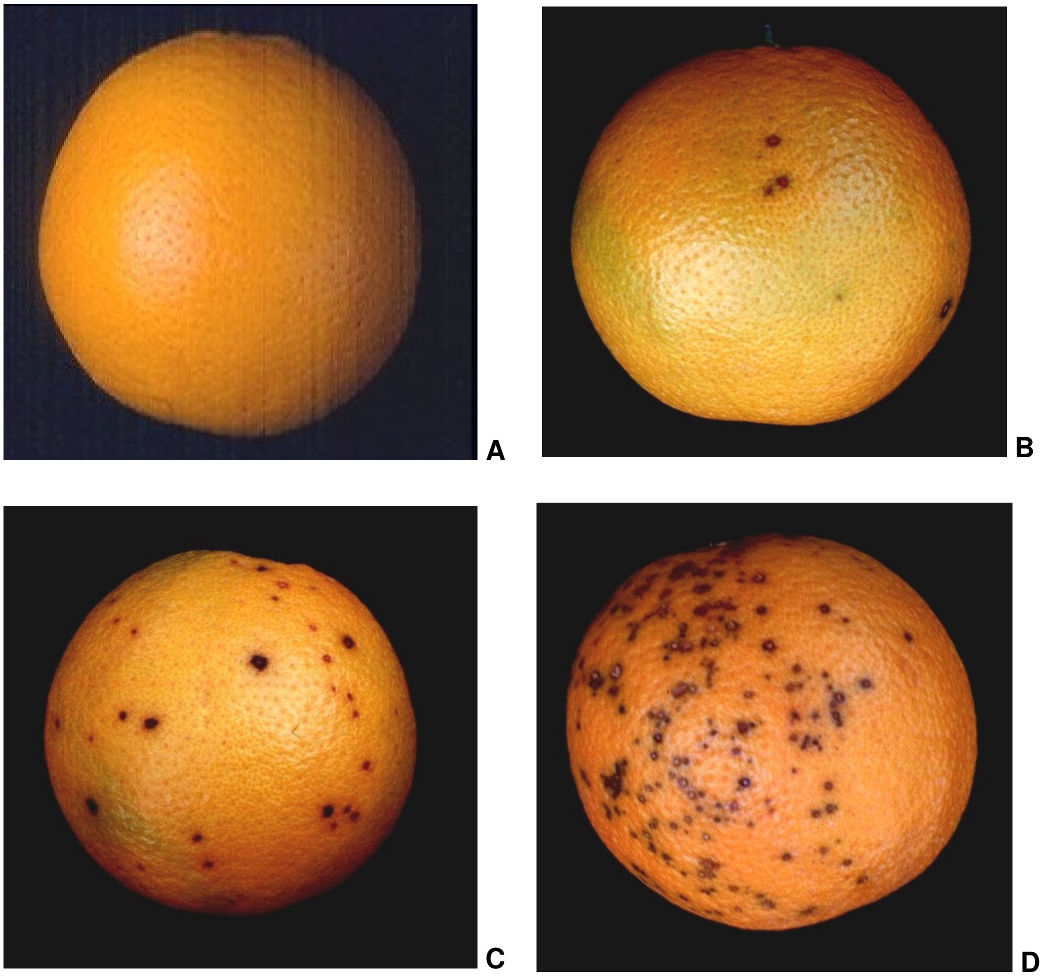


Figure 7.1. Rating used to evaluate level of fruit infection, where A: 0 = clean; B: 1 = 1-5 spots per fruit; C: 2 = 6-50 spots per fruit; D: 3 = > 50 spots per fruit.

Weather data consisting of mean monthly maximum and minimum temperature and total monthly rainfall for the Burgersfort area was obtained from the South Africa Weather Service.

7.4 Results

No ascospores resembling those of *G. citricarpa* could be discerned on the discs of the spore trap for the entire evaluation period of October to February for the 2001/2002, 2002/2003 and 2003/2004 seasons. Some fungal spores frequently observed on the disks include *Alternaria*, *Aspergillus*, *Bipolaris*, *Chaetomium*, *Cladosporium*, *Epicoccum*, *Penicillium* and *Stemphylium* species. A large number of unknown elongated to subglobose hyaline to dematiaceous spores and numerous pollen grains were observed, without any attempt to identify them. Apart from the spores and pollen grains, numerous miscellaneous particles, consisting of mainly dust particles, were also observed.

Leaf litter treatment applied during the 2001/2002 season resulted in significantly less CBS in the mulched rows than the non-mulched rows (Table 7.1). CBS index was 87.5% lower in the mulched rows compared to the non-mulched. Both treatments resulted in lower CBS than the control trees. The layer of wheat mulch remained sufficiently intact to cover the soil effectively and about a 5 cm thick mulch layer remained at the end of the growing season (Fig. 7.3)

The mean minimum and maximum temperatures per month during September to March were very similar for the four years, with mean minimum and maximum temperature of 12.7 to 13.4℃ and 20.8 to 23.6℃, respectively (Fig. 7.4). Season 2003/2004 had slightly higher mean monthly maximum temperature of 26.9℃ for December 2003 and 26.2℃ for January 2004, compared to the second highest of 24.7℃ for February 2005. A bigger difference was observed for rainfall between seasons. Total rainfall during September to March was recorded as 877.2, 597.4, 516.6, 492.5 and 160.6 mm, respectively, for the 2003/2004, 2000/2001, 2001/2002, 2002/2003 and 2004/2005 seasons.

No significant differences in results were obtained between years for a specific treatment repeated in the same orchard (Table 7.1). No significant differences were evident between mulching and no mulching for the 2002 to 2005 seasons, and both were significantly lower than the control for each year (Table 7.1). Leaf litter treatments reduced CBS incidence by 95.9% to 97.2% compared to the control for the 2002 to 2005 seasons.

No significant differences were evident when percentage infected fruit per tree were compared between fruit borne within the canopy and on the outside, as well as for fruit on top, middle or bottom part of tree (Table 7.2). The same observations were evident when percentage infected fruit per tree were compared for the aspectual distribution within a tree, with no significant differences in CBS occurrence for northern, eastern, southern or western part of the tree (Table 7.3). No CBS was present on chemically sprayed fruit in the adjacent orchard blocks at the time of assessment (results not shown).

CBS infection occurred mostly in the same trees for the 2002 to 2005 seasons. In rows receiving wheat mulch 46.7% of the trees bearing symptomatic fruit were infected each year during the three-year period, whereas 91.7% of the trees were infected each year in rows were leaf litter was removed (results not shown).

7.5 Discussion

This study confirmed that sanitation practices, such as leaf litter removal and mulching of leaf litter with wheat straw can decrease the primary inoculum of CBS and contribute to better management of the disease in a commercial orchard. Leaf litter removal or mulching can provide an alternative to chemical control and improve control in an integrated approach. Regardless of the prevailing climatic conditions each year, the number of infected fruit at harvest was on average reduced by 89% and 96% by leaf removal and mulching, respectively, compared to the control.

The mean minimum and maximum temperatures per month during September to March were very similar for the four years. In contrast, differences in rainfall between seasons were observed. Total rainfall during September to March was similar for 2000/2001 to 2002/2003 seasons, while 2003/2004 season received about 40% more rain than the previous seasons. In contrast, season 2004/2005 was the driest, receiving about 80% less rain than the previous season.

CBS-symptomatic fruit can be as high as 60% or more at harvest in orchards where no control measures were applied (Sutton & Waterson, 1966; Brodrick, 1969). In this study up to 40.8% of fruit in control rows were infected, indicating that the disease pressure in the area was severe and serious economic losses could occur without CBS control. In a similar study, no control measures resulted in up to 40.9% of fruit infected to some extent with CBS, whereas mulching with buffalo grass increased clean fruit at time of harvest by 21% compared to no treatment (Schutte & Kotzé, 1997). The higher level of control

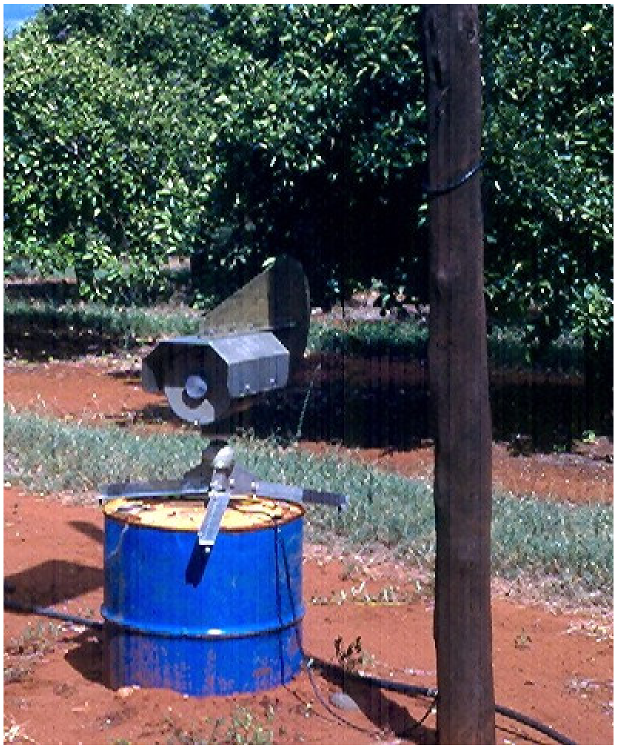
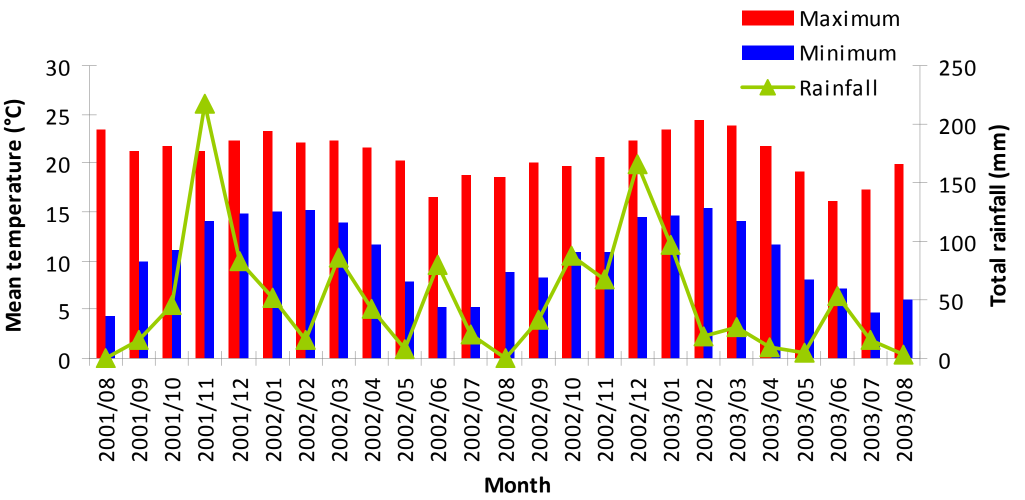


Figure 7.2. Quest volumetric spore trap used to capture spores during October to February.

Figure 7.3. Layer of wheat straw mulch on the orchard floor at the end of the season (March).



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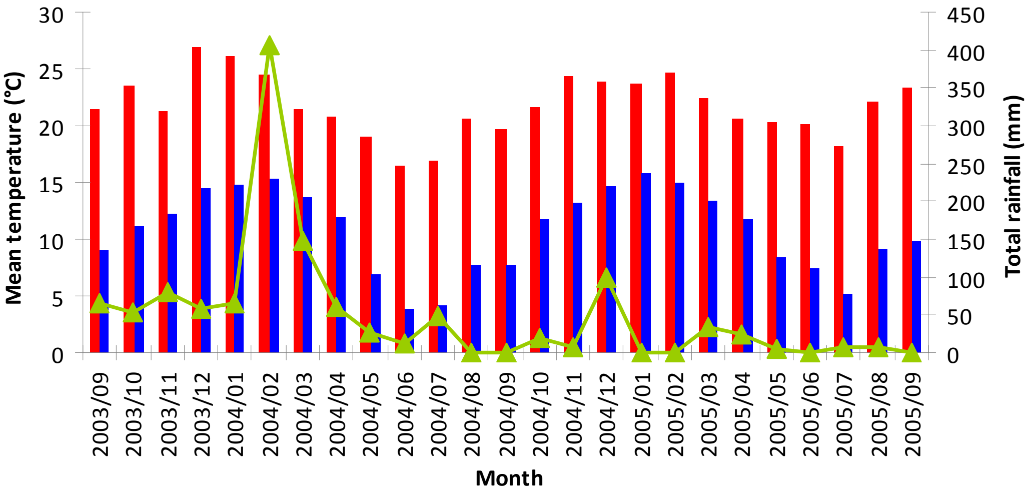

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Figure 7.4. Prevailing climatic conditions in Burgersford area, Mpumalanga, A: August 2001 to August 2003 and B: September 2003 to September 2005.

Table 7.1. Incidence and severity of citrus black spot in a commercial Valencia orange orchard where leaf litter was either left undisturbed (control), removed or removed together with mulching with wheat straw[a]

Parameter	Control	Non-mulched	Mulched
2001/2002			
Infected trees (%)	100 a	87.2 b	23.3 c
Infected fruit (%)	52.6 a	12.3 b	2.3 c
CBS-index	16.9 a	4.8 b	0.6 c
2002/2003			
Infected trees (%)	100 a	15.0 b	17.9 b
Infected fruit (%)	40.8 a	1.7 b	1.4 b
CBS-index	11.5 a	0.4 b	0.4 b
2003/2004			
Infected trees (%)	100 a	13.8 b	12.8 b
Infected fruit (%)	38.2 a	1.5 b	1.2 b
CBS-index	10.7 a	0.4 b	0.3 b
2004/2005			
Infected trees (%)	100 a	12.5 b	9.0 b
Infected fruit (%)	33.5 a	1.0 b	1.4 b
CBS-index	9.8 a	0.3 b	0.4 b
Mean			
Infected trees (%)	100 a	32.1 b	15.8 b
Infected fruit (%)	41.3 a	4.1 b	1.6 b
CBS-index	12.2 a	1.5 b	0.4 b

[a] Values are the mean of 48 fruit per tree with 12 to 80 trees per treatment; Values followed by the same letter in a row do not differ significantly according to Student's *t*-least significant difference ($P \leq 0.05$)

Table 7.2. Vertical and horizontal distribution of citrus black spot-infected fruit in trees in a commercial Valencia orange orchard from which leaf litter was either left undisturbed, removed or removed together with mulching with wheat straw

Treatment	Percentage infected fruit per tree[a]				
	Vertical distribution			Horizontal distribution	
	Top	Middle	Bottom	Internal	Peripheral
2001/2002					
Control	17.7 a A	17.2 a A	17.7 a A	25.9 a A	26.7 a A
Non-mulched	4.8 a B	4.8 a B	5.5 a B	9.7 a B	5.4 a B
Mulched	0.4 b B	6.0 a B	3.4 B	5.2 a B	4.6 a B
2002/2003					
Control	13.9 a A	12.7 a A	14.2 a A	20.3 a A	20.5 a A
Non-mulched	5.0 a B	4.5 a B	2.1 b B	6.3 a B	5.4 a B
Mulched	3.0 a B	2.5 a B	2.5 a B	4.5 a B	3.6 a B
2003/2004					
Control	12.7 a A	12.7 a A	12.8 a A	17.2 A	21.0 A
Non-mulched	4.0 a B	4.0 a B	3.2 a B	5.3 a B	5.9 a B
Mulched	2.9 B	5.0 a B	1.3 b B	5.2 a B	4.0 a B
2004/2005					
Control	11.5 a A	10.4 a A	11.5 a A	17.9 a A	15.6 a A
Non-mulched	4.0 B	1.5 B	2.1 B	4.5 a B	3.0 a B
Mulched	4.0 a B	3.5 a B	3.8 a B	6.7 a B	4.6 a B
Mean					
Control	14.0 a A	13.3 a A	14.1 a A	20.3 a A	21.0 a A
Non-mulched	4.5 a B	3.7 a B	3.2 a B	6.4 a B	4.9 a B
Mulched	2.6 a B	4.3 a B	2.8 a B	5.4 a B	4.2 a B

[a] Values are the mean of 16 fruit (vertical) or 24 fruit (horizontal) per tree with 12 to 80 trees per treatment; Values followed by the same letter in a row within vertical or horizontal distribution (lower case) or in a column within a season (upper case) do not differ significantly according to Student's *t*-least significant difference ($P \leq 0.05$).

Table 7.3. Aspectual distribution of citrus black spot-infected fruit in trees in a commercial Valencia orange orchard from which leaf litter was either left undisturbed, removed or removed together with mulching with wheat straw

Treatment	Percentage infected fruit per tree[a]			
	North	East	South	West
2001/2002				
Control	16.5 a	12.5 a	11.6 a	12.0 a
Non-mulched	4.4 a	3.3 a	2.9 a	4.2 a
Mulched	3.1 a	1.5 a	2.4 a	2.8 a
2002/2003				
Control	14.2 a	10.1 a	7.1 a	9.4 a
Non-mulched	5.0 a	2.4 a	1.4 a	2.8 a
Mulched	4.0 a	1.2 a	0.6 a	2.2 a
2003/2004				
Control	12.7 a	8.9 a	8.5 a	8.2 a
Non-mulched	4.9 a	2.5 a	1.5 a	2.3 a
Mulched	3.8 a	2.1 a	0.8 a	2.5 a
2004/2005				
Control	10.2 a	7.6 a	7.3 a	8.3 a
Non-mulched	3.8 a	1.1 a	0.8 a	1.9 a
Mulched	4.8 a	2.1 a	2.1 a	2.3 a
Mean				
Control	13.4 a	9.8 a	8.6 a	9.5 a
Non-mulched	4.5 a	2.3 a	1.7 a	2.8 a
Mulched	3.9 a	1.7 a	1.5 a	2.5 a

[a] Values are the mean of 12 fruit per tree with 12 to 80 trees per treatment; Values followed by the same letter in a row do not differ significantly according to Student's *t*-least significant difference ($P \leq 0.05$).

obtained in this study was mainly due to persistent leaf litter removal in the specific orchard for three years before commencement of treatments. Although the study of Schutte & Kotzé (1997) was only over two years, the authors noted a decrease of disease occurrence due to mulching from the first to the second year.

The persistent removal of leaf litter from the entire estate (46 blocks on about 215 ha combined) since 1998 has dramatically reduced the ascospore inoculum within the orchard and no ascospores of *G. citricarpa* could be detected on the discs of the volumetric spore trap for the full evaluation periods of October to February for the 2001/2002, 2002/2003 and 2003/2004 seasons. The spore trap was not operational for the last season, as the solar panel, generating energy for the trap, was stolen in the winter of 2004. A shortcoming of this study is that no data on ascospore levels within the orchard were collected before commencement of leaf litter removal and that no spores were trapped in the control block.

The source of inoculum, other than ascospores, in this particular orchard was investigated after no ascospores were captured during two consecutive seasons. No infected fruit remained on the trees after harvest (July), and very few dead twigs or branches were found which could harbour pycnidiospores. It is unlikely that pycnidiospores were the source of inoculum, as distribution of infected fruit in the trees, as well as spots on the fruit were random, indicating air-borne inoculum rather than water-borne inoculum as source for infection. It is possible that the low level of ascospores present in the orhard was not effectively captured with the spore trap.

A similar reduction in ascospore levels were obtained in a study on black rot of grape, caused by *G. bidwellii*, when overwintering mummified berries were covered with wheat straw (Becker & Person, 1993). The severity of black rot on clusters was significantly reduced by up to 62% when mummified berries were removed compared to control and number of ascospores released was often reduced to undetectable levels (Becker & Person, 1993). A significant reduction in *V. inaequalis* inoculum and scab symptoms on apple was achieved with various leaf litter management strategies, including shredding of leaf litter, application of urea and/or biocontrol products to leaf litter, leaf sweeping with leaf ploughing within rows (Sutton *et al*., 2000; Vincent *et al*., 2004; Holb *et al*., 2006; Gomez *et al*., 2007).

Studies on *G. bidwellii* and *V. inaequalis* indicated that the correct timing of mulching and/or leaf litter removal is of utmost importance and is linked to the epidemiology of the disease. Similar to chemical control programmes, sanitation practices aiming at reducing/eliminating the inoculum in overwintering leaf litter has to be applied before the onset of the critical infection period. In SA the critical fruit infection period is from October to January when newly set fruit are highly susceptible and infective mature ascospores are released at the commencement of the rainy season (Kotzé, 1981). These high levels of CBS control obtained through sanitation in this study can be ascribed to thorough leaf litter removal or confinement of the inoculum prior to the onset of the critical infection period.

The type of mulching material is important as the decay rate of material with a high carbon/nitrogen ratio immobilises nitrogen and can result in a temporary nitrogen shortage (Casale *et al*., 1995). Wheat has a carbon/nitrogen ratio of about 100 and ratios above 100 are considered to be too high and unsuitable for use as mulch (Handreck & Black, 1994). Since citrus trees normally produce nearly 80% of their roots in the top 50 cm of soil (Cahoon *et al*., 1956), the effect of decomposing wheat mulch on citrus growth and soil nutrient levels should be evaluated in future studies.

This type of sanitation practices would most likely be applied in organic orchards as the cost for manual removal of leaf litter and even mulching is very high. Leaf removal in the specific orchard near Burgersfort was terminated after the 2004/2005 season mainly due to labour cost implications. Manual removal of leaf litter in the 215 ha estate was also very difficult to complete within such a short time (August to beginning of October). Nevertheless, reduction in CBS can be achieved by persistent and entire removal, inactivation or immobilisation of overwintering inoculum residing in infected leaf litter on the orchard floor.

7.6 References

Becker, C.M. & Pearson, R.C. 1993. Evaluation of cultural control strategies for managing black rot (*Guignardia bidwelli*) of grapes. *Phytopathology* **83**: 1377.

Brodrick, H.T. 1969. Physiological studies with *Guignardia citricarpa* Kiely. DSc. thesis, University of Pretoria, Pretoria.

Cahoon, G.A., Hardy, R.B. & Miller, D.B. 1956. Declining citrus root systems. *California Agriculture* **10**: 3-12.

Casale, W.L., Minassian, V., Menge, J.A., Lovatt, C.J., Pond, E., Johnson, E. & Guillemet F. 1995. Urban and agricultural wastes for use as mulches on avocado and citrus and for delivery of microbial biocontrol agents. *Journal of Horticultural Science* **70**: 315-33.

De Wet, T.H. 1987. Control of citrus black spot at Lisbon Estate with particular reference to resistance of *Guignardia citricarpa* Kiely to benomyl. M.Sc. (Agric) dissertation, University of Pretoria, Pretoria.

European Union. 1998. Commission Decision of 8 January 1998 recognizing certain third countries and certain areas of third countries as being free of *Xanthomonas campestris* (all strains pathogenic to *Citrus*), *Cercospora angolensis* Carv. et Mendes and *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*). *Official Journal of the European Community* **L 015**: 41-42.

Faber, B.A., Downer, A.J. & Menge, J.A. 2003. Effects of mulch on avocado and citrus. *Proceedings of the Fifth World Avocado Congress* 2003: 719-724.

FAO. 2010a. FAO stat Agriculture data, http://faostat.fao.org/site/567/default.aspx#ancor. (Accessed 22 February 2010).

FAO. 2010b. FAO stat Agriculture data, http://faostat.fao.org/site/535/default.aspx#ancor. (Accessed 22 February 2010).

Gomez, C., Brun, L., Chauffour, D. & De le Vallée, D. 2007. Effect of leaf litter management on scab development in an organic orchard. *Agriculture, Ecosystems and Environment* **118**: 249-255.

Handreck, K.A. & Black, N.D. 1994. *Growing media for ornamental plants and turf*. University of New South Wales Press, Randrick.

Herbert, J.A. & Grech, N.M. 1985. A strain of Guignardia citricarpa, the citrus black spot pathogen, resistant to benomyl in South Africa. *Plant disease* **69**: 1007.

Holb, I.J., Heijne, B. & Jeger, M.J. 2006. Effect of integrated control measures on earthworms, leaf litter and Venturia inaequalis infection in two European apple orchards. *Agriculture, Ecosystems and Environment* **114**: 287-298.

Kotzé, J.M. 1963. Studies on the black spot disease of citrus caused by *Guignardia citricarpa* Kiely, with particular reference to its epiphytology and control at Letaba. DSc. (Agric) thesis, University of Pretoria, Pretoria.

Kotzé, J.M. 1981. Epidemiology and control of citrus black spot in South Africa. *Plant Disease* **65**: 945-950.

Janisiewicz, W.J. & Korsten, L. 2002. Biological control of postharvest diseases of fruits. *Annual Review of Phytopathology* **40**: 411-441.

Miles, A.K., Willingham, S.L. & Cooke, A.W. 2004. Field evaluation of strobilurins and a plant activator for the control of citrus black spot. *Australasian Plant Pathology* **33**: 371-378.

SAS. 1999. SAS/STAT user's guide. SAS Institute Inc, Cary, North Carolina.

Schutte, G. C. & Kotzé, J. M. 1997. Grass mulching as part of an integrated control programme for the control of citrus black spot. *Citrus Journal* **7**: 18-20.

Schutte, G.C., Beeton, K.V. & Kotzé, J.M. 1997. Rind stippling on Valencia oranges by copper fungicides used for control of citrus black spot in South Africa. *Plant Disease* **81**: 851-854.

Schutte, G.C, Mansfield, R.I., Smith, H. & Beeton, K.V. 2003. Application of Azoxystrobin fro control of benomyl-resistant *Guignardia citricarpa* on Valencia oranges in South Africa. *Plant Disease* **87**: 784-788.

Sutton, B.C. & Waterson, J.M. 1966. *Guignaridia citricarpa*. CMI Description of Pathogenic Fungi and Bacteria. No 85. CAB International, Wallingford.

Sutton, D.K., MacHardy, W.E. & Lord, W.G. 2000. Effects of shredding or treating apple leaf litter with urea on ascospore dose of *Venturia inaequalis* and disease buildup. *Plant Disease* **84**: 1319-1326.

Swart, S.H. & Kotze, J.M. 2007. Seasonal variations in ascospore availability of *Guignardia* spp. under different climatic conditions. *South African Journal of Science* **103**: v.

Vincent, C., Rancourt, B. & Carisse, O. 2004. Apple leaf shredding as a non-chemical tool to manage apple scab and spotted tentiform leafminer. *Agriculture, Ecosystems and Environment* **104**: 595-604.

Wolstenholme, B.N., Moore-Gordon, C. & Ansermino, S.D. 1996. Some pros and cons of mulching avocado orchards. *South African Avocado Growers' Association Yearbook* **19**: 87-91.

CHAPTER 8

General discussion

Citrus black spot (CBS), caused by *Guignardia citricarpa* Kiely, gained prominence as an economical important disease of citrus in the 1990's due to the phytosanitary risk associated with infected plant material. Restrictive trade barriers have been introduced to more effectively regulate the movement of citrus fruit from CBS-infected production regions to CBS-free countries in the Mediterranean and European Union (EU), as well as in Chile, Japan, United States of America and New Zealand (European Union, 1998; Paul *et al.*, 2005; Everett & Rees-George, 2006; Lemon & McNally, 2010). A whole consignment of fruit may be rejected, if, during inspection at packinghouses or ports, one spot on one fruit within the consignment is found (European Union, 1992; Bonants *et al.*, 2003). Consequently, CBS has a great impact on global citrus trade and is of great concern to affected growers.

The disease originated in south east Asia (Smith *et al.*, 1997) and has spread world-wide to summer rainfall production areas mainly through infected, but symptomless nursery trees (Kiely, 1949; Wager, 1952; Calavan, 1960; Kotzé, 1981). The main source of inoculum of CBS in infected orchards is ascospores produced only on leaf litter (Kiely, 1948; Kotzé, 1981). Pycnidiospores, when present on symptomatic fruit or twigs within a citrus tree, may also be a source of inoculum. Symptomatic CBS fruit may contain pycnidia with viable pycnidiospores and are regarded by the EU as a source of inoculum for CBS-free areas, therefore justifying their phytosanitary regulations. Furthermore, infected symptomless fruit may develop symptoms during transport or storage, increasing the possibility of symptomatic fruit reaching European markets.

Symptomatic CBS fruit or peel lying on the ground underneath citrus trees is not considered by us to be a source of inoculum. Only pycnidiospores are produced on symptomatic fruit and these spores have a relative short viability period. The current study clearly demonstrated that pycnidiospores of *G. citricarpa* from various sources failed to infect mature detached green leaves or leaf litter under controlled and field conditions. This is the first report on artificial inoculation of leaf litter with pycnidiospores of *G. citricarpa*. Symptomatic fruit or peel lying on the ground underneath citrus trees therefore cannot lead to infection and colonisation of freshly detached leaves and leaf litter by *G. citricarpa* and do not contribute to the production of subsequent inoculum in an orchard.

Therefore, commercial fruit are not considered to be a high risk for introduction of the pathogen into areas free of CBS.

Since *G. citricarpa* cannot infect freshly detached citrus leaves or leaf litter as shown in the current study, leaves have to be infected by the pathogen while still on the tree. Therefore, the inoculum produced on the leaf litter, thus depends on the level of infection of young leaves while attached to the tree (Kiely, 1948; Wager, 1952; Kotzé, 1963; McOnie, 1964c; Whiteside, 1967). Infected young citrus leaves forms a vital part in the survival of the pathogen and the period of susceptibility of citrus leaves to *G. citricarpa* was investigated in the current study.

The current study provided the first scientifically-founded data, substantiated by molecular identification of the pathogen, on the duration of the susceptibility to CBS of newly emerging citrus leaves monitored over time. The study indicated that the susceptibility period of citrus leaves to infection by the black spot pathogen was up to 10 months, considerable longer than previously perceived. Citrus trees can produce more than one new leaf flush per year. This implies that some part of the leaves on a citrus tree will be susceptible to *G. citricarpa* throughout the year. This, together with the long susceptibility period of newly formed leaves, makes chemical control of leaf infections unpractical. Therefore, apart from protecting susceptible fruit, control should also focus on reducing inoculum in the orchard.

Although sanitation practices in citrus orchards whereby infected late hanging fruit are removed before the new crop sets and pruning of dead and possible infected twigs are widely practiced within SA, no sanitation practices are currently employed to reduce inoculum from leaf litter (Kotzé, 1981). The current study showed that leaf litter removal or mulching can provide an alternative to chemical control. Sanitation through leaf litter management can also improve control in an integrated approach. Environmental and human health concerns have led to increasing restrictions on the use of chemical fungicides and greater focus on alternative non-chemical control measures that can contribute to disease control and resistance management in an integrated approach (Janisiewicz & Korsten, 2002).

Regardless of the prevailing climatic conditions each year, control achieved with litter management in the current study resulted in control equal to that achieved with the industry standard for fungicides (Schutte *et al*., 2003). This study confirmed the findings of Schutte & Kotzé (1997) that sanitation practices, such as leaf litter removal and mulching of leaf litter with wheat straw can decrease the primary inoculum of CBS and contribute to better management of the disease in a commercial orchard. This type of sanitation practices would most likely be applied in organic orchards as the cost for manual removal of leaf litter and even mulching is very high. Leaf removal in the orchard used for this study, was terminated mainly due to labour cost implications. Also, manual removal of leaf litter in the 215 ha estate was very difficult to complete in less than three months (August to beginning of October). Nevertheless, reduction in CBS can be achieved by persistent and entire removal, inactivation or immobilisation of overwintering inoculum residing in infected leaf litter on the orchard floor.

Once *G. citricarpa* has infected young citrus leaves, the pathogen usually remains latent as a small knot of mycelium directly under the cuticula (McOnie, 1967), until leaf drop and senescence (Kiely, 1948; Kotzé, 1981). After leaf drop, the pathogen is able to grow saprophytically and produce spores on the dead leaves within 40 to 180 days, depending on the temperature and frequency of wetting (Kotzé, 1981). The rate and severity of spore production, especially ascospores, on newly formed leaf litter in an orchard will provide valuable information on availability of inoculum and potential infection events.

The Kotzé Inoculum Monitor (KIM) was successfully applied to capture ascospores of *G. citricarpa* from naturally formed citrus leaf litter. Ascospores were captured from leaf litter collected during October to March each year with peak ascospore availability between December to February. Ascospore production was seasonal with most spores captured from leaf litter collected between October and February each year and no spores collected during the winter months. This seasonal production and maturation of spores has been reported for *G. citricarpa* as well as numerous other fungi (Pady, 1957; Kotzé, 1963; McOnie, 1964a, b; Chatterjee & Hargreave, 1974; Smith, 1996; Guerin *et al*., 2001; Rossi *et al*., 2001; Swart & Kotzé, 2007). The peak ascospore production recorded in the current study also corresponds to the period of reported fruit susceptibility in SA (Kotzé, 1981), starting from flowering (September to October) up to five months later (February to March).

The study using the newly developed KIM provided supporting information on ascospore maturity not previously accessible with the field-based volumetric sucking-type spore traps, such as the Hirst and Burkard versions. The KIM has the advantage over field-based spore traps of providing information on the presence of mature, ready to be dispersed, ascospores on leaf litter before a natural spore release event. Another advantage of the KIM is that variations in external factors such as temperature, water (dew/rain) and wind are eliminated, making data from different samples from the same orchard over time or from different orchards more comparable. Inoculum densities between orchards can be compared and the potential CBS risk can be assigned to these orchards, which in turn will contribute to improved management of the disease.

Various citrus production regions in South Africa have officially been declared free of CBS and include some of the regions in the Northern Cape, Free State, North West and all the citrus producing regions within the south-western Western Cape Province (European Union, 1998; Mabiletsa, 2003; APHIS, 2009, Shea, 2010). To verify and maintain the pest-free status of a production region, extensive monitoring work is required. In the absence of symptomatic fruit or sporulating fruiting bodies of *G. citricarpa* on leaf litter, detection techniques relies on isolations and DNA amplification with species-specific primers from symptomless plant tissue. Generally, detection of the pathogen from symptomless fruit or leaf material has a low success rate due to the restricted growth of the pathogen in latently infected tissue. An artificial leaf wilting method was optimised in the current study to provide an alternative detection method for *G. citricarpa* from symptomless leaves.

Alternate wetting and drying of leaf litter and variation in temperature have been reported to provide optimal conditions for spore formation and maturation (Kiely, 1948; Kotzé, 1981). Kiely (1948) described an artificial wetting and drying technique to induce sporulation of the CBS pathogen on freshly detached mature green leaves. However, few researchers have applied the technique with success (Wager, 1952; Kotzé, 1963; McOnie, 1964c, 1967; Whiteside, 1967). Most attempts to replicate the artificial leaf wilting described by Kiely in the current study failed. Results achieved were too variable and the method was found as not suitable for application in routine surveys.

After several adaptations from the original method described by Kiely (1948), formation of visual fungal fruiting structures on treated leaves developed after six to 14 days, in the current study. This is significantly faster than in the field under natural conditions or reported from Kiely’s wilting treatment. Furthermore, detection of the pathogen was improved considerably when combining the artificial leaf wilting with polymerase chain reaction (PCR) with species-specific DNA primers compared to PCR results of untreated green leaves or treated leaves without PCR. The artificial leaf wilting technique was very reliable, fast and effective in enhancing growth and sporulation of the CBS pathogen in latently infected citrus leaves. The wilting treatment in combination with PCR can be used to monitor citrus nurseries and orchards throughout the year, especially for CBS-free orchards to verify and maintain its pest-free status. Larger samples rather than smaller ones should be used due to natural variation in level of infection in leaves. This is the only detection method not dependant on season for sample collection and can greatly enhance the detection of the CBS pathogen throughout the year.

Some of the outcomes of this study have been included in a pest risk assessment (PRA) on CBS that have been presented to the EU. This study supports the PRA of South Africa stating that the risk associated with fruit for introduction of *G. citricarpa* is low. It is also my opinion that the current EU phytosanitary regulations pertaining to *G. citricarpa* on fresh citrus fruit imported into the EU are without adequate technical justification and are unnecessarily restrictive and disruptive to trade relevant to risk. In accordance with the International Plant Protection Convention principles of technical justification and minimal impact, failure to overturn current phytosanitary regulations pertaining to *G. citricarpa* on fresh citrus fruit imports would constitute an unjustified technical barrier to trade. It is recommended that the current EU phytosanitary regulations pertaining to *G. citricarpa* in association with fresh fruit should be re-evaluated.

Some aspects of the pathogen-host interactions require further clarification and future work should focus on:

- asco- and pycnidiospore production on leaf litter, and possibilities to reduce or inhibit especially ascospore production on the leaf litter without the need for labour intensive removal or confinement of the leaf litter.
- re-evaluation of leaf and fruit inoculations in the field as greenhouse studies may not be representative of field conditions.
- refined optimal as well as extreme conditions for infection of susceptible host material.
- survival of *G. citricarpa* in latently infected citrus plants. Can the pathogen be eradicated from an infected tree and to what extent does the pathogen move within citrus tissue?
- interaction between *G. citricarpa* and *G. mangiferae* isolates in the same host tissue. Do these two fungi compete for space and nutrients, and does *G. mangiferae* influence *G. citricarpa* in any way with disease expression?
- the underlining mechanisms of symptom development and the conditions required for formation of different symptom types on fruit.
- improved detection methods that can distinguish between *G. citricarpa* and *P. citriasiana*, the causal agent of citrus tan spot.

8.1 References

APHIS. 2009. Designated pest(s) free areas that meet APHIS requirements for pest-free areas listed in 7 CFR 319.56-5, Revision 20091102_006. Available from http://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/ DesignatedPestFreeAreas.pdf (Accessed 2 April 2010).

Bonants, P.J.M., Carroll, G.C., De Weerdt, M., Van Brouwershaven, I.R. & Baayen, R.P. 2003. Development and validation of a fast PCR-based detection method for pathogenic isolates of the Citrus Black Spot fungus, *Guignardia citricarpa*. *European Journal of Plant Pathology* **109**: 503-513.

Calavan, E.C. 1960. Black spot of citrus. *Californian Citrograph* **46**: 4, 18, 22-24.

Chatterjee, J. & Hargreave, F.E. 1974. Atmospheric pollen and fungal spores in Hamilton in 1972 estimated by the Hirst automatic volumetric spore trap. *CMA Journal* **110**: 659-663.

European Union. 1992. Commission Directive 92/103/EEC of 1 December 1992 amending Annexes I to IV to Council Directive 77/93/EEC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. *Official Journal of the European Community* **L363**: 1-65.

European Union. 1998. Commission Decision of 8 January 1998 recognizing certain third countries and certain areas of third countries as being free of *Xanthomonas campestris* (all strains pathogenic to *Citrus*), *Cercospora angolensis* Carv. et Mendes and *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*). *Official Journal of the European Community* **L015**: 41-42.

Everett, K.R. & Rees-George J. 2006. Reclassification of an isolate of *Guignardia citricarpa* from New Zealand as *Guignardia mangiferae* by sequence analysis. *Plant Pathology* **55**: 194-199.

Guerin, L., Froidefond, G. & Xu, X.-M. 2001. Seasonal patterns of dispersal of ascospores of *Cryponectria parasitica* (chestnut blight). *Plant Pathology* **50**: 717-724.

Janisiewicz, W.J. & Korsten, L. 2002. Biological control of postharvest diseases of fruits. *Annual Review of Phytopathology* **40**: 411-441.

Kiely, T.B. 1948. Preliminary studies of *Guignardia citricarpa* n.sp.: the ascigerous stage of *Phoma citricarpa* McAlp. and its relation to black spot of citrus. *Proceedings of the Linnean Society of New South Wales* **73**: 249-291.

Kiely, T.B. 1949. Black spot of citrus in New South Wales coastal orchards. *Agricultural Gazette of New South Wales* **60**: 17-20.

Kotzé, J.M. 1963. Studies on the black spot disease of citrus caused by *Guignardia citricarpa* Kiely, with particular reference to its epiphytology and control at Letaba. DSc (Agric) thesis, University of Pretoria, Pretoria.

Kotzé, J.M. 1981. Epidemiology and control of citrus black spot in South Africa. *Plant Disease* **65**: 945-950.

Lemon, N. & McNally, A. 2010. USDA confirms new citrus disease in Florida. United States Department of Agriculture, Animal and Plant Health Inspection Service, News Release 8 April 2010.

Mabiletsa, P. 2003. Republic of South Africa, citrus annual 2003. *Global Agriculture Information Network Report* **SF3037**: 1-11.

McOnie, K.C. 1964a. Apparent absence of *Guignarida citricarpa* Kiely from localities where citrus black spot is absent. *South African Journal of Agricultural Science* **7**: 347-354.

McOnie, K.C. 1964b. Orchard development and discharge of ascospores of *Guignardia citricarpa* and the onset of infection in relation to the control of citrus black spot. *Phytopathology* **54**: 1448-1453.

McOnie, K.C. 1964c. Source of inoculum of *Guignardia citricarpa*, the citrus black spot pathogen. *Phytopathology* **54**: 64-67.

McOnie, K.C. 1967. Germination and infection of citrus by ascospores of *Guignardia citricarpa* in relation to control of black spot. *Phytopathology* **57**: 743-746.

Pady, S.M. 1957. Quantitative studies of fungus spores in the air. *Mycologia* **49**: 339-353.

Paul, I., Van Jaarsveld, A.S., Korsten, L. & Hattingh, V. 2005. The potential global geographical distribution of citrus black spot caused by *Guignardia citricarpa* (Kiely): likelihood of disease establishment in the European Union. *Crop Protection* **24**: 297-308.

Rossi, V., Ponti, I., Marinelli, M., Giosuè, S. & Bugiani, R. 2001. Environmental factors influencing the dispersal of *Venturia inaequalis* ascospores in the orchard air. *Journal of Phytopathology* **149**: 11-19.

Schutte, G. C. & Kotzé, J. M. 1997. Grass mulching as part of an integrated control programme for the control of citrus black spot. *Citrus Journal* **7**: 18-20.

Schutte, G.C, Mansfield, R.I., Smith, H. & Beeton, K.V. 2003. Application of Azoxystrobin fro control of benomyl-resistant *Guignardia citricarpa* on Valencia oranges in South Africa. *Plant Disease* **87**: 784-788.

Shea, K. 2010. Notice of determination of pest-free areas in the Republic of South Africa. Department of Agriculture, Animal and Plant Health Inspection Service, Docket No. APHIS-2009-0037. *Federal Register* **75**: 6347-6348.

Smith, I.M., McNamara, D.G., Scott, P.R. & Holderness, M. 1997. *Quarantine pests for Europe*. CAB International, Wallingford.

Smith, J.H. 1996. A study of the effect of various disease control programs on spore releases of the citrus black spot pathogen *Guignardia citricarpa* Kiely. *Proceedings of the International Society of Citriculture* **1**: 351-352.

Swart, S.H. & Kotze, J.M. 2007. Seasonal variations in ascospore availability of *Guignardia* spp. under different climatic conditions. *South African Journal of Science* **103**: v.

Wager, V.A. 1952. The black spot disease of citrus in South Africa. *Science Bulletin of the Department of Agriculture of the Union of South Africa* **303**: 1-52.

Whiteside, J.O. 1967. Sources of inoculum of the black spot fungus, *Guignardia citricarpa*, in infected Rhodesian citrus orchards. *Rhodesia, Zambia and Malawi Journal of Agricultural Research* **5**: 171-177.