IMPACT ASSESSMENT OF CITRUS BLACK SPOT, *GUIGNARDIA CITRICARPA* KIELY, IN SOUTHERN AFRICA AND AN ALTERNATIVE APPROACH IN MANAGEMENT STRATEGIES

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DEDICATION

This thesis is dedicated to my parents and siblings, more especially my late mother Ester Ndahekelekwa Halueendo and brother Laban Kauko Iniko Halueendo. I love you all.

DECLARATION

I, the undersigned, hereby declare that the thesis submitted herewith for the degree M. Inst. Agrar. to the University of Pretoria contains my own independent work and has not been submitted for any degree at any other university.

Keumbo Lorna Maija Ester Halueendo

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Citrus black spot (CBS) caused by *Guignardia citricarpa* is responsible for economic losses in Southern African countries such as South Africa, Mozambique, Swaziland, Zimbabwe and Namibia. Black spot is considered to be a phytosanitary disease for the European Union and the United States of America markets. Exporters to these countries incur losses throughout the supply chain due to phytosanitary restrictions. For these reasons, the occurrence and management practices of CBS and its impact on growers in Southern Africa were investigated through a survey using a questionnaire. In the study, it was found that when CBS was present it was primarily managed by using chemicals and general orchard sanitation. In addition, growers in some of the surveyed countries or production regions follow spraying programs that are based on disease forecasting models and this practice has proven very effective in managing the disease. Furthermore, furfural, a sugarcane waste product was assessed for its efficacy in controlling *G. citricarpa*. The efficacy of the product as a contact or a fumigant was demonstrated *in vitro* and *in vivo* on fresh leaves, leaf litter and fruit lesions as well as in soil. A molecular study, using a Polymerase Chain Reaction protocol was conducted to assess the survival of the pathogen in the soil after exposure to furfural. The product however only proved efficient under natural conditions. The non-target effect of furfural on the soil micro-flora was also assessed. The product proved suitable for soil applications as it is not phytotoxic and has minimal non-target effects on bacterial populations. Furfural proved to control *G. citricarpa*, by breaking the life cycle, thus reducing the disease incidence. The application of furfural on a larger scale (irrigation or spraying) will therefore improve the control of CBS in developing countries.

CHAPTER 1

GENERAL INTRODUCTION

Citrus (*Citrus* L.), particularly the commercially important varieties, are generally believed to have originated from species native to southeastern Asia and the Malay Archipelago (Davies & Abrigo, 1994). Currently, citrus is grown in over 100 countries on six continents, primarily between the latitudes of 40º N and 40º S (Ismail & Zhang, 2004; Anon., 2006). The crop is especially grown in areas with sufficient rainfall, where there is enough water for irrigation and where the temperatures do not drop too low to kill the trees (Whiteside, 1988). Citrus fruits are consumed and appreciated around the world for their nutritional value, particularly vitamin C. The skin and fruit pulp can also be used for livestock feed and to prepare compost (Opeke, 1982). It has also been reported that water extracts from composted orange peels has been used to control certain plant diseases whereby the disease control rate has been equivalent to that provided by chemicals (Kupper *et al*., 2005). On the other hand, investigations into the potential use of citrus seeds as sweetening agents and as an alternative to sugar were being investigated (Opeke, 1982).

The world's major citrus- producing countries include Brazil, United States of America (USA), China, Argentina, Australia, Cuba, Egypt, India, Israel, Italy, Japan, Mexico, Morocco and Spain (Whiteside, 1988; United States Department of Agriculture/USDA, 2008). Brazil is the leading country in citrus production and mostly produces oranges for processing. The USA is the second largest producer of oranges and the largest producer of grape fruits. China is ranked as the third largest producer of oranges and fourth in overall production followed by countries such as Mexico, Italy, Japan, Egypt, Argentina, Turkey, Israel, South Africa and Morocco (Food and Agriculture Organization/FAO, 2002 ;Anon., 2006). For these countries, citrus is an important earner of foreign exchange and plays a major role in economic growth and development as well as job creation.

According to FAO (2007), world's citrus production for 2004/05 declined as a response to reduced citrus crops in Brazil and USA, the world's largest producers. Despite improved production in the Mediterranean, the production remained low due to severly impact of hurricanes on grapefruit production in Florida and Cuba. For 2004/05, global citrus export contracted slightly due to smaller orange crops in Spain and USA and lower shipments of grapefruit (FAO, 2007). Total world citrus exports for 2004/05 were estimated at 9.2 million tons, a 4% decrease from the 2003/04 level (Anon, 2006). The main exporting countries include the Mediterranean rim, the USA, Spain and the Southern Hemisphere countries such as South Africa, Australia and Argentina (Anon, 2006).

It is suggested that citrus was first introduced to Africa from India during AD 700-140 (Anon, 2006). Today Egypt, Algeria, Morocco and South Africa are the largest citrus producing countries in Africa (Davies & Abrigo, 1994). In southern Africa, citrus production areas range from latitudes of 17° to 34° S and are mostly linked to river valleys where the environmental conditions are more conducive for optimal growth (Barry, 1996). For 2004/05 production year, Angola produced the most citrus tonnes in the Southern African Development Community (SADC) followed by Tanzania and South Africa (FAO, 2007).

Citrus fruits are normally available all year-round, due to the fact that the crop is harvested from October to June in the Northern Hemisphere and from April to November in the Southern Hemisphere. Oranges make up the largest portion of world citrus production, which is about 65%, followed by mandarins at 19%, lemons and limes at 11% and grapefruits at 5% (Ismail & Zhang, 2004). Citrus fruits are mostly marketed or consumed as fresh, processed or as concentrated juice (Whiteside, 1988). It is estimated that world citrus consumption per capita stands at 12.2 kg annually for oranges, 1.8 kg of lemons and limes and 0.7 kg of grapefruits (Ismail & Zhang, 2004).

However, like any other crop, commercial citrus production is hampered by diseases, which affect the roots, leaves and fruits and alter production quality and quantity. One of

the most important diseases is Citrus black spot (CBS), which is also a phytosanitary barrier to trade. The disease is caused by the fungus, *Guignardia citricarpa* Kiely, anamorph *Phyllosticta citricarpa* (McAlp) (Schutte, 1995). There is, however, two strains of *Guignardia* associated with citrus. *Guignardia citricarpa* is a pathogenic strain and causes citrus black spot, while the other strain *G. mangiferae* is non-pathogenic and does not cause citrus black spot symptoms (Meyer *et al*., 2001). The pathogenic strain, *G. citricarpa* attacks fruits and leaves of citrus and causes lesions that affect marketability of the fruit and may consequently cause the heavily infected fruits to fall from the tree (Kiely, 1948).

Black spot is considered to be a cosmetic disease that causes lesions on the fruit rind, thus spoiling its marketability. The fungus survives on dead leaves on the orchard floor, (which is the main source of inoculum in citrus black spot epidemics) or on fruit lesions (McOnie, 1964). However, fruits are not considered to be significant in disseminating the pathogen as the pycnidiospores on fruit lesions are water borne as compared to the air borne ascospores on leaves (Smith, 1996). In addition to leaf litter and fruits, soil may also be a source of inoculum for CBS, which can be disseminated by the movement of people, animals and equipment between orchards. However, the soil as inoculum source has not been inclusively shown or proven (Truter, M., unpublished data).

With globalisation and trade liberalisation among countries, it is important that international regulations and standards are put in place to control the movement of products in order to protect human, animal, plant and environmental health. However, some regulations and standards may be used as unfair barriers to trade. It is therefore crucial that any barrier imposed on a particular product is scientifically proven, to determine the potential risk it may have on other countries' agricultural activities. In the case of CBS, it is considered important to identify the correct strain of *Guignardia* that represent the phytosanitary risk, to avoid institution of unfair trade barriers on the movement of citrus between countries.

Global efforts to retain the spread of the disease have resulted in strict quarantine regulations and to an extent the containment of the disease. In countries where the disease is endemic, effective management practices such as chemical use, orchard and packhouse sanitation, as well as biological control have resulted in a reduction of inoculum and sustained growth of the industry (Kotze, 1981). On the other hand, areas where the disease does not occur such as the EU and citrus growing parts of USA, have implemented strict measures under which citrus from CBS infested areas are exported to the regions (Smith *et al.*, 2002). These measures include the export of only citrus fruits and seeds and no other vegetative material; that fruits should originate from CBS-free areas or orchards, and lastly the fruits should originate from orchards where approved treatments to contain the disease were used and where the fruits are symptomless during quality and phytosanitary inspection (Smith *et al.*, 2002). Due to the above-mentioned reasons, it is therefore of utmost importance that CBS is properly managed to maintain global trade.

The main objectives of this study therefore include:

1. the assessment of the occurrence, distribution and management practices of CBS in Southern Africa, specifically in South Africa, Namibia, Swaziland and Mozambique;
2. the assessment of the presence and persistence of *G. citricarpa* in the soil;
3. the assessment of "Cropguard" or furfural as an alternative control approach to break the life cycle of *G. citricarpa,* thus reducing the inoculum and controlling the pathogen on leaf litter and in the soil and
4. the assessment of cropguard's non-target effect on other soil micro-flora.

In order to achieve the objectives of the study, different aspects of the disease were investigated. Chapter two provides an overview of the pathogen *G. citricarpa* and the disease (CBS) in terms of the origin, current distribution, epidemiology and different management practices currently used in the industry.

Chapter three covers a survey which was done to determine the status of CBS, in terms of its prevalence, control strategies and the impact the disease has on the industry in Southern Africa including countries such as South Africa, Namibia, Swaziland and Mozambique.

In Chapter four, the efficiency of furfural or Cropguard, a by-product of sugar processing from sugar cane, as an alternative control measure of the pathogen, on appressoria development, on fresh fruits and leaves, on leaf litter and in the soil was assessed. The non-target effect of furfural on other soil micro-flora is also determined in this section as well as the survival of *G. citricarpa* in soil. There is no published data on the survival or dissemination of *G. citricarpa* in soil. This is therefore the first study of its kind to investigate this aspect of the disease.

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CHAPTER 2

LITERATURE REVIEW

2.1 CITRUS BLACK SPOT

Citrus black spot (CBS) is one of the most important diseases in major citrus production areas of the world, such as Asia, Australia, South America and Southern Africa (Anon., 2002). The disease is primarily important as a pre-harvest disease and affects the rind of the fruit and causes cosmetic lesions on the skin (Paul *et al*., 2005). The lesioned or spotted fruits are normally downgraded, thus rendering them unsaleable (Seberry *et al*., 1967). Though the disease may cause different types of fruit spots preharvestly in severely affected orchards, it may occasionally produce red spots postharvestly (Meyer *et al*., 2001). Black spot also affects leaves and causes spots especially on lemons. Almost all commercially important citrus cultivars are susceptible to CBS (Kotze, 1981). Black spot is critically important in summer rainfall areas and citrus production and export would not be economically viable if the disease is not controlled effectively. The disease spreads slowly, similar to other wind-borne fungal diseases and can build up to serious epidemic levels if not controlled effectively (Smith, 1996).

2.2 PATHOGEN

Citrus black spot is caused by a fungal pathogen, *Guignardia citricarpa*, Kiely. The pathogen was first discovered and described by Benson in New South Wales in 1895, who produced drawings of spotted oranges and lemons of the typical symptoms of CBS (Schutte, 1995). The sexual stage of *G. citricarpa* was discovered by Kiely in New South Wales, Australia in 1948 (Kiely, 1948). The imperfect or asexual stage was named *Phoma citricarpa*, McAlp, which was later changed in 1966 to *Phyllostictina citricarpa*, McAlph. However, the name was changed to *Phyllosticta citricarpa* in 1973 with the

renaming of the conidial stage (Schutte, 1995). The life cycle of the pathogen is laid out in section 2.7 of this chapter.

2.3 ECONOMIC IMPORTANCE OF CITRUS BLACK SPOT

Economically, citrus black spot is of great importance. The disease does not cause decay, but rather cosmetic lesions that render the fruits unmarketable. In countries such as China, Australia and South Africa, *G. citricarpa*, is considered as of phytosanitary importance due to its role in international trade (CABI/EPPO, 1998). Few spotted fruits can lead to the rejection of a whole consignment (Mutengwe, personal comunication, July 2006). Whenever this happens, the consignment is normally re-packed and diverged to a less sensitive market which is usually associated with huge economic losses.

2.4 ORIGIN AND GLOBAL DISTRIBUTION OF CITRUS BLACK SPOT

Citus black spot was discovered in orange orchards in Sydney (Australia) in 1897. The disease has also been reported in South America (Brazil and Argentina), Asia (Bhutan, China, Indonesia, Taiwan and the Philippines) and Africa (Mozambique, Nigeria, Swaziland, Zambia, Zimbabwe and South Africa) (Everett & Rees-George, 2006; Paul *et al.*, 2005). Prior to this study, there were no documented reports or publication regarding the presence of CBS in Namibia (Paul *et al.*, 2004). However, it has been reported that some Namibian farmers obtain citrus nursery trees from a nursery in the Brits area of South Africa's North Western Province, where the disease occurs (Le Roux, personal communication, June 2005). Before this study, the question on whether the pathogen can survive and establish in Namibia remained.

The occurrence of CBS in Japan and New Zealand is controversial in terms of whether the pathogen is present in these countries or not. Though the pathogen is believed to occur in Japan, it does not cause fruit spots but rather fruit decay (Paul *et al*, 2004). Furthermore, what was thought to be *G. citricarpa* from Japan was later identified as *G. mangiferae*, which is non-pathogenic. Citrus black spot was previously recorded to be

present in New Zealand, which was based on morphological identification of an isolate. However, the isolate was later identified on a molecular basis to be the endophytic strain, *G. mangiferae*. Furthermore, CBS symptoms have never been observed in New Zealand which might imply the absence of the disease from that country (Everett & Rees-George, 2006). Also the disease does not occur in citrus-producing areas in Mediterranean and European countries, such as Greece, Israel, Italy, Spain, Portugal, France and Turkey (Anon, 2002; Meyer *et al*., 2001). According to Cook (1975), CBS has also not been found in citrus-producing areas of the United States of America and Chile.

The presence of citrus black spot in South Africa was first discovered by Doidge in 1929, along the coastal regions of Natal near Pietertmaritzburg. According to Wager (1952), black spot spread slowly in Pietermartizburg, whereby it fluctuated from season to season and caused considerable damage in 1940. Later it was found in certain areas of the then Eastern and Northern Transvaal. The disease was also found in, Mpumalanga, Limpopo Province, North-Western Province, Gauteng and Eastern Cape (Paul, 2005). In Mozambique CBS has been reported in areas between Maputo and Swaziland, at Morrumbene, Machipanga and at Nicaudala (Le Roux *et al*. 2003). The disease has also been reported in Swaziland, although there are no data on the losses caused to the industry (Schutte, 1995; Paul *et al*., 2005).

It has been reported that some areas in countries where the disease occurs are free of CBS. This is observed in China where the disease is only found in the provinces of Sichuan, Yunnan, Guangdong, Fujian and Zhejiang. The same trend is observed in the Northern Cape and all the areas in Southwestern Western Cape of South Africa (Paul *et al.*, 2005).

2.5 PHYTOSANITARY ISSUES / REGULATIONS

Globalisation and trade liberalisation have opened doors for the free flow of products and most countries are signing trade agreements for the exchange of goods and services. This not only presents chances for development and economic growth, but also risks of

introducing foreign diseases and pests. Globally, there is great concern over the introduction of pests and pathogens into countries where they do not occur. Though, the pre-harvest fruit symptoms are normally identified prior to packing the export consignment, *G. citricarpa* can still be latently present on some of the fruits and the symptoms may develop while the fruits are in transit (Obagwu, 2003). However, there is low risk of infection from fruit lesions, since pycnidiospores on the fruits are only water borne and not wind-borne (CABI/EPPO, 1998). Despite this, measures are still instituted to prevent or minimise the entry of infected fruits into disease free areas. Whenever instituting phytosanitary measures to prevent the spread of CBS, respective countries should provide scientific evidence as this can sometimes be used as unnecessary barriers to trade (World Trade Organization/WTO, 2007).

2.6 SYMPTOMS

Citrus black spot symptoms are more common on fruits and seldom develop on leaves. Fruit symptoms were first described by Benson in 1895 whereby he referred to the way the spots increased in size to grow together and render the fruits unsaleable or cause heavily infected fruits to fall from the tree (Kiely, 1948). Leaf symptoms often develop on lemons (cv. Eureka) and rarely on Valencia oranges (Kiely, 1948). When present, leaf symptoms represent small necrotic spots with a grey centre surrounded by a dark brown ring and a yellow halo (Fig. 2.1). These lesions will occasionally develop pycnidia of which, the majority are without fruiting structures of *G. citricarpa* (Kiely 1948; Obagwu, 2003).

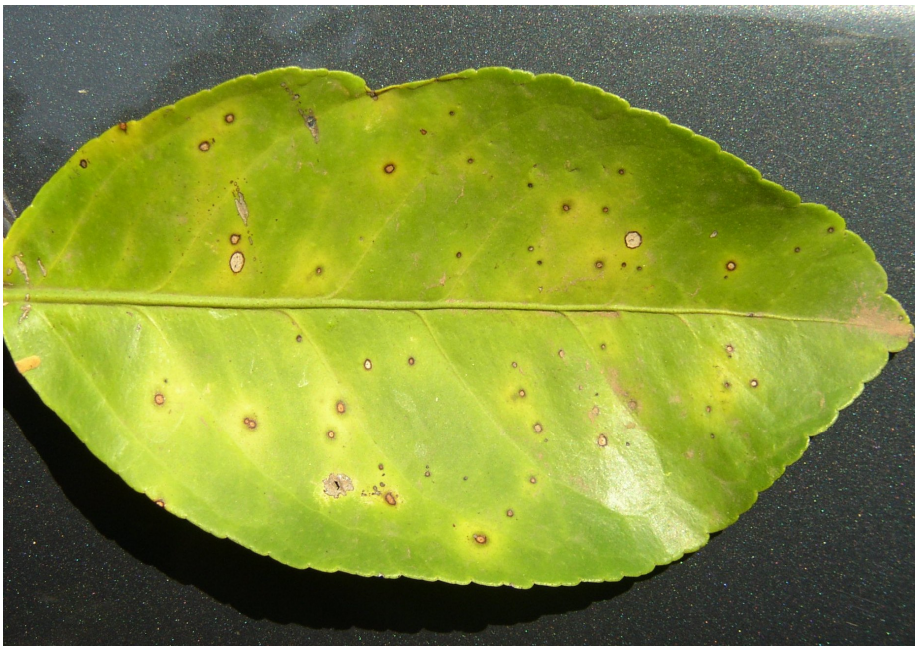

Fig. 2.1: Citrus black spot (*Guignardia citricarpa*) symptoms on lemon cv. Eureka leaf.

(Picture: Courtesy of Dr T. Regnier)

On the other hand, fruit lesion development is characterised by fruit maturity and ambient temperature (Whiteside, 1965). Symptomless mature fruits developed symptoms after being exposed to average temperatures of more than 21°C. In the orchard however, fruit symptoms have been observed on the sunny sides of the trees. Kiely (1948), classified fruit symptoms into three categories: hard spots, freckle spots and virulent spots.

The hard spots represent the typical symptoms which appear at the beginning of fruit maturity and thus are mostly preharvest. These symptoms normally develop on the side of the fruit exposed to sunlight. Hard spots can sometimes appear before the fruit colour changes from green to orange (Figure 2.2). However, on coloured orange fruits, the lesions appear as small (several millimetres in diameter), circular and brown areas with slight depressions. The lesions later sink in the centre and this turns grey-white from where the pycnidia may develop. The rim of the lesion is usually black surrounded by a ring of green rind tissue (Kiely, 1948; Kotze, 1988).

Figure 2.2: Citrus black spot (*Guignardia citricarpa*) symptoms on lemon cv. Eureka fruit.
(Picture: Courtesy of Dr. T. Reigner)

When the environmental conditions remain conducive for disease development, formation of hard spots stops and are replaced by freckle spot formation. Freckle spots are similar to hard spots except that they develop after the fruit colour change or after fruit picking, thus postharvest.

Virulent spots on the other hand develop late in the season (about two to three weeks after the onset of freckle spots) (Kiely, 1948), on fully matured fruits under warmer temperatures. These necrotic spots are usually sunken and brown to brick red at the edges. False melanose develops a few months after the fruit has developed resistance to the pathogen (Kotze, 1988). Moreover, thepathogen can also cause latent infection on the twigs (Kotze, 1981).

2.7 EPIDEMIOLOGY AND DISEASE CYCLE

The citrus black spot pathogen's reproductive cycle is characterised by two stages. *Phyllostica citricarpa* is the imperfect stage that produces pycnidiospores, while *Guignardia citricarpa* is the sexual stage producing ascospores (Kiely, 1948).

The pycnidiospores are produced in small globular, black structures called the pycnidia, which develop on fruit lesions, rarely on leaf lesions and in great numbers on dead leaves on the orchard floor (McOnie, 1965). Each pycnidia stalk bears pycnidiospores. The pycinidiospores are also found on dead twigs from where they are disseminated onto young leaves and fruits (CABI/EPPO, 1998). The pycinidiospores are disseminated in water, by splashing raindrops or irrigation water (Whiteside, 1965). However, these are not considered to be the main source of inoculum, though sometimes they can be if infected out-of season fruits are left to hang on the tree after blooming and fruit set. This happens in such a way that, the pycnidiospores on the fruits are washed onto young fruits down the canopy (Kotze, 1988). On the contrary, in Brazil, pycnidiospores role in CBS epidemiology is as important as that of the ascospores. This is due to the fact that rain in Brazil is not confined to one season and flowering sometimes occurs more than twice a year (Paul, 2005).

On the other hand, ascospores that also develop on dead leaves on the orchard floor are considered to be the main source of inoculum for CBS (Kellerman & Kotze, 1977). Ascospores are produced in perithecia, which are externally similar to pycnidia except that they produce ascospores in groups of eight in each tube. The ascospores develop in about 40-80 days after the leaves have dropped. However, perithecia development is depended on the frequency of wetting and drying of the leaves (Kotze, 1988). Once the perithecia are mature, they swell up and eject the ascospores that are disseminated in air currents or water for shorter distances (Whiteside, 1965). Ascospores can be ejected vertically up to 1 cm before they are disseminated by wind (CABI/EPPO, 1998).

Nursery trees with latent infections also pose a risk of introducing CBS to disease free areas. The pathogen infects the tree, that is either the leaves or twigs, and remains latent. Citrus black spot epidemiology is mostly influenced by factors such as inoculum availability, suitable climatic climatic conditions for infection, the phenological stage in the growth cycle of the plant, the fruit age as related to its susceptibility to infection and symptom development and subsequent ascospore development (Magarey & Borchert, 2003). Climatic conditions play a major role in the amount of infection, the development of the fungus on fallen leaves, spore dissemination and germination and fungal penetration of fruits and leaves (Whiteside, 1965). However, other important factors in black spot development are summer rains which coincide with conducive temperatures (21-28ºC) for CBS infection (Lee & Huang, 1973).

The disease cycle is initiated with the development of pycnidiospores and ascospores on dead leaves on the orchard floor. Under favourable conditions, the ascospores develop and mature and are disseminated by air currents onto fruit or leaf surfaces (Kotze, 1988). As little as 3mm of rainfall can trigger ascospore discharge from the ascocarps. Once arrived on the plant surface, especially the leaves, the spores germinate and form germ tubes which penetrate the cuticle of the susceptible leaf or fruit (Whiteside, 1965). The fungus forms a mycelial mass between the cuticle and the epidermal wall, where it stays latent for months (Kotze,1988) until the leaves have fallen from the tree and have been subjected to wet and warm conditions to enhance the development of the fungus and spore release (Whiteside, 1965). On the other hand, continuous heavy rain may reduce the number of ascospores in the air through a washing out effect (CABI/EPPO, 1998). The impact of rainfall patterns on perithecia development was investigated by Lee and Huang (1973), who demonstrated that moderate precipitation favours the formation and development of perithecia (Table 2.1).

No published information is available regarding the survival of CBS in the soil. However, Peck *et al*., (2001), conducted a study whereby they demonstrated the survival of *Mycosphaerella pinodes* and *Phoma medicaginis* var *pinodella* (pea's black spot pathogens) in South Australia soils for several years.

Table 2.1: Effect of sprinkling water to detached citrus leaves on the formation and development of perithecia of *Guignardia citricarpa* (Source: Lee and Huang, 1973)

Treatment	Days of sprinkling water	Number of leaves treated	% leaf area with perithecia
A	1st- 7th day	29	35.3
B	1st- 14th day	29	18.9
C	1st- 21th day	33	18.9
D	1st- 28th day	30	9.2
E	22th- 28th day	29	45.7
F	22th- 35th day	30	31.7
G	22th- 42 th day	56	26.3

2.8 CONTROL MEASURES

Efforts have been made and are continuously being improved to manage CBS. Different control measures such as; quarantine, sanitation, cultural practices, chemical and biological controls are being used either separately or in an integrated approach. However, knowledge of the pathogen's biology (life cycle), the environmental influence on disease development and the mode of action of different fungicides play a crucial role in the effective control of CBS (Agrios, 1997). Citrus black spot may cause enormous losses where it is not controlled or when insufficient control measures such as spraying programmes are not followed (Seberry *et al*., 1967).

2.8.1 *Quarantine*

Citrus black spot is introduced to clean production areas by means of infected plant material, especially propagating materials. In an effort to prevent such incidences, plant quarantine regulations and trade requirements in plant materials are instituted between countries or regions. In establishing new orchards, certified trees from black spot-free nurseries or areas should be used. Furthermore, nursery trees' green leaves should be tested for latent infection as this also poses a risk of introducing the pathogen to CBS-free areas (Whiteside, 1965). This procedure aids in prohibiting the introduction of plant

material with a latent infection to disease free areas. In cases where the disease is accidentally introduced to an unaffected area, efforts should be made to prevent or retard its spread and establishment (Kotze, 1981).

However, CBS is spread by means of various ways including windborne ascospores and arguments have revolved around the nature of their dissemination. On the other hand, the movement of fruits does not introduce black spot to disease free areas because ascospores are not produced on the fruits but rather pycnidiospores that are not a major source of inoculum (Kotze, 1981). The pycnidiospores are proven to cause infections only at very high concentrations of (> 3.5 x 10^5 spores / ml) (Mayers, 1986).

2.8.2 *Sanitation*

Sanitation involves the removal of all sources of inoculum from the orchard. This is especially true for mature out-of-season fruits that might be infected with CBS and that can release pycnidiospores during wet periods, representing a potential inoculum source as the pycnidiospores are washed down the canopy onto young fruits (Kotze, 1981). In addition, infected dead branches or old trees also present a potential source of inoculum from which pycnidiospores are also washed down the canopy. In an effective orchard sanitation programme, infected plant material should be cut off, removed and burned. The leaf litter, which is the source of ascospore inoculum, can also be removed from the orchard floor (Paul, 2005).

Establishing nurseries in CBS free areas can also reduce disease incidences and dissemination. This is very important because nursery trees with latent infections have been reported to spread CBS in countries such as South Africa and Zimbabwe (Whiteside, 1965). This happens when infected twigs are grafted on healthy trees or infected nursery trees are planted in disease free areas (CABI/EPPO, 1998). Another way of preventing or reducing the introduction of *G. citricarpa* to disease free areas is by removing leaves from the nursery trees prior to selling (CABI/EPPO, 1998).

2.8.3 *Chemical control*

Chemicals play an important role in the control of CBS. The most crucial time for black spot infection is the early or first part of the fruit's development on the tree, which starts from November to January, depending on the region and cultivar type (Kotze, 1981). This is the critical time that determines the application rate of protective fungicides. However, due to global climate changes, the critical infection period can be altered from year to year. It is therefore important that growers consider climatic factors such as: the first major rains as well as the prevailing temperatures because these determine the time for ascospores discharge and thus the critical period for infection. Continuous monitoring of the climate and thus ascospores release is crucial because the fruit remains susceptible for the first few months of development, more especially from anthesis up to 16 weeks later (Kellerman & Kotze, 1977). Whenever a severe infection is predicted, about five sprays are applied during the 4-5 fruit susceptibility months (Kotze, 1988).

Protective chemicals such as copper fungicides (copper hydroxide and copper oxychloride) and mancozeb (dithiocarbamate), azoxystrobin, carbendazim, fosetyl-A, zineb, pyraclostrobin and trifloxystrobin are used in such a way that the application coincides with the critical period of infection (Nel *et al*., 2003). The chemicals are used in mixtures of protectant and systemic fungicides to ensure most effective control of the disease. Schutte *et al.* (2003), conducted a study on Valencia Oranges in South Africa, whereby the efficacy of chemical mixtures such as mancozeb and azoxystrobin and mineral oil was compared to benomyl, mancozeb and mineral oils. They found that mancozeb, azoxystrobin and mineral oil mixtures controlled the disease better than the mixture of benomyl, mancozeb and mineral oil.

In order to control the infection that may have escaped the protective fungicides, benzimidazole fungicides, such as benomyl, are applied as postinfection treatments (Kotze, 1988). Due to the build up of pathogen resistance to benomyl, it has since been recommended not to use this fungicide which subsequently resulted in the fungicide being deregistered for use on citrus. The chemical is however still being used in some countries in Africa, (in South Africa it is used in areas where pathogen resistance has not

been recorded), Asia and the Pacific regions, Europe and Central Asia regions and in North America (Anon, 2007; Kellerman, personal communication, August 2006).

Copper fungicides have been used successfully in controlling black spot. Despite this, it was later discovered that these fungicides affect the physiology and ultimate appearance of the fruit. Brown to dark brown lesions may develop on the fruit rind, which affects market appeal (Kotze, 1981). To overcome this, copper fungicides were later replaced by dithiocarbamates, which prevented physiological damage.

Even though chemicals represent the front line of defense against plant pathogens (Agrios, 1997), a major problem still lies with resistance development. This is being addressed with the minimal use of chemicals, mixing and integrating its use with other control measures. An important aspect of reducing the use of chemicals lies in disease forecasting (Kotze, 1988). Knowledge of the biology of the pathogen, weather conditions and the host plant biology plays an important role in predicting the critical infection period, thus when to apply the fungicides. In addition to the rational use of resources, disease forecasting also aids in minimising environmental degradation through the reduced use of chemicals.

2.8.4 *Spraying programmes*

Generally, CBS control sprays commences in October with the first rain through to January/February. This is when the ascospores are released and there is usually 50% petal fall (Table 2.2).

2.8.5 *Use of oils and other additives*

Oils such as mineral oil are being used as chemical additives and are generally not considered as treatments on their own. On the other hand, mineral oil is known to be an effective additive. A study by Schutte *et al*. (2003) compared the effectiveness of mineral oil to different adjuvants in the mixtures of azoxystrobin and mancozeb which proved that fruits sprayed with the mixture of mineral oil did not develop any symptoms.

2.8.6 *Use of biofertilisers*

Concerns arising from the extensive use of chemicals have sparked interests in the focus on alternative approaches to control CBS. The use of biofertilisers is aimed at reducing environmental degradation with chemicals, thus promoting agricultural sustainability. Kupper *et al*. (2005) have reported the use of water extracts from organic matter and biofertilisers as being one such approach. These extracts have a large composition of microbial dynamics of bacteria, filamentous and yeast-like fungi and actinomycetes. The mode of action of biofertilisers is through microbial metabolites and cells or by direct or indirect action of nutrients.

Furthermore, the microbial community of biofertilisers can be complemented with the addition of recommended biocontrol products to make it more effective. In countries, such as South Africa, composted orange peels have been evaluated for CBS control and yielded control rates equivalent to mancozeb (Kupper *et al*., 2005).

Table 2.2 Fungicides registered for control of citrus black spot, dosage and application schedules in South Africa (Source: Nel *et al*., 2003). Latest version from the National Department of Agriculture

Chemical name	Dosage (per 100L water or as indicated)	Application schedule
Azoxystrobin		First spray: early to mid November Second spray: mid January Used in higher rate if the previous season's infestation levels were high
*Benomyl +	25g + 200g+0.5L mineral oil	Applied once between 1 and 20 December, for early cultivars
Mancozeb	50g + 200g + 0.5L mineral oil	
Carbendazim +	27.5ml + 200g +0.5L mineral oil	Early cultivars: One spray between 1 and 20 December
Mancozeb	55ml + 200g + 0.5 L mineral oil	Late cultivars: Trees of 20 years and younger: One spray between 1 and 20 December Old, susceptible trees: One spray between 1 and 20 December. Fruits for processing: early cultivars and Valencias of 20 years and younger: One spray in early December Valencias older than 20 years: One spray in early December
Copper hydroxide	350ml	Normal season: 3 applications at 30 -35 day intervals
Copper oxychloride	150- 200g	Wet season: 4 applications at 30 -35 day intervals
Mancozeb	150-200g	Normal season: 3 sprays at 25 day intervals starting in third week of October. Wet season: 2 sprays at 25 day intervals starting at full petal fall
Maneb / Zinc oxide	200ml	Applied from 100% petal fall of 4- 5 sprays at 25-28 day intervals
Pyraclostrobin	200ml	4 -5 sprays at 25-28 day intervals starting at 100% petal fall
Zineb	200g	Normal season: 5 applications at 19-21 day intervals, starting in third week in October. Wet season: 6-7 applications at 19-21 day intervals , starting at 100% petal fall

*** Only registered for use in areas where resistance has not been recorded**

2.9 CONCLUSION

Citrus black spot is of major economic and social importance in citrus producing countries where the disease occurs, which is mostly in summer rainfall areas. Though the disease seldom causes post-harvest fruit decay, it causes superficial blemishes that render the fruits unmarketable.

Even though chemicals are used to manage the disease, other control measures such as exclusion of the pathogen are very important. For instance, strict quarantine measures through inspections that are implemented to prevent introduction of the pathogen to disease free areas are vital, as this can be detrimental to the local citrus industry. This is especially important when considering the latent infection of the pathogen in nursery trees. Such infections go unnoticed until the plants have matured and thus develop symptoms with ascospores on dead leaves and pycnidiospores on fruits and fresh leaves.

Citrus black spot establishment, prevalence and severity are highly depended on various factors such as, climatic conditions, source of inoculum and citrus cultivars. It is therefore important that countries have to consider their prevalent climatic conditions and that of the importing country, specific areas of origin of the plant materials and the disease prevalence in the importing country when instituting pathogen exclusion measures. Furthermore, responsible international bodies, such as the World Trade Organisation (WTO) should oversee accordingly to prevent the institution of unnecessarily trade barriers among member countries.

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CHAPTER 3

IMPACT ASSESSMENT OF CITRUS BLACK SPOT IN SOUTHERN AFRICA

ABSTRACT

Citrus black spot (CBS) caused by *Guignardia citricarpa* is responsible for considerable losses in countries such as South Africa, Mozambique, Swaziland and Zimbabwe. The occurrence and management practices of CBS and its impact on growers in Southern Africa were studied. Questionnaires were formulated to suite specific countries such as South Africa, Namibia, Mozambique and Swaziland. In this study it was confirmed that CBS occur in all these countries including Namibia where the disease has never been reported before. In all countries surveyed the disease is managed by means of chemicals such as mancozeb, cabrio, benomyl, flint dithane, strobirulins, azoxystrobin, carbendazim, abamectin, citrex oil sporekill, derosal, copper hydroxide, copper oxychloride, pyraclostrobin and trifloxytrobin as well as general orchard sanitation. However, in South Africa, most growers in the Marble Hall area for instance spray chemicals according to a regionally managed CBS forecasting model. This trend has proven effective in managing the disease for over more than ten years and reducing the incidence to almost 0%, as all the interviewed growers did not experience any CBS on their orchard during the time of the survey. To the contrary, the disease is prevalent in areas where a similar disease control strategy is not used, such as in Nelspruit. The low incidences of CBS occurence in the Tsumeb district, Namibia, may be partially attributed to the unpredictable rainfall patterns of few heavy showers separated by long period of no rain which prevent major disease outbreaks. In Mozambique, CBS is only reported to be controlled at commercial level while subsistence farmers do not manage the disease, which may have led to the prevalence of CBS in the country. Citrus black spot is also managed in Swaziland by mainly strobilurins due to resistance development when using Benomyl. It is considered important that to compliment the current management practices, training of all interested parties, such as subsistence farmers on basic citrus production and specifically on CBS management practices will play a major role in sustaining the industry as well as the livelihood of the people in the region.

3.1 INTRODUCTION

The citrus industry in Southern Africa has been under threat for the past century, from several devastating diseases (Le Roux *et al*., 2003). Among them is CBS, which causes considerable losses in countries such as South Africa, Mozambique, Swaziland and Zimbabwe. Black spot is considered to be a phytosanitary disease, especially for market access to the European Union (EU) and the United States of America (USA). Exporters to these countries incur losses throughout the supply chain due to phytosanitary restrictions (Paul *et al.*, 2005). It was reported that, the management of the disease especially in South Africa costs between R2 000 and R2 500 per hectare (Groenewald, 2002). However, losses in packhouses of up to 10% are normally incurred despite effective spraying programs (Groenewald, 2002).

What is notable is that the exact losses and CBS cost estimates are not known. In order to more effectively manage the disease and make strategic decisions on an industry basis, it is essential to have certain critical information regarding control strategies and the economic impact of the disease on the grower and industry at large. Impact assessment studies have been widely used in such studies, to determine the effects of proposed or ongoing projects on the intended communities.

An impact assessment (IA) study is defined as a form of evaluation which covers the effects of a project output on targeted beneficiaries (Phophi, 1999). Boroush *et al.* (1980) defined IA as a perspective approach that analyses short and long-term effects that arise from the interaction of technologies and societal systems.

Impact assessment is used to initiate public awareness about the effects of social, economical and technological changes caused by the societal side effects, which in turn prompt the improvement of mechanisms to manage new technologies or approaches (Boroush *et al.*, 1980). This helps policy makers to take into account the known options and arrive at social choices on future technological or project developments. Impact assessment can be conducted through modified peer review, use of surveys, cost-benefit methods, cost-effective analysis, case studies and partial indicators of impact or mathematical programming (Phophi, 1999). There are many types of impact assessments such as, risk assessment, social impact assessment, environmental impact assessment and economic impact assessment. This chapter covers the social impact of CBS on citrus growers as well as local communities in South Africa, Namibia, Mozambique and Swaziland.

During this study, a survey was initiated to determine the distribution of CBS in Southern Africa. Questionnaires were formulated to also collect information on the current management programs, disease severity and CBS social impact on the farmers.

3.2 SAMPLED COUNTRIES

3.2.1 *South Africa*

South Africa situated on the southern part of the continent is one of the 50 wealthiest countries in the world and is home to about 45 million people (Anon, 2006). The climate is generally dry and more than 67% of the country is semi-arid to arid, receiving less than 810 mm of rain annually (Encarta encyclopaedia, 2005). Due to the aridity, only 13.5% of land is used for crop production, of which only 3% is of high potential (Anon, 2006). South Africa is classified as a middle-income country with an abundance of resources. The economy mainly depends on mining, fishing, forestry, industries and agriculture (Anon, 2006). The agricultural sector is one of the largest, contributing about 2.6% to GDP. It is estimated that agricultural exports constituted 8% of total exports for the past five years (Anon, 2006). Exported agricultural products include sugar, grapes, citrus, nectarines, wine and deciduous fruits. Maize and livestock (meat and dairy products) are some of the major agricultural products for the country (Anon, 2006).

South Africa's citrus industry

It has been reported that the first citrus trees (lemons and oranges) were planted in South Africa in 1652, shortly after Jan van Riebeeck's arrival. However, the modern SA citrus industry only came into being in 1926 with the establishment of the South African Co-operative Citrus Exchange (Stanbury, 1996). Citrus is currently grown in various regions of South Africa, such as the Northern Province, Mpumalanga, Kwa-Zulu Natal, North-Western Province, Eastern Cape, Western Cape as well as new plantations in the Northern Cape (Urquhart, 1999). Generally, farms range from 0.5 to 500 ha in size, with some larger farms of 6000 ha.

According to the Food and Agricultural Organization (FAO) of the United Nations (2001), South Africa is currently ranked number twelve on the list of the world's largest citrus producers (Helm, 2006). For the 2004/05 production year, the country produced about 1.9 million tones with the gross export value of R2, 6 billion (Anon, 2007). More than 60% of the production is exported, placing the country as the world's third largest exporter of fresh citrus fruit, following Spain and the United States (Helm, 2006; Paul *et al.*, 2005; Stanburry, 1996). In addition, South Africa is the leading exporter of fresh citrus in the Southern Hemisphere (SA Fruit Journal, Feb/March 2006). The

country exports citrus to more than sixty countries, where the biggest export markets include, Europe, the Middle East, Japan, the Far East, USA and other African countries (Perishable Products Export Control Board / PPECB, 2007; Mabiletsa, 2002).

The export window in South Africa starts from April to October. It is estimated that about 1, 1 million tonnes of South Africa's citrus fruits are exported annually (Helm, 2006). According to the International Trade Commission (ITC) (2006), South Africa exported 1,127,630 tonnes of citrus to various countries in 2004. Generally, the exports earn about 92% of the farm income (Stanburry, 1996), which is about R3.2 billion a year. According to 2005 statistics, citrus exports made up 2.46% of gross value of agricultural products (Urquhart, 1999). In addition to being a foreign exchange generator, the industry also has social benefits, as it employs around 100,000 people on the farms and packhouses and in other related industries (SA Fruit Journal Feb/ March, 2006).

Citrus black spot in South Africa

Citrus black spot was first discovered in South Africa by Doidge in 1929, along the coastal regions of Natal near Pietertmaritzburg (Kotze, 1981). According to Wager (1952), the disease spread slowly in Pietermaritzburg, whereby it fluctuated from season to season and caused considerable damages in 1940. The disease was also found in, Mpumalanga, Limpopo Province, North-Western Province, Gauteng and Eastern Cape (Paul, 2005).

The Western Cape is currently the only area in South Africa which is declared to be black spot free, which is attributed to its winter rainfall climate, under which the pathogen cannot complete its life cycle (Mabiletsa, 2002). The current distribution of CBS in South Africa is presented in Figure 3.1 (Paul *et al*., 2005).

Table 3.1: Citrus exports by South Africa in 2004 (Source: International Trade Commission, 2006)

IMPORTING COUNTRY	QUANTITY (in tonnes)
Netherlands	184, 954
United Kingdom	124, 892
Japan	87, 346
Saudi Arabia	67, 267
Russian Federation	82, 366
Spain	66, 881
United States of America	36, 947
Belgium	50, 106
United Arab Emirates	47, 602
Italy	75, 193
Mozambique	73, 483
Hong Kong	43, 725
Canada	32, 630
France	19, 577
Greece	16, 597
Germany	17, 259
Ukraine	20, 935
Malaysia	9, 780
Oman	7, 066
Republic of Korea	5, 579
Singapore	7, 213
China, Taiwan Province of	5, 077
Bahrain	3, 895
Kuwait	4, 189
Mauritius	4, 785
TOTAL	**1,127,630**

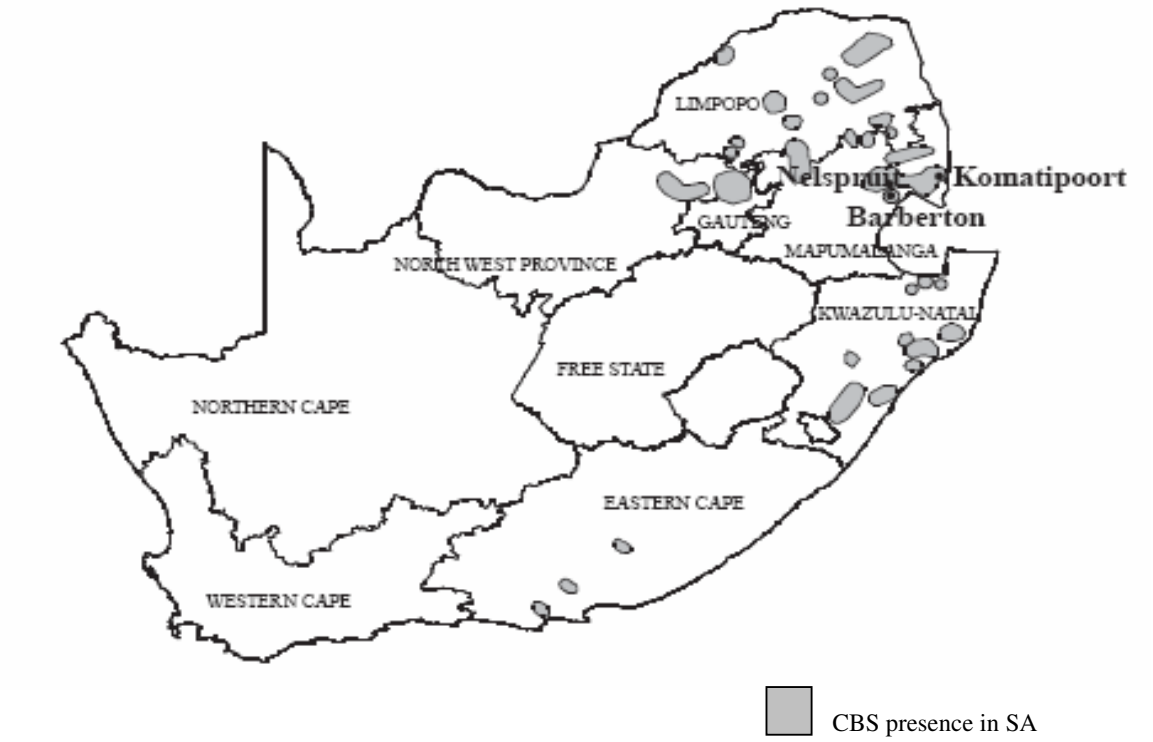

Figure 3.1: Citrus black spot distribution in South Africa (2005).

(Source: Paul et al., 2005)

The presence of CBS in South Africa has affected the industry, especially the export market. South Africa has been trading with Europe, being its major export market, since 1930 and black spot was not introduced into the EU (Mabiletsa, 2002). Further, Mabiletsa reported that with the introduction of sanitary and phytosanitary measures, *G. citricarpa* was declared a sanitary pathogen and the EU has since introduced a zero tolerance for black spot. The USA, Japan and Korea have followed b introducing a zero tolerance for CBS.

In order to ensure CBS-free fruit exports for the EU and USA markets, the Perishable Products Control Board (PPECB) officials conduct packhouse inspections for CBS (PPECB, 2007). On the other hand, officials from the Directorate of Plant Health and Quality of the National Department of Agriculture conduct inspections on the imported plant products at the ports of entry.

3.2.2 *Namibia*

Namibia situated in the south- western part of Africa is one of the driest countries on the continent. The climate is arid to semi-arid in most parts of the country, except for the far northeast, where the climate is sub-tropical. The country receives an annual rainfall of 200mm on average and the

humidity is less than 10% in winter and ranges from 50% to 80% in summer (Namibia Holiday and Travel, 2004). Crop production is therefore depended on irrigation, since only 2% of the country receives adequate rain (Hoffmann, 2006).

The economy of Namibia mainly depends on mining, fishing, tourism and agriculture. Although the agricultural sector contributes less than 10% to the country's GDP, it is the largest employment provider and 70% of the population depends on agriculture for their livelihood (Anon, 2006). However, the biggest part of the agricultural economy has been distorted to favour livestock production leaving only a small percentage for crop production (United Nations Institute for Namibia). According to the International Trade Commission (ITC) statistics, Namibia exported about 170 tonnes of citrus to Germany in 2004 (ITC, 2006). There is no statistical data available on annual production and most of the produce is marketed locally either as fresh or processed.

Citrus black spot in Namibia

Prior to this study, there were no documented reports or publication regarding the presence of CBS in Namibia. It has, however, been reported that some farmers obtain citrus trees from a nursery in the Brits area of South Africa's North Western Province, where the disease occurs (Le Roux, personal communication, June 2005). Based on that, it is very important to establish the country's status on CBS incidences. At the inception of the country's independence in 1990, officials from the Plant Quarantine Division of the Ministry of Agriculture, Water and Forestry have been conducting inspections on all the imported plant products at the ports of entry, to prevent the entry of infected materials. Infected plant materials are treated to prevent the introduction of diseases, destroyed or returned to the country of origin (Government Notice, 2005).

3.2.3 Mozambique

Mozambique, situated on the southeastern cost of Africa is currently one of the poorest countries in the world (Anon, 2004). The Mozambique government has recently embarked on projects to improve and stabilise the country after the 1977-1992 civil war (Anon, 2004). The political stability since 1994, coupled with international donations, made the country increase its economic growth rate, reducing the inflation to single digits in 1990s and double digits in 2000-2003. However, according to 2001 estimates, about 70% of the population still lives under the poverty line (Anon, 2004). The agricultural sector remains the employer for most of the workforce and contributes 22% to the GDP. Agricultural products include cotton, cashew nuts, sugarcane, cassava (tapioca), corn, coconuts, sisal, citrus and tropical fruits, potatoes, sunflowers, beef and poultry (Anon, 2004).

Mozambique citrus industry

Following the collapse of the government-run citrus orchard in Maputo province in the nineties, the citrus production also collapsed, which left 235 people unemployed. The market has since recovered with the establishment of a 700 ha citrus estate in 2002. The estate rehabilitated more than 30,000 grapefruit and orange trees in three years and currently employs about 300 permanent and contract staff. Today, this is the only major commercial citrus producer in Mozambique that produces high quality products for exports. Generally citrus exports from this estate have never been rejected internationally (TechnoServe, 2006). The company has improved the production, especially for star ruby grapefruit from 15 000 cartons in 2003 to 45,000 cartons in 2005. Citrus and citrus products are parts of the export commodities for the country. The major citrus importing countries are Spain, Germany, United Kingdom, France and Austria, Table 3.2 (International Trade Commission/ITC, 2006).

Table 3.2: Citrus exports by Mozambique in 2004 (Source: International Trade Commission)

IMPORTING COUNTRY	QUANTITY (in tonnes)
Spain	337
Germany	384
United Kingdom	183
France	64
Austria	28
TOTAL	**996**

Citrus black spot in Mozambique

Citrus black spot has also been reported in Mozambique (Whiteside, 1965), although there are no available statistical data on losses due to the disease. The presence of the disease was also confirmed through the survey conducted by Le Roux *et al*. (2003), where CBS was observed on Valencia in several regions in Mozambique, such as between Maputo and Swaziland, at Morrumbene, at Machipanga on the Zimbabwean border and at Nicaudala. There are no regulations to govern the entry of plants and plant materials into the country. However, inside the country, inspections of plant products are sometimes in place and reside mainly under the control of the Agricultural Institute in Maputo.

3.2.4 *Swaziland*

Swaziland, situated on south-eastern Africa is one of the poorest countries in the region. The country heavily depends on South Africa from where it receives nine-tenth of its imports and sends

most of its exports to. Most of Swaziland's foreign exchange is generated from sugar and wood pulp exports, with the sugar industry being the most important generator of foreign currency (Anon, 2006). Although the agricultural sector only contributes about 17% to the Gross Domestic Product (GDP), it is the main source of income for more than 70% of the population, especially in rural areas (Anon, 2006). The main agricultural products include maize, sugar, cotton and citrus.

Swaziland citrus industry

About 1,875.4 hectares of land in Swaziland is planted with different citrus varieties such as oranges, grapefruit, limes and the 'easy peeler' varieties (Anon, 2006). This area has shrunk from 1,919.7 hectares cultivated during the 2003 season. The decrease is attributed to the closure of one of the major citrus estates (Anon, 2006). This has also decreased the production volumes by 4.7%, from 74.418 tonnes to 70.920 tonnes (Anon, 2006). Most of the citrus produced in Swaziland is exported through the Swaziland Citrus Board via the port of Maputo to the EU, Eastern Europe, the Middle and Far East, Japan and some African countries (Anon, 2006; ITC, 2006) (Table 3.3).

Table 3.3: Citrus exports by Swaziland, 2004 (Source: International Trade Commission, 2006)

IMPORTING COUNTRY	QUANTITY (in tons)
Netherlands	13,609
Japan	5,443
United Kingdom	4,714
Spain	947
Russian Federation	775
Italy	427
Germany	324
France	240
Mauritius	232
China, Taiwan Province	292
Poland	178
Ireland	85
Kenya	48
Serbia and Montenegro	17
Czech Republic	18
Romania	28
TOTAL	**27, 404**

Citrus black spot has also been reported in Swaziland (Schutte, 1995; Paul *et al*., 2005). However, there is no available data on the incurred losses or management practices.

3.3 MATERIALS AND METHODS

Questionnaires (Appendix A and B) were formulated and sent for verification to Dr Hennie le Roux, Citrus Research International (CRI) and for statistical evaluation to Mrs Hester Vermeulen, Department of Agricultural Economics and Extension, University of Pretoria. In addition, the questionnaire was submitted to the University of Pretoria's ethical committee for clearance.

Due to the status of the citrus industries in the different countries, such as South Africa, Namibia, Swaziland and Mozambique, the questionnaires were developed to suit the respective countries, given the differences in economic and social status. For South Africa, Namibia and Swaziland, the questionnaire was translated to Afrikaans to accommodate Afrikaans speaking farmers. In the case of Mozambique, the questionnaire was translated to Portuguese, the main spoken language in the country. Because of a lack of sufficient knowledge about CBS for small-scale growers, the questionnaire was also simplified to ensure adequate response.

A list of citrus growers was obtained from CRI for South Africa. Initially fifteen questionnaires were e-mailed to major growers in the main production provinces (as per list from CRI) in South Africa. Secondly, the questionnaires were distributed to growers during the farmers' information day at Marble Hall on 11/04/2006. Due to a lack of responses, the questionnaires were used on a one-on-one interview basis targeting growers in Marble Hall and Nelspruit areas. The entire process took about eight months.

In Namibia, questionnaires were distributed by an Agricultural Extension Officer in the Tsumeb district, Otjikoto Region, in the north central part of the country. This exercise took about five months to be completed.

In the case of Mozambique, the researcher sent a questionnaire to a citrus commercial estate in Maputo province, which was posted back upon completion. On the other hand, questionnaires were distributed to growers (small-scale / subsistence) in Massalela, and Nhaquila provinces, by the Agricultural Extension Officer and took about two months to be completed.

The same procedure was followed in Swaziland, whereby questionnaires were distributed to growers by the Agricultural Extension Officer and the process took four months to be completed.

3.4 RESULTS

Although there was generally poor response from the growers, some of the questionnaires were adequately completed to give the researcher a good overview of individual growers' status of CBS aspects.

About eleven commercial growers in South Africa returned the questionnaires. These were mainly from Marble Hall, Nelspruit and Malelane areas. According to growers' responses, these areas receive an average annual rainfall of 501-750 mm. The total area planted to citrus range from 5 to 1374 hectares with annual production of 70 to 5000 tonnes. About 5 – 200 permanent and 60 – 1900 part-time workers are employed, which also includes labour for other farm activities than citrus. Planted citrus cultivars include navels, oranges, lemons, soft citrus, and cultivars Nova, Tomangos, Shamoutis, Turkey, Star Ruby and Proteas.

The interview with six commercial growers from Marble Hall revealed that CBS is well known in the area. Though Marble Hall is a CBS area, most growers have not experienced the disease. It appeared that growers applied effective management practices (especially spraying programmes) which are based on a regional disease forecasting model which is used to predict the time of spore release. Management practices include the use of chemicals, such as mancozeb with mineral oil, cabrio, benomyl, flint dithane, strobilurins, azoxystrobin, and carbendazim. The monthly spraying programme starts at the beginning of October to mid-January. Orchard sanitation also forms part of the management practices whereby infected fruits are buried underground at about 25 cm and leaf litter is regularly removed from under and between the trees, thereby successfully removing the inoculum source.

The interview also revealed that about 83% of the interviewed growers are certified either for Globalgap (100%) or both Globalgap and Natures Choice (20%). Globalgap is a private sector body which sets global standards for certification of agricultural products on a voluntary basis, by bringing together like-minded parties to commonly harmonise Good Agricultural Practice (G.A.P.). The certificate mostly covers all the process of certified production inputs and farming activities until the exit point when the products leave the farm (Anon, 2008). Nature's Choice is Tesco's farm integrated scheme, which sets environmental standards and specifies shape, size, taste, variety and

shelf life requirements (Anon, 2008). In addition, farm owners ensure that all graders in packhouses are trained to identify CBS fruit symptoms and remove them.

Nelspruit in Mpumalanga Province is also a CBS area but has a general higher reported incidence of the disease. Six growers were interviewed and only three supplied valuable information. All three growers are also certified to Globalgap (100%) while one (33%) is registered for both Globalgap and Natures Choice. Two of the responded three growers from the area were experiencing CBS at the time of the survey. Though they also mostly depend on chemicals to manage the disease, not all the interviewed growers spray as recommended in the disease forecasting models. The spraying programme starts in October to December. Different chemicals such as azoxystrobin, mancozeb and strobilurin are sprayed. Most growers in this region also follow orchard sanitations, when dead and dry twigs or trees are removed from the orchard and burned. The summary of the information obtained is presented in Table 3.4

In Namibia, the survey was conducted in the Tsumeb district, in the north central of the country. Only 40% of the 10 questionnaires were returned. This area receives an average annual rainfall of 501-750mm, though the citrus plantations are under irrigation. The sizes of the plantations range from 0.5 – 30 ha with an annual production of 26 – 251 tonnes. A total of 14 – 39 permanent workers and 53 – 70 part-time workers are employed on the farms, which also includes labour for other farm productions than citrus. Citrus growers in Tsumeb mostly plant navels, Valencias (cv. delta, midnight and late Valencia) and soft citrus (cv. minneola and nova). Although growers did not report lemon cultivars in their responses, the researcher noted Eureka lemon trees during the field visit (after the questionnaires were returned).

According to the growers' responses, CBS occurs in the area, where it has been prevalent for the past 43 years. At the time of the survey, only one of the interviewed growers reported the presence of the disease on his farm. The researcher observed and collected spotted fruits and leaves mostly of lemon cv. Eureka from the plantation during the field visit. The collected samples were taken to South Africa for analysis, following standard quarantine requirements as stipulated by the National Legislation and germ-plasm transfer agreements, (Import permit number: P0023983). The pathogen was isolated and its identity confirmed using the ISO 17025 accredited PCR test method (Meyer *et al*., 2005) (Fig. 3.1) by Ms M. Wilmott of the Department of Microbiology and Plant Pathology, University of Pretoria.

Table 3.4: Summary of critical data obtained from a citrus black spot survey in South Africa

Farm name	Citrus cultivars	Area planted (ha)	Production (tons)	Rainfall (mm)	CBS knowledge	CBS pervalence	Current CBS status	Management	Type of chemicals & spraying program
MbH 1		110	5000		Yes		Absent	Chemicals, sanitation & disease forecasting	Mancozeb & Cabrio (Oct., Nov. Jan.)
MbH 2	Navels & Valencia	85.58	300	501-750	Yes	1994	Absent	Chemicals, sanitation & disease forecasting	Benomyl &Flint Dithane (Nov. & Dec : Nov. Dec., Jan)
MbH 3		38	1600	501-750	Yes	2005, very low	Absent	Chemicals, sanitation & disease forecasting	Mancozeb with mineral oil (Dec),2nd / 3rd spray depend on severity
MbH 4		100	4350	501-750	Yes	14 years ago, not serious	Absent	Chemicals, sanitation & disease forecasting	
MbH 5		160	2 600	250 - 500	Yes		Absent	Chemicals, sanitation & disease forecasting	Mancozeb & Flint-strobilurins (Beg. Nov./01 & mid Jan./18)
MbH 6	Oranges Lemons Soft citrus	1373,9	160	501-750	Yes	2002	Absent	Chemicals, sanitation & disease forecasting (not always follow it)	Azoxystrobin (mid. Nov.-mid Dec: Jan 4 valencia) Carbendazim (mid. Nov-mid Dec) Mancozeb(mid. Nov-mid Dec & Jan)
EC 1		45		250 - 500	No	Never	Absent	Chemicals	Benomyl & Mancozeb + oil (Nov & Jan)
Rsb 1	Lemons Nova Navel Valencia	200		501-750	Yes	Never	Absent	Chemicals & orchard sanitation	Abamectin & Citrex oil(Aug) Abamectin (Oct. & Dec.) Flint, Sporekill, Dithane and Citrex oil (Jan.)
Nsp 1	Tomangos Shamoutis Navels Turkeys Valencias	109	264	501-750	Yes	1968	Present	Chemicals, sanitation & disease forecasting	Azoxystrobin 1st (8-22 Nov.) 2nd (5-20 Dec) Mancozeb 1st (8-22 Nov.) 2nd (5-20 Dec.)
Nsp 2	Star Ruby	5		501-750	Yes	Never	Never experienced	Chemicals & disease forecasting	Strubiline (Nov. & Dec.)
Nsp 3	Navels Shamouties Proteas Valencia	800		501-750	Yes	30 yrs back	Present	Chemicals & sanitation	Azoxystrobin (22 Oct. & 6 weeks later) Mancozeb (1 Oct. & six weels later). Depend on market: Canada(less mancozeb), Europe(end with mancozeb)

KEYS: MbH :Marble Hall

EC : Eastern Cape

Rsb : Rustenburg

Nsp : Nelspruit

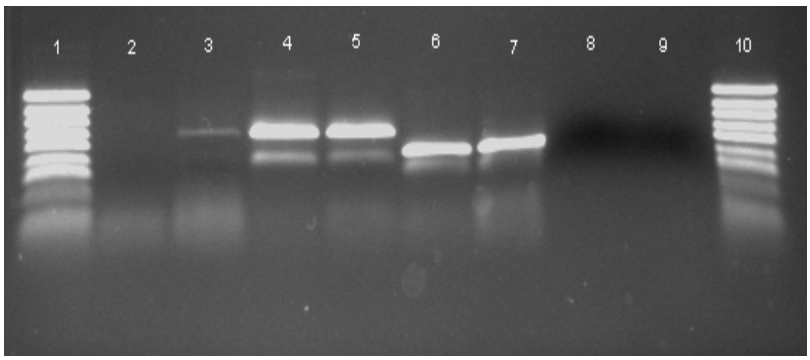

Figure 3.2: Agarose gel of PCR of *Guignardia citricarpa* on lemon fruit samples from Namibia.

1 & 10- Ladder base pair; 2 & 3 – Fruit lesions; 4 & 5- *Guignardi citricarpa* isolates; 6 & 7 *Guignardia mangiferae* isolates; 8 - *Colletotrichum*; 9 – Negative control

Management practices in the Tsumeb area include exclusion of the pathogen and the use of chemicals, such as mancozeb, benomyl and strobilurin (pyraclostrobin). However, the monthly spraying programmes which mostly start from December to February are not depended on weather or disease forecasting but rather on farmers' knowledge of the critical time of infection. On the other hand, since independence in 1990, officials from the Plant Health section of the Ministry of Agriculture and Forestry have been conducting strict inspections at the ports of entry on imported nursery trees which are mainly from South Africa, to prevent the entry of infected materials (Rhodes, personal communication, November 2006). The importers are required to be in possession of a phytosanitary certificate from the country of origin before the plant materials are allowed entry into the country (WTO, 2002).

One of the interviewed growers from Tsumeb does not produce for export (Britz, personnal communication, January 2007) and none are certified to any international standard such as Globalgap or Nature's Choice. Currently growers are not really affected by the disease, due to the fact that they do not export and that the disease is not prevalent.

The survey in Mozambique was conducted in Massalela (Inhambane province) and in the south of Maputo province. Nine questionnaires were completed and returned. These comprise eight small scale (subsistence) growers and one big (commercial) grower. The average annual rainfall ranges from 501 – 750 mm. The size of the commercial farm is 700 ha where 254 permanent and 100 part-time workers are employed. On the other hand subsistence growers have no knowledge of the plots sizes, but rather the numbers of trees they keep, which range from 55 – 285 trees per grower.

Planted cultivars for both subsistence and commercial growers include tangerine, Valencia (cv. Delta and Valencia late) and pomelos (cv. Marsh and Star Ruby).

According to the information provided by interviewed growers, CBS has been experienced since 1992 at the earliest. It is a major constraint in citrus plantations especially for subsistence growers, which sometimes cause the fruits to drop from the trees. Despite the presence of the disease, no management practices have ever been put in place, especially at a subsistence farming level. This can be attributed to the economic status of the country. In some cases growers have to travel long distances to fetch water for watering the trees.

The interviewed commercial farmer in Maputo province reported the presence of CBS which has been managed since 2002. According to the responses from the interview, management practices include chemical use and orchard sanitation throughout the year. Growers do not consult disease forecasting models for spraying. However, preventive chemicals such as benomyl or derosal are sprayed right after the first rain. The chemicals are sprayed in a mixture with mineral oil to improve the efficiency and calibrated Eagle and Heavy duty sprayers are used to effectively cover the whole leaf surfaces. The spraying programme starts in August to February and sometimes varies depending on the rainfall patterns.

Since the presence of CBS has only been visually reported in Mozambique, fresh fruits and leaves as well as dry leaves with typical CBS symptoms were collected from plantations in Inhambane province. Samples were transported to South Africa, following standard quarantine requirements as described before (Import permit number: P0023984). The pathogen was isolated and its identity confirmed by using the ISO 17025 accredited PCR test method (Meyer *et al*., 2005) (Fig. 3.3) by Dr L. Meyer of the Department of Microbiology and Plant Pathology, University of Pretoria.

Only one questionnaire was received from Swaziland, which represents 20% of the five questionnaires that were dispatched. The surveyed area receives an annual rainfall of 501- 750 mm on average. A total of 77 permanent and 380 part-time workers are employed on a 700 ha farm where pomelos (cv. Marsh, Star Ruby and Pomelit / X202), Valencia (cv. Amanzi and Olinda) and lemons (cv. Eureka and Lisbon) are planted.

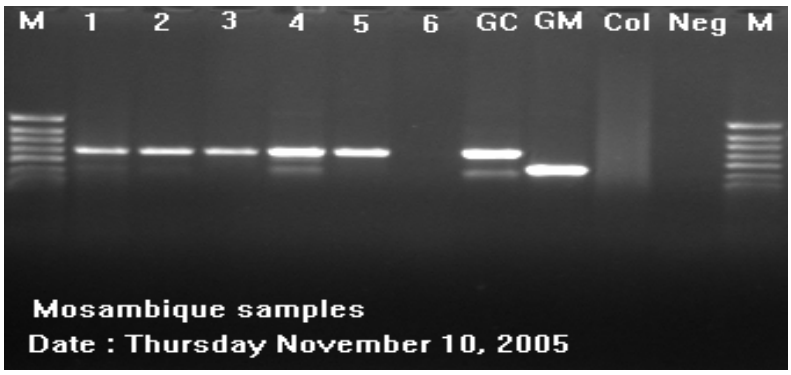

Figure 3.3: Agarose gel of PCR of *Guignardia citricarpa* on lemon fruit and leave samples from Mozambique.

M- Ladder base pair,**1 & 2** – Fruit lesions, **3** – Fruit clean surface, **4** – Dry leaves, **5** – Green leaves, **6** – Blank, **GC** – *Guignardia citicarpa*, **GM** – *Guignardia mangiferae*, **Col** - Colletotrichum, **Neg** – Negative control.

According to the provided information from the survey, CBS was first discovered in Swaziland in the late 60s, when it did not cause severe losses. However, in the mid-80s losses due to CBS were incurred and spraying was commenced. A mixture of benomyl and dithane was used mainly on grape fruits exported to Japan. Infected old Valencias were sprayed with benomyl only when the symptoms became severe. However, after benomyl was de-registered, other chemicals such as, copper hydroxide, copper oxychloride, mancozeb, pyraclostrobin, and trifloxytrobin became important and part of the disease management programmes.

It was not until CBS became a phytosanitary disease, that growers started following a spraying programme. Thus strobilurins were used and benomyl replaced. It was also noted that though the growers have access to weather stations and data, the spraying programmes are independent of the weather or disease forecasting information. The interviewed grower indicated that he is currently not registered for any of the standards, such as Globalgap or Nature's Choice.

3.5 DISCUSSION

Marble Hall is situated in one of the CBS hotspot areas. However, the results from both the interview and survey have proven that the disease is effectively managed and does not constitute a major problem for the growers. Most of the growers spray as per recommendation by the consultants. The effectiveness of the practice of spore trapping was demonstrated in the study conducted by Truter *et al*. (2004), whereby the Kotze Quest Inoculum Monitor (K-QIM) was used

to evaluate the number of trapped ascospores after submerging Valencia and Eureka leaf litter in water at different temperatures for different periods of time (Truter *et al*. 2004). The study showed the ideal environmental conditions under which ascospores are discharged. The knowledge of weather conditions and time that initiate ascospores discharge is very crucial in determining the beginning of a spray programme. This practice not only serves in preventing infection but also ensure rational use of the rather costly chemicals that in turn leads to the sustainability of citrus production. In addition, this serves in preventing pathogen resistance development to chemicals as well as slowing down environmental degradation. In addition to disease forecasting, following general orchard sanitation such as burning infected fruits and twigs and removing litter between and under the trees also contribute to the effective management of the disease in the area.

Some of the interviewed growers in Nelspruit experienced severe CBS infections in their orchards at the time of the survey. In addition to highly suitable climates for CBS establishment in the area (Paul *et al*., 2005), the disease severity and prevalence may also be attributed to the fact that not all growers spray at appropriate times. Missing spraying at the time of spore release has adverse effects on inoculum build up on specific farms (where the spraying programme is not followed) which later spread to other farms in the region. This phenomenon is very crucial in the overall management of CBS. It is therefore important that CBS is successfully managed at a regional level.

There has never been published information on CBS in Namibia and this survey is the first positive confirmation of the presence of CBS in the country. What is very important is the fact that growers are aware of CBS and have reported (during the survey) the first incidences to have occurred as far back as 43 years ago, though it was never severe. One of the growers reported the presence of the disease in the orchard at the time of the survey and the pathogen status was confirmed by PCR, thus the pathogen is still prevalent in the area, but at low levels. The presence of lemon cultivars which are reported to be more susceptible to infection than other citrus (Kotze, 1988) in the visited orchard, might be contributing to the prevalence of the disease. Furthermore, Namibia being a dry country, evaporation rates are high which keeps humidity at low levels, as compared to the other countries studied. This may also be a contributing factor that prevents CBS from reaching epidemic levels. This is accomplished by the effective orchard hygiene practices that growers follow thereby further preventing build up of the disease pressure.

In Mozambique, the poor management of CBS, especially by subsistence growers may be attributed to the lack of knowledge and resources. This is in line with the survey results by Le Roux *et al*. (2003), which reported that a number of mandarin trees belonging to subsistence farmers between

Machipanga and Inchope on the Zimbabwean borders have been neglected and riddled with CBS. Furthermore, it has also been observed that most of the citrus trees are intercropped with cassava or coffee and coconut trees (Fig.3.4) which also makes management difficult. No interactions could happen between the commercial and subsistence farmers as they are situated more than 400 km from each other.

Figure 3.4: Tangerine trees growing between coconut trees in Inhambane province, Mozambique. **(Picture: Courtesy of Dr Regnier)**

On the other hand, the good management of CBS on the commercial farm was instrumental in accessing the European and Middle Eastern markets. The citrus Estate also makes use of the three- to four-week production window competitive advantage that Mozambique has over other African countries, to increase citrus sales (TechnoServe, 2006).

Citrus black spot also occurs in Swaziland (Schutte, 1995; Paul *et al*., 2005), though it never caused severe losses as per interviewed farmers. This may also be attributed to effective spraying programmes whereby benomyl was replaced with strobilurins due to resistance development.

For almost all the surveyed countries (specific regions), the average annual rainfall is in the same range of 501-750 mm. Given the occurrence of CBS in some of the areas, this implies that the rainfall is conducive to disease dispersal, development and prevalence. The minimum and

maximum winter and summer temperatures are in the range of <10°C, 10 - 15°C and 16 – 21°C, 21 – 30°C and sometimes > 30°C. These conditions, especially when the summer temperatures of 16-27°C, coincide with the rains can lead to ascospores development and subsequently disease development (Paul, 2005). This shows that the environmental conditions in all the surveyed areas are conducive for disease development. Therefore one of the discrepancies in disease prevalence during the survey can be attributed to differences in disease management practices, based on Marble Hall using disease forecasting models which result in more targeted disease control. This is in comparison to Nelspruit where disease spray program is based on generic spraying, thus the disease is more prevalent.

Eventhough most of the surveyed orchards are situated in areas that receive the same amount of rainfall, the disease prevalence may also vary due to differences in the levels of humidity and the amount of day light. The low incidences or poorly reported CBS status in Namibia might be attributed to the generally higher temperatures and low rainfall as well as higher evaporation rates. This may shorten the period of wetness of the leaf surfaces, thus making it less favourable for pathogen germination and penetration. Furthermore, all growers make use of integrated management practices to some extend, where chemicals and orchard sanitation are employed. This is a holistic approach in the control of any plant pathogen, in terms of minimising environmental degradation, resistance development as well as the rational use of resources. However, due to the prevalence of the disease in certain areas, much still needs to be done in order to improve the control strategies and maintain the industry in the region.

Considering the number of people employed at different farms, the citrus industry has a major impact on the livelihood of the people in the region. It is therefore important that the industry is sustained at the national as well as regional levels.

3.6 CONSTRAINTS

It is important to highlight some of the constraints experienced during this study. It took the researcher about three to four months to get the final version of the questionnaire accepted and validated and eight months to interview the growers. In all the countries, more constraints were encountered during the interviews. Firstly, to locate and get hold of citrus growers was a major problem since stakeholders, such as the National Department of Agriculture and Citrus Growers Association constantly withheld the growers' contact details thus making it difficult to locate. Secondly, getting the information from the growers was another major obstacle because of the lack

of willingness to collaborate and share what growers regard as sensitive information. It took about 1 to 1, 5 hours to interview one farmer.

In the case of South Africa, attempts were made to forward the questionnaires to the growers by e-mail, which proved fruitless. Based on that, a second strategy was used whereby the researchers personally visited growers and interviewed them. This exercise proved to be slightly more effective but with some growers not wiling to give the necessary information for the study.

For other countries surveyed such as Namibia, Mozambique and Swaziland the Extension Officers who distributed the questionnaires experienced the same obstacles and only few questionnaires were completed and returned. This was especially true for Namibia and Swaziland. However, growers from Mozambique participated well. In general, very little industry support for this project was experienced throughout this study.

3.7 CONCLUSIONS AND RECCOMMENDATIONS

The occurrence of CBS in Southern Africa has been reported for many decades (Paul *et al*., 2005). The survey results indicated the prevalence of the disease in different countries such as South Africa, Namibia, Mozambique and Swaziland. Growers use similar practices, i.e. the use of chemicals and orchard sanitation. On the other hand, in some countries or regions in the same country, growers still make use of benomyl, a curative fungicide, which has been de-registered due to resistance development of *G. citricarpa*. This is due to the fact that, it is only being used where resistance has not been observed.

It is recommendable that all growers use disease forecasting models for more effective timing of spraying for better management of CBS. Disease forecasting as demonstrated with the conducive conditions for spore release and strapping by Truter *et al*., (2004), will aid growers in their spraying programmes. This does not only play a major role in reducing the disease incidences but is also crucial in preventing environmental degradation from the continuous use of chemicals. With disease prediction, the chemicals are only sprayed when deemed necessary. Secondly, this approach also prevents resistance development of *G. citricarpa* from excessive use of specific chemicals.

Training of growers, especially in the case of subsistence growers in Mozambique is crucial. Knowledge of specific, non-costly CBS management practices such as orchard sanitation will help to reduce the inoculum source, thus breaking the life cycle of the pathogen. On the other hand, most

of the subsistence growers' citrus plantations in Mozambique are intercropped with cassava or coconut trees and no orchard sanitation is implemented. This may have a major impact on management strategies by creating microenvironments that are conducive for disease development, such as humidity.

The citrus industry and CBS do not only have an impact on citrus growers but also on local communities where people are employed in the industry. It is therefore very important that the disease is properly managed to sustain the industry which in turn ensures the livelihood of the people. This is particularly true for growers who solely depend on citrus for a livelihood (especially the subsistence growers). Proper and successful management of CBS will lower incidence levels in the region, which in turn ensures increased production. On the other hand, this will reduce the time spent on managing the disease so that growers can take part in other activities from where they can also make a living.

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CHAPTER 4

USE OF FURFURAL TO CONTROL THE PATHOGEN

ABSTRACT

The effect of furfural in controlling *Guignardia citricarpa*, the causal agent of citrus black spot was assessed. Different application approaches using furfural such as direct contact or as a fumigant were demonstrated in *vitro* and *in vivo*. The product was evaluated on conidial development, in fresh leaves, leaf litter, and fruit lesions as well as in the soil. The Polymerase Chain Reaction (PCR) was used to determine the survival of the pathogen in the soil after exposure to furfural for a period of between one and four weeks. The non-target effects of the product on the general soil micro-flora were also assessed. Furfural presented the potential of controlling *G. citricarpa*, by breaking the life cycle, thus reducing the disease incidence. The product also proved suitable for soil applications due to its minimum non-target effects on bacterial and fungal populations and it also promoted growth of yeasts. The future commercial use of furfural as an alternative to other chemicals should be investigated.

4.1 INTRODUCTION

The citrus black spot (CBS) pathogen, *Guignardia citricarpa* Kiely, causes different types of fruit symptoms to occur ranging from red freckled lesions, black and red spots to classic hard spot (Kiely, 1948). In Southern Africa, fruit lesions which may produce pycnidiospores do not contribute to the epidemic development of the disease as it does not represent a major inoculum source and it is waterborne (Whiteside, 1965). The leaf symptoms which seldom appear are represented by small necrotic spots with a gray centre surrounded by a dark brown ring and a yellow halo (Kotze, 1988). Infected leaves only produce ascospores when decomposing on the orchard floor. The ascospores are the main source of inoculum in CBS epidemics (Kellerman & Kotze, 1977) and are typically dispersed by air currents and deposited on leaf and fruit surfaces (Whiteside, 1965). Various control measures have been used to prevent the introduction of the pathogen, reduce inoculum and subsequently prevent infection (Korf *et al*., 2001). These include the use of chemicals, quarantine and cultural practices (Kotze, 1981).

Current control is mostly focused on preharvest or protective field sprays using fungicides such as mancozeb, azoxystrobin and benomyl (in areas where resistance to benomyl has not been reported)

(Kellerman, personal communication, August 2006). The spraying programmes, especially in South Africa are timed to coincide with the critical infection period, which has resulted in improved management of the disease. This trend has also been instrumental in securing market access, such as the European Union (EU) and the United States America (USA). However, a global move away from pesticides has resulted in a search for alternative disease control approaches. Studies have been conducted to discover and integrate natural products (biocontrol) into the existing control measures, such as the use of chemicals in order to reduce environmental degradation. Kupper *et al.* (2005) demonstrated that the use of biofertilisers, produced from the anaerobic digestion of cattle manure showed some potential in controlling CBS disease.

In addition to biofertilisers, water extracts from organic matter had also been proven effective in controlling CBS (Kupper *et al*., 2005). They further reported that applying water extracts from composted orange peels to the soil or plant, reduced or controlled the disease incidence to rates similar to mancozeb. Other natural or biocontrol products, such as garlic and *Coprosma repens* have also shown some potential in inhibiting the growth of *G. citricarpa* (Obagwu, 2003).

Furfural or 2-furaldehyde, is a well-known by product of agro-wastes obtained from sugar cane bagasse (de Carvahlo *et al.*, 2004), cereal or pulping wastes. It is found in a number of dietary sources. Because of its formation during thermal decomposition of carbohydrates, 2-furaldehyde is also found in numerous processed foods and beverages (Maga, 1979), in some fruits and vegetables and is also added as a flavouring agent to some foods. Furfural is produced commercially by hydrolosis of pentosans (found in baggase, a waste product from the sugar milling process) under specific conditions of temperature and pressure (Anon., 2007). Due to global environmental concerns from the public, it is important to note the potential effect of this product. According to Ettinger (1954), virtually no degradation occurred in a solution of 2-furaldehyde in distilled water over 30 days, suggesting that hydrolysis is not an important process at environmental pH. Although some emission to the atmosphere is expected from wood burning, no atmospheric effects are expected given the short half-life for reaction with hydroxyl and other radicals and possible photodegradation of 2-furaldehyde. Also the low volatilisation of the compound from water and soil would not be expected to add significantly to atmospheric levels.

In laboratory analyses, furfural has been found to have some nematicidal activities (Spaull, 1997). Soil-borne diseases as well as other plant diseases were also found to be controlled by this product (Gilreath *et al*., 2003). This study was therefore undertaken to investigate the use of furfural as an alternative disease control option for *G. citricarpa*.

4.2. EFFECTS OF FURFURAL ON THE GROWTH AND DEVELOPMENT OF *GUIGNARDIA CITRICARPA*

4.2.1 MATERIALS AND METHODS

Effect of furfural in growth medium on the pathogen

Pathogen isolation

Guignardia citricarpa (GC-m155), was originally isolated from symptomatic Eureka lemon fruit, purified, preserved and identity confirmed by M. Truter (Residing mycologist at the Department of Microbiology and Plant Pathology, University of Pretoria at the time of the study). The isolate was maintained on half strength Potato Dextrose Agar (PDA) (Biolab, Johannesburg, South Africa) and prepared for inoculation studies by either scraping off the spores with a sterile glass rod after six to 14 days of incubation at 23 ºC or punching a five mm diameter disc from the edge of actively growing cultures.

After PDA was autoclaved and cooled down to 50°C, 0.04% Tritron X 100 (Merck, Johannesburg) and furfural (Sigma, Johannesburg) was aseptically added to the medium to give final concentrations of 2, 4, 6, 8 or 10 % (v:v). The agar was then poured into 90 mm Petri-plates. Discs of 5 mm with mycelia and spores of the fungus were placed at the centre of each PDA plate after the agar had set. Petri-plates of only PDA were used as controls. After six days of incubation at 23°C, mycelial growth was observed and measured with a digital caliper (Absolute Digimatic-Mitutoyo Corp., Japan). The experiment was carried out with five replicates per furfural concentration. Data were expressed as percentage inhibition of mycelial growth according to the method described by Plaza *et al.* (2004).

Control of conidia germination and appresoria development

The furfural effect was also tested in terms of inhibiting conidia germination of the pathogen. Disposable multiwells plates (Corning®) (96 wells ELISA plate) with a flat bottom and well capacity of 0.37 ml were used for this experiment. Four wells per treatment were used and replicated twice per plate. Each well was aseptically filled with 0.25 ml of the different prepared concentrations of furfural (0.1, 0.5, 3.5, 7 and 8 %, v:v) and freshly squeezed orange juice (2%) was used as control. Twenty-five micro-litres of *G. citricarpa* pycnidiospore suspension (10^3 spore ml^{-1}) were added to the solution in each well. The plates with the solutions/pycnidiospore suspensions

were incubated at 25°C (±2°C) in darkness. Germination rate and appressoria development was measured after 96 hours. Pycnidiospore germination and growth potential were assessed at 400x magnification under an inverted microscope (Nikon TMS-F). A spore was considered germinated if the length of the germ tube was equal to or longer than the spore, or when an appressorium was present either sessile or attached to a germ tube. The experiment was designed as a split-plot in two blocks. The results were expressed as percentage conidia germination and appressoria development.

Volatile effects of furfural on the pathogen

The volatile effect of furfural was assessed on the *in vitro* growth of *G. citricarpa* cultures. Glass cover slips were fixed to the inside of Petri-plate lids using a drop of glycerol. Drops of furfural concentrations of 8% and 10% v:v were placed on the cover slip by using a dropper.

Freshly picked (50) symptomatic Valencia leaves were collected from CBS infected trees in an orchard in Brits (North Western Province, SA) and surface disinfected by dipping in 70% ethanol for 2 minutes. After the leaves were air dried, 0.5 cm discs were aseptically cut with a sterile cork borer. Three hundred discs were used in total. Five discs were placed on the surface of each PDA in the Petri- plate. Twenty of these plates were exposed for four days to 8% furfural as a volatile and a similar number was exposed to 10% furfural for six days as described above (using the drop in the lid method). The remaining plates were used as control. The presence or absence of fungal growth was recorded. The fungicidal property of furfural was confirmed by transferring the leaf discs after six days exposure to the volatile onto fresh PDA plates and incubating it further for six days at 23°C. The presence or absence of mycelium growth from the leaf discs was again recorded and data were expressed as percentage positive or negative mycelial growth.

Valencia orange and Eureka lemon fruits with CBS lesions were harvested from the same orchard in Brits and surface disinfected with 70% ethanol. After air drying, the lesions were aseptically removed with a sterile scalpel and transferred onto Petri- plates containing PDA (10 sections per Petri- plate). A total of 50 lesions from Valencia oranges and 50 lesions from lemons were used in this experiment. Plates were exposed for four days to 8% and 10% furfural on the paper discs stuck to the lids. Fifty lesions of both fruit types were also used as control and exposed to paper discs impregnated with distilled water. The fungicidal effect of furfural was confirmed by aseptically transferring lesions showing no fungal growth onto fresh PDA plates and incubation for six days at 23 ±2°C. The presence or absence of fungal growth from the cut lesions was recorded and data expressed as percentage of lesions presenting pathogen growth.

Control of *Guignardia citricarpa* on leaf litter by furfural

Eureka leaf litter was used to test the effect of furfural (Illovo, Johannesburg) on *G. citricarpa* under controlled conditions and in the field. Four bags (± 500g) of litter were collected from a CBS infected orchard at Brits in October 2005. The presence of pycnidia on the dry leaf surface was observed under the microscope at x 100 enlargement and the presence of *G. citricarpa* was confirmed by PCR as described by Meyer *et al.* (2005). The leaf litter was used throughout the study.

The litter was surface disinfected with 70% ethanol as before and placed into an empty sterile Petri-plate. Two leaves were used for each concentration of furfural tested which was diluted with distilled water to obtain a final concentration of 2, 4, 6, 8 and 10%. Leaves were sprayed with five millilitres of furfural and left to dry at room temperature (23 ± 2°C). Two leaves sprayed with sterile distilled water were used as control. The spraying was done for three consecutive days after which the litter was left to dry for four days. After four days, pieces of leaf litter with lesions were aseptically cut from the sprayed leaves using a sterile scalpel and placed on the surface of half strength PDA (five pieces per plate). The plates were incubated at 25°C for seven days. The presence or absence of mycelial growth was recorded. The trial was done in duplicate.

The *in vivo* experiment of furfural on leaf litter was conducted at the Eureka and Valencia orchard at the University of Pretoria Experimental Farm over a period of four months. Green leaves (6), leaf litter (6) and soil samples (collected from underneath six trees at three different spots around the trees but within the canopy) were collected to determine the absence or presence of *Guignardia* spp. in the orchard. This was confirmed by PCR according to the method described by Meyer *et al.* (2005). The PCR proved negative for *G. citricarpa* but positive for *G. mangiferae*. This knowledge was crucial in ensuring that the orchard was free from CBS to avoid re-contamination of the litter (the CBS infected litter from Brits, which was placed under the trees and sprayed with furfural) during the experiment.

A total of 48 plastic grids (350mm diameter, 10mm mesh) with litter were prepared (Fig. 4.1). The litter, just enough to cover the grid surface, was secured between two plastic grids, which were fastened with cable ties. In the orchard, 12 trees were randomly selected and marked, six for the treatment and six for the control. Under each marked tree, four prepared grids were placed.

Figure 4.1: Plastic grid with Citrus black spot infected leaf litter under a tree.

Previous results showed that furfural solutions at concentrations of 8% and 10% were the most effective in controlling the pathogen (unpublished data). However, at 10% concentration, the product does not effectively dissolve in distilled water as compared to 8%. For this reason, 8% furfural was used to spray the litter. To enhance proper dissolution, 0.1% Tween 80 (Sigma, SA) was added to the mixture. A high-pressure sprayer, Polyspray 3 (Efekto, SA) was used. For the treatment, the litter was sprayed with 150ml 8% furfural for three consecutive days at monthly intervals, for four months. The control was similarly sprayed with distilled water. After the first month, one grid was removed from each tree (just before the rest were sprayed). Another grid was removed from each tree a month later and the assessment repeated as described. Grids removed after four months thus had four sprays of furfural. The grids were taken to the laboratory for spore counting using the Kotze Inoculum Monitor according to the method of Truter *et al*. (2004). The method basically consist of soaking the grids (with the litter secured between) in tap water at 40°C ± 2°C for about 5 minutes after which it was air-dried on a paper towel for ten minutes. The aim for soaking the litter was to remove excess soil and to facilitate spore release. The ascospores were trapped on a microscope slide and counted under a stereomicroscope.

Use of furfural to control *Guignardia citricarpa* in the soil

The soil trials were conducted in two locations. One trial was set up in the greenhouse (with temperatures of 20-29ºC) at the Department of Microbiology and Plant Pathology, University of Pretoria. Pots (12.5 cm) with 500g of soil each were kept on greenhouse benches (Fig. 4.2). Each treatment and control was replicated thrice.

Figure 4.2: Pots with soil samples in the greenhouse (soil collected from a citrus black spot infested orchard).

The second soil trial was conducted on a CBS infected farm in Brits (Fig. 4.3). Thirty pots with the same amount of soil (500g) were kept under Eureka trees (five pots under each tree, for six trees). Treatments and controls were similarly replicated. For all the treatments 8% furfural was used. The different treatments and controls of the experiment are described in Table 4.1.

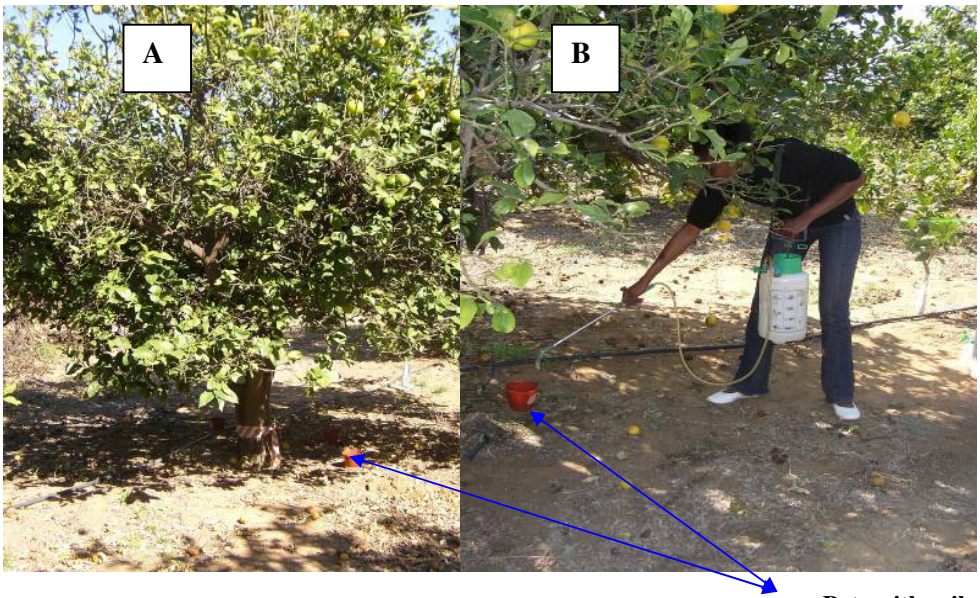

Figure 4.3: Trial under lemon cv. Eureka trees in the orchard in the Brits area, North Western Province, South Africa.

(A) Pots with soil samples; (B) Soil sprayed with 8% furfural to control *Guignardia citricarpa*.

Table 4.1: Different controls and furfural treatments for the soil experiments in the greenhouse and on the farm to control *Guignardia citricarpa*, causal agent of citrus black spot

GREENHOUSE EXPERIMENT	
Controls	**Treatments**
Soil(500g)	
Soil(500g)+Water	Soil (500g) + furfural (8%)
Soil(500g)+Inoculum(10^4 spores/ml)	
Soil(500g)+Inoculum(10^4 spores/ml)+ Water	Soil (500g) + Inoculum (10^4 spores/ml) + Furfural (8%)
	Autoclaved soil(500g)+Inoculum(10^4 spores/ml) + furfural (8%)
FARM / FIELD EXPERIMENT	
Soil(500g)	Soil (500g) + furfural (8%)
Soil(500g)+Water	Soil (500g) + Inoculum (10^4 spores/ml) + furfural (8%)
Soil(500g) + Inoculum (10^4 spores/ml)	

To ensure positive contamination, the soil was also inoculated with *G. citricarpa* spore suspension at 10^4 spores/ml for 500g. The suspension was prepared under aseptic conditions from three weeks old *G. citricarpa* PDA plates. Spores were harvested from the culture with a moistened sterile swab. The spores on the swab were suspended in 20 ml autoclaved water in Bijon bottles. The harvesting was repeated, until the solution was concentrated to give a final concentration if applied at 10^4 spores/ml. The suspension was filtered through sterile cloth gauzes to remove undesirable particles from the culture. The inoculum concentration was adjusted to 10^4, by counting spores with a haemacytometer and adjusting the final concentration according to requirements.

In each pot, 50 ml of the inoculum was added for the treatment while the same volume of distilled water was added to each control pot. Due to the distance from Pretoria to Brits (120 km), the sampling from the trial on the farm was done at two weekly intervals as compared to the weekly sampling from the greenhouse trial. At each sampling, one pot was removed from each treatment from where sub samples were taken for analysis. The remaining soil in the other pots was drenched again with the same volume of furfural and water. The procedure was repeated until all the pots were removed. Twenty soil samples were tested for the presence of *G. citricarpa* using a modified PCR method.

For DNA extraction, the EPICENTRE SoilMaster™ DNA Extraction Kit was used. Soil samples of 300 mg were used and extraction buffer (Meyer *et al*., 2005) was added. After incubation, the tubes were centrifuged at 13000 rpm's. After precipitation with a DNA Precipitation Solution (Meyer *et al*., 2005), the remaining pellet was washed and suspended in 100µl of TE Buffer. A RAPD PCR amplification reaction was performed and the PCR analysis was done by loading the DNA onto a 1% horizontal agarose gel stained with 10% v/v ethidium bromide (Merk). The hyper ladder IV (Bioline) was also loaded onto the gel to estimate the size of the products. Due to very fine bands obtained from the normal PCR on samples kept on the farm (Brits), a nested PCR was performed on these samples according to the method described by Meyer (2006). Electrophoresis was performed at 100 V for 1 hour and visualised under UV illumination.

Soil samples were also taken from the greenhouse and farm trials for the assessment of non-target effect of furfural on soil micro-flora. The soil was assessed for both fungi and bacteria. Two growth media, Malt Extract Agar (MEA) (Biolab, Midrand), amended with chloroamphenicol (0.1%) (CAPS, Johannesburg) and Standard Nutrient Agar (STD) (Biolab, Midrand), amended with cycloheximide (0.1%) (Biolab) were prepared in Petri-plates and used for fungi and bacteria respectively.

About 90 ml of 0, 1% sterile water agar (WA) (Biolab) was prepared in volumetric flasks for soil suspension. The dilutions were carried out in 9 ml of ¼ strength sterile Ringer's solution (Oxoid, Johannesburg) in test tubes. Ten gram of soil from each sample was mixed in 9 ml sterile Ringer's solutions and serially diluted. Samples for fungal determination (on MEA^{+}) were diluted up to 10^{-5} while for bacterial samples (on STD^{+}) were diluted up to 10^{-6}. The Petri-plates were incubated at 25ºC for three days, after which fungi and bacteria in each plate were counted and recorded.

Statistical analysis

Analysis of variance (ANOVA) among means was performed with one-way ANOVA using the Excel data analysis (two factors without replication, and single factor). Differences at $P \leq 0.05$ were considered to be significant.

4.2.2 RESULTS

The study demonstrated the potential of furfural in inhibiting the *in vitro* growth of *G. citricarpa*. At the furfural concentration of 2 %, the mycelial growth was inhibited by 77.6% while at 4, 6, 8 and 10% there was 100% inhibition (Fig. 4.4).

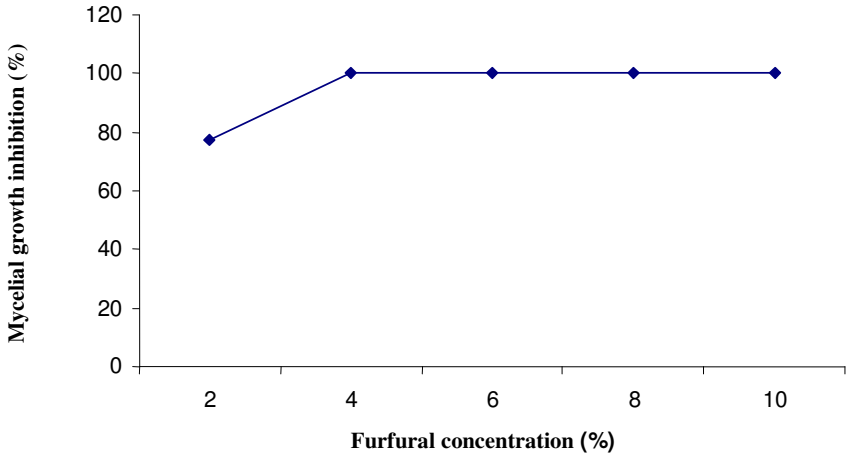

Figure 4.4: Inhibition of mycelial growth of *Guignardia citricarpa* by furfural incorporated into the growth medium.

When furfural was tested in terms of inhibition of conidia germination and appresoria development of *G. citricarpa*, it was demonstrated that the product had an inhibitory effect on preventing germination and appresoria formation. At the concentration of 0.1%, there was 3.6 % conidia germination as compared to 15.9% for the control. However, 0.5; 3.5; 7 and 8% furfural completely inhibited conidia development. On the other hand, only 7 and 8% furfural inhibited appresoria development completely, while the concentrations of 0.1, 0.5 and 3.5, only allowed appresoria development up to 12.2, 8.9 and 8.1% respectively, compared to 62.37% for the control (Table 4.2).

Table 4.2: Effect of furfural on the germination and appressoria development of pycnidiospores of *Guignardia citricarpa*

Furfural concentration (%)	Conidia germination (%)	Appresoria development (%)
0	15.9 ± 2.1	62.4 ± 5.0
0.1	3.6 ± 1.01	12.2 ± 1.9
0.5	0	8.9 ± 0.9
3.5	0	8.1 ± 0.1
7	0	0
8	0	0

Furfural tested as a biofumigant showed the potential of retarding mycelium growth and development of the pathogen. Fungal growth was completely inhibited at the two concentrations (8 and 10%) tested, which demonstrated the fungicidal effect of the product. When the fungus was

transferred to fresh PDA medium, there was 4- 20% growth for cultures which were exposed to 8% furfural, while the cultures exposed to 10% did not grow at all (Table 4.3).

When the leaf litter was sprayed with different furfural concentrations, it was noted that there were some inhibitory effects. The fungus fully grew on the agar when the litter was sprayed with distilled water. Some fungal growth was also observed from one of the five plated pieces (20% fungal growth) after seven days of incubation at 25°C, when the leaf litter was sprayed with 2% and 4 % furfural. However, 6%, 8% and 10% proved to be 100% effective in inhibiting the fungus (Fig. 4.5).

Table 4.3: Presence or absence of *in vitro Guignardia citricarpa* growth from citrus leaves and fruits after exposure to volatile furfural

Treatment	Plant Material	Concentration of furfural (%)	Mycelial growth (growth+ or no growth)
At transfer*	Naturally infected fresh leaf	0	+ (96%)
		8	-
		10	-
	Orange with CBS lesions	0	+ (98%)
		8	-
		10	-
	Lemon with CBS lesions	0	+ (100%)
		8	-
		10	-
Six days after transfer to a fresh medium	Naturally infected fresh leaf	8	+ (4%)
		10	-
	Orange with CBS lesions	8	+ (10%)
		10	-
	Lemon with CBS lesions	8	+ (20%)
		10	-

*Data recorded after four days exposure to volatile furfural

Positive if at least one lesion or disc presented any fungal growth

Numbers in brackets are the percentage of leaf or fruit sections showing mycelial growth.

Data are the means of three independent trials

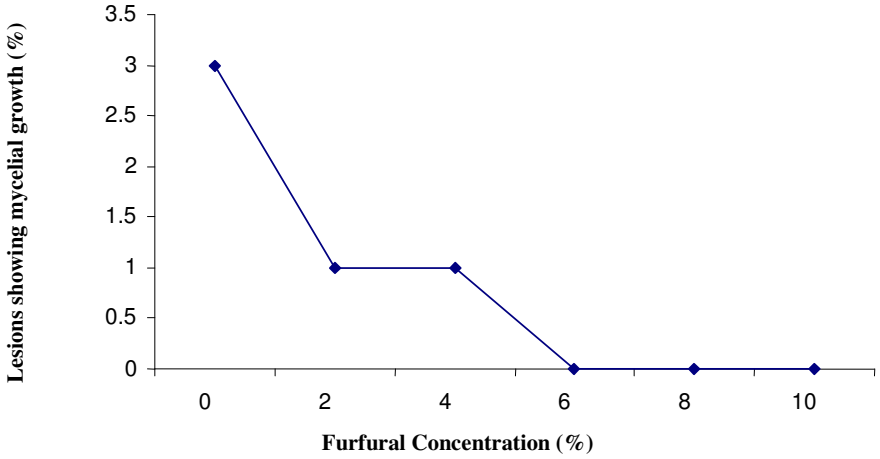

Figure 4.5: Fungal growth on half strength Potato Dextrose Agar from leaf litter pieces with *Guignardia citricarpa* lesions sprayed with distilled water and different furfural concentrations.

***Value expressed as average of three replicates**

% lesion developing: Number of litter pieces from which mycelia grew (out of five pieces per plate)

The results also demonstrated the efficacy of furfural in controlling *G. citricarpa* ascospores on leaf litter in the field. When the dry leaf litter was sprayed with 8% furfural, the number of countable ascospores was less as compared to the litter sprayed with distilled water (Fig. 4.6). However, it was noted that, *G. citricarpa* ascospores were thus overgrown by *Colletotrichum* spp. (data not presented).

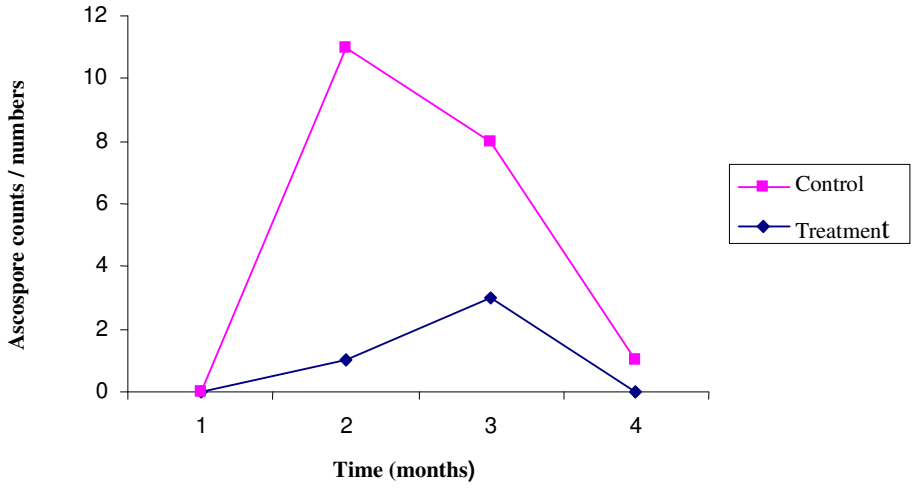

Figure 4.6: Number of ascospores on leaf litter after sprayed with 8% furfural and distilled water.

***Values expessed as average of six replicates**

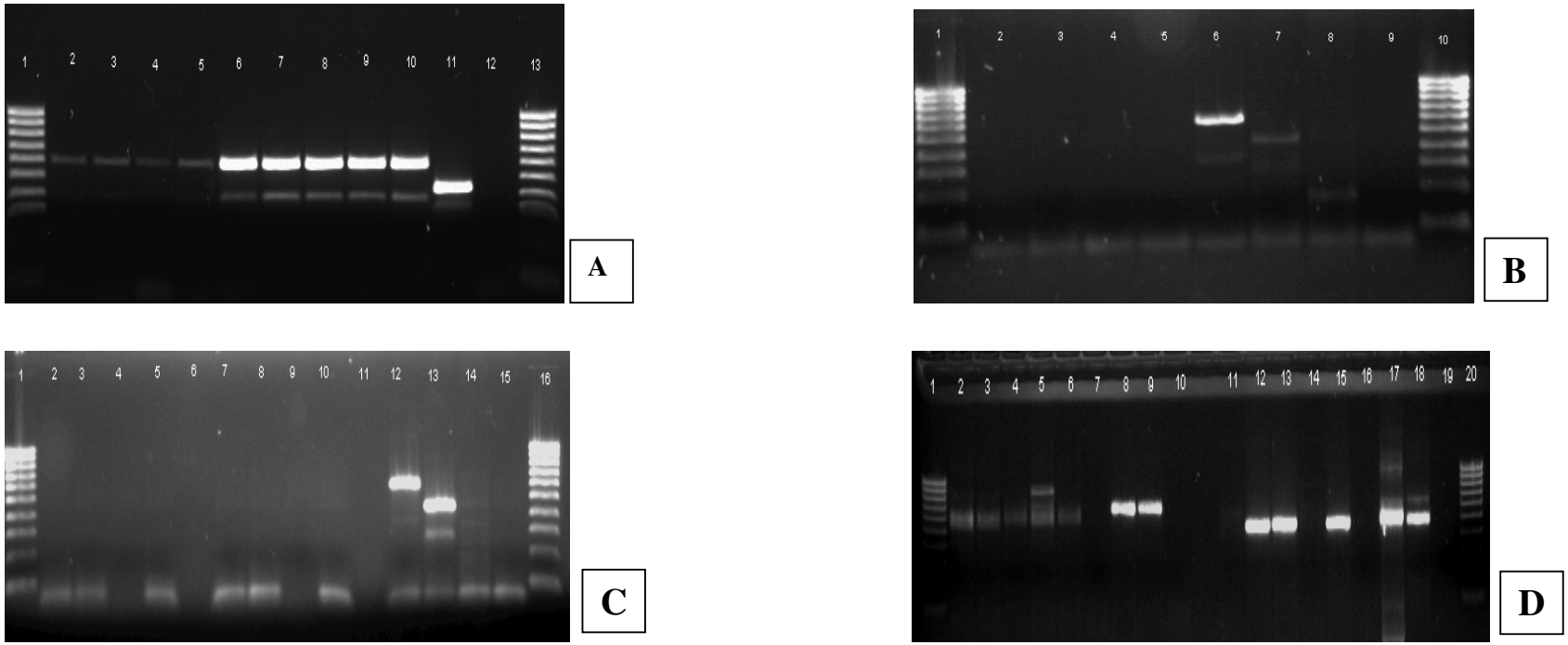

Figure 4.7 (A): Agarose gel of PCR product of the soil samples taken from the greenhouse after one week. 1 & 13: Ladder; 2: Soil + Inoculum; 3: Soil + Inoculum + furfural; 4: Autocalved soil + Inoculum + furfural; 5-10: *G. citricarpa* isolates; 11: *G. mangiferae*; 12: *Colletotrichum gloeosporioides*. **(B): Agarose gel of PCR product of soil sample kept in the greenhouse after two weeks. 1 & 10: Ladder; 2: Soil + Water; 3: Soil + Inoculum + Water; 4: Soil + Inoculum + furfural; 5: Autoclaved soil + Inoculum + furfural; 6:** *G. citricarpa*; **7:** *G. mangiferae*; **8:** *Colletotrichum gloeosporioides*. **; 9: Negative control (C): Agarose gel of PCR product of soil sample kept in the greenhouse after three and four weeks. 1&16: Ladder; 2: Soil + Inoculum[3]; 3: Soil + Inoculum + Water[3]; 4: Soil + Inoculum + furfural[3]; 5: Autoclaved soil + Inoculum + [3]; 7: Soil + Inoculum[4]; 8: Soil + Inoculum + Water[4]; 9: Soil + Inoculum + furfural[4]; 10: Autoclaved soil + Inoculum + furfural[4]; 12:** *G. citricarpa*; **13:** *G. mangiferae*; **14:** *Colletotrichum gloeosporioides*. **; 15: Negative control (D): Agarose gel of ITS and NestedPCR product of soil sample kept on the farm after two and four weeks. 1 & 20: Ladder; 2: Soil + Water[2]; 3: Soil + Inoculum[2];4: Soil + Inoculum + Furfural[2] 5:Soil + Water[4]; 6: Soil + Inoculum[4], 7: Soil + Inoculum + furfural[4];8:** *G. citricarpa*; **9:** *G. citricarpa*; **10:Blank ; 11: Soil + Water[2]; 12: Soil + Inoculum[2];13: Soil + Inoculum + Furfural[2]; 14:Soil + Water[4]; 15: Soil + Inoculum[4], 16: Soil + Inoculum + furfural[4];17:** *G. citricarpa*; **18:** *G. citricarpae*; **19:Blank ****(2-9: ITS PCR ; 11 – 18: Nested PCR)**

The effect of furfural on the pathogen survival in the soil was confirmed with the PCR. It was found that *G. citrica*rpa was still present in the soil after it was kept in the greenhouse for a week at the temperatures of 20°C to 29°C (Fig. 4.7 A). However, when the PCR was performed after two weeks, no *G. citricarpa* was detected in the soil (Fig 4.7 B). The same negative result was observed at three and four weeks (Fig. 4.7 C). On the other hand, positive PCR indicated the presence of *G. citricarpa* in the soil which was kept in the field both at two and four weeks (Fig. 4.7 D). However, no further study was conducted to determine the viability of the pathogen.

Under greenhouse conditions, some significant differences in the number of fungal colonies were observed over time (Table 4.4). The difference between treatments varied according to the period of exposure. No fungal colonies were observed when the soil was drenched with furfural throughout the experiment. The control soil showed a decrease in the number of fungal colonies in the second week which increased and then decreased in the third and fourth week respectively. When the soil was drenched with water, or only inoculated with *G. citricarpa* the number of fungal colonies decreased in the first three weeks (when compared to 43000 cfu's found in the original soil), and then increased in the fourth week. Furthermore, the difference in the colony numbers was not significant when the soil was inoculated and drenched with water throughout the experiment.

Furfural decreased the number of bacterial colonies in the soil as compared to the control soil (Table 4.5). The number of colonies increased in the second week and later decreased and increased in the third and fourth week respectively, when the soil was inoculated with the pathogen *G. citricarpa*. Furthermore, the presence of the inoculum in the soil increased the number of bacterial colonies after two and four weeks. On the other hand, when the inoculated soil was treated with furfural, the colonies decreased in the second week and then increased in the third week. A decrease in the number of colonies was also observed in the fourth week. Overall, furfural was able to completely kill bacterial populations over time, when the soil was previously autoclaved and inoculated with *G. citricarpa.*

Four weeks after the treatment under natural conditions, especially in a heavy infected citrus orchard, an application of furfural did not completely kill the fungi. An increase in the number of colonies at four weeks was due to the presence of yeast (Table 4.6).

Table 4.4: Total fungal colony counts in the soil after one, two, three and four weeks of exposure to furfural in the greenhouse

	Total number of fungal colonies (cfu's/g soil) *			
	Time (in weeks)			
Treatnment	**1**	**2**	**3**	**4**
Soil	3.5×10^4 Aa	3.4×10^4 Aa	4.4×10^4 Aa	2.1×10^4 Ab
Soil + Water	4×10^4 Aa	2..9 $\times 10^4$ Bb	2..2 $\times 10^4$ Bb	3.6×10^4 Aa
Soil + Furfural	0 Ba	0 Ca	0 Ca	0 Ba
Soil + Inoculum	3.3×10^4 Aa	2.3×10^4 Bc	1.8×10^4 Bc	2.8×10^4 Ab
Soil + Inoculum + Water	3.5×10^4 Aa	4.8×10^4 Aa	4.2×10^4 Aa	3.5×10^4 Aa
Soil + Inoculum + Furfural	0 Ba	0 Ca	0 Ca	0 Ba
Autoclaved soil + Inoculum + Furfural	0 Ba	0 Ca	0 Ca	0 Ba

* Values expressed as averages of three replicates for each dilution series.

For each week, within each column or row, means followed by the same upper-case or lower-case letter, respectively, do not differ significantly at $P \leq 0.05$.

Table 4.5: Total bacterial colony counts in the soil after one, two, three and four weeks of exposure to furfural in the greenhouse

	Total number of bacterial colonies (cfu's/g soil) *			
	Time (in weeks)			
Treatment	**1**	**2**	**3**	**4**
Soil	8.1 x 10^5 Ba	6..9 x 10^5 Ba	6.9 x 10^5 Ba	8.6 x 10^5 Ca
Soil + Water	1.5 x 10^6 Ba	2.2 x 10^6 Ba	8 x 10^5 Bb	2.6 x 10^5 Ba
Soil + Furfural	1..9 x 10^5 Cb	2..8 x 10^5 Cb	2.7 x 10^5 Cb	7.7 x 10^5 Ca
Soil + Inoculum	7.2 x 10^6 Aa	8 x 10^6 Aa	3.8 x 10^6 Ab	6.6 x 10^6 Ab
Soil + Inoculum + Water	8.2 x 10^4 Da	9.9 x 10^4 Da	6.1 x 10^4 Da	8.2 x 10^4 Ea
Soil + Inoculum + Furfural	1.4 x 10^5 Ca	9.8 x 10^4 Db	1.9 x 10^5 Ca	9.8 x 10^4 Db
Autoclaved soil + Inoculum + Furfural	3 x 10^3 Ea	6.6 x 10^2 Ea	6.6 x 10^2 Ea	0 Fb

* Values expressed as averages of three replicates for each dilution series.

For each week, within each column or row, means followed by the same upper-case or lower-case letter, respectively, do not differ significantly at $P \leq 0.05$.

Table 4.6: Total fungal colony counts in the soil after two and four weeks of exposure to furfural on the farm

	Total number of fungal colonies (cfu's/g soil) *	
	Time (in weeks)	
Treatment	**2**	**4**
Soil	8.1×10^4 Aa	4.2×10^4 Ca
Soil + Water	8.8×10^4 Aa	1.0×10^5 Ba
Soil + Inoculum	9.3×10^4 Aa	5.9×10^4 Ca
Soil + Furfural	6.6×10^2 Bb	1.8×10^5 Aa
Soil + Inoculum + Furfural	0 Bb	4.5×10^4 Ca

* Values expressed as averages of three replicates for each dilution series.

For each week, within each column or row, means followed by the same upper-case or lower-case letter, respectively, do not differ significantly at $P \leq 0.05$.

No significant difference was observed in the number of bacterial colonies in the untreated soil, both after two and four weeks (Table 4.7). Similar results were observed when the soil was inoculated with *G. citricarpa*. Two weeks after treatment with furfural the number of bacterial colonies decreased significantly even when the soil was previously inoculated with *G. citricarpa*. However, a significant increase in bacterial colonies was recorded after four weeks of treatment with furfural.

Table 4.7: Total bacterial colony counts in the soil after two and four weeks of exposure to furfural on the farm

	Total number of bacterial colonies (cfu's/g soil) *	
	Time (in weeks)	
Treatment	**2**	**4**
Soil	3.1×10^6 Ba	2.8×10^6 Ba
Soil + Water	6.3×10^6 Aa	1.1×10^6 Cb
Soil + Inoculum	2.7×10^6 Ba	2.1×10^6 Bb
Soil + Furfural	1.4×10^5 Db	1.0×10^8 Aa
Soil + Inoculum + Furfural	3.4×10^5 Cb	9.0×10^7 Aa

* Values expressed as average of three replicates for each dilution series.
For each week, within each column or row, means followed by the same upper-case or lower-case letter, respectively, do not differ significantly at $P \leq 0.05$.

4.2.3 DISCUSSION

Throughout the study, furfural proved to be effective in controlling the growth and development of *G. citricarpa*. The inhibitory effect of furfural was also reported by Liu *et al.* (2005) on several microorganisms and in a previous study by Bringmann and Kûhn (1980) which showed that concentrations as low as 0.17mM was toxic to *Pseudomonas putida*. According to Khan (1995), the mode of action of furfural on mycelial growth inhibition could be due to an interaction with the fungal cell wall, causing a degradation of the protein structure. Most filamentous fungi have a mechanic-sensitive ion channel (Shaw and Hoch, 2000) which could be influenced by furfural and consequently disturb further growth of the pathogen.

The noted difference in preventing pycnidiospores germination at 0.5%, 3.5%, 7% and 8% and appresoria development at 7 % and 8 % as compared to the control and 0.1% furfural concentration proved that furfural can be used successfully to control the pathogen. The application of furfural as a protective agent applied before spore germination may play an important role in preventing or

minimising the disease severity. Applying the product as a leaf litter application in orchards will also aid in controlling the disease incidences and ultimately reducing the inoculum levels over time.

The volatile properties of furfural and its potential in controlling *G. citricarpa* pathogen were also demonstrated. The furfural concentrations of 8% and 10% were fungicidal to the pathogen on litter. This was demonstrated when the leaf discs were transferred onto fresh medium after being exposed to furfural and no further mycelial growth was observed. No fungal growth was also observed when the naturally infected fresh leaves and oranges and lemons with lesions previously exposed to 10% furfural, were transferred onto a fresh medium. This implies the fungicidal effect of furfural at the concentration tested. However, the pathogen survived in fruit lesions and fresh leaves after being exposed to 8% furfural. In this regard, furfural was fungistatic, implying its effectiveness in slowing down fungal growth without completely eradicating it. The less effective activity of furfural at 8% on fruits and fresh leaf material may be attributed to the ineffective penetration of the volatile product into the plant tissue. The concentration of the volatile in actual contact with the pathogen could be lower than that required to be fungicidal. Similar observations have been reported with essential oils on the growth of *Penicillium digitatum* Sacc and *italicum* Wehmer (Plaza *et al*., 2004).

Furfural also proved to be effective *in vitro*, when applied to leaf litter with *G. citricarpa* lesions. Although at lower concentrations (2% and 4%) furfural did not fully prevent fungal growth, there was more than 50% disease control. However, the higher tested furfural concentrations of 6%, 8% and 10% successfully controlled *G. citricarpa* on leaf litter. This finding demonstrates the effectiveness of the product in preventing the inoculum build up or reducing the inoculum levels on the orchard floor, which will also have the same effects in the soil.

Given the fact that there is no proven method of managing *G. citricarpa* on fallen leaves/litter (Kotze, 1988), except its possible removal from the orchard floor to reduce the amount of ascospore inoculum (Paul, 2005), this finding may create an opportunity towards the reduction of the disease severity or breaking the life cycle of the pathogen.

Furfural effectiveness in controlling the pathogen on litter in the field was also demonstrated. The product proved effective at 8% in controlling *G. citricarpa*, whereby numbers of ascospores recorded showed a decline with the number of sprays. However, the presence of mixed spores on the leaf surface often resulted in rapid overgrowth by *C. gloeosporioides*, as was observed during this study. This often makes the counting of ascospores and isolation of CBS difficult, which explains the importance of a selective medium for CBS for successful isolation of the pathogen

from different materials. The alternate wetting of the litter may also have caused decomposition, which might have killed the ascospores. This is in line with the study conducted by Lee and Huang (1973), whereby large quantities of precipitation and successive rainy days decomposed the litter before the perithecia developed and matured, thus eradicated latent *G. citricarpa.*

The study further demonstrated the potential of furfural in controlling *G. citricarpa* in the soil. The effects however differed between the controlled and natural environments. When the inoculated soil was kept in the greenhouse for a week, *G. citricarpa* was still detected in the inoculated samples by PCR but not after two weeks. This shows that after a week, the pathogen could still be detected in the soil kept under greenhouse conditions.

Comparatively, the results from the samples kept on the farm (under natural conditions) demonstrated the effects of the product in controlling *G. citricarpa* in the soil over a four week period. After four weeks exposure to furfural, *G. citricarpa* was not detected by the PCR in the treated soil as compared to the positive PCR in the untreated soil. However, the viability of the pathogen was not assessed or determined during the current study. This finding is in line with a previous study regarding other pathogens' survival in the soil (Peck *et al*., 2001). The study revealed the survival of field peas' black spot pathogens, *Mycosphaerella pinodes* and *Phoma medicaginis* var *pinodella* in South Australian soils for several years.

Pycinidiospores that may be present in the soil are disseminated by means of water such as splashing raindrops or irrigation water (Whiteside, 1965). Eventhough this mode of dissemination is not likely to reach the tree canopy to infect the fruits and leaves, the pathogen may still be spread by soiled equipment, such as spades and shavels coming into contact with the canopy. In addition, the pathogen may also be spread from orchard to orchard in the soil through farm equipment and on workers' boots or feet. No study has ever been done on the survival of *G. citricarpa* in the soil under natural environmental conditions. This is the first report on this aspect.

The demonstrated potential of furfural in controlling *G. citricarpa* in the soil may provide an option that could be combined with other approaches towards a hurdle effect by breaking the life cycle of the pathogen and reducing the inoculum over time. Most control strategies have been aimed at protecting the fruit and leaves from infection (Kellerman & Kotze, 1977). However, pycnidiospores are not considered to be the main source of inoculum in CBS epidemics (Kotze, 1988), but their presence and build up in the soil may prevent an effective disease eradication

strategy. It is therefore crucial that in infected orchards, soils should be treated to reduce potential inoculum.

Furfural has previously been reported to control root-knot nematodes, *Meloidogyne spp*, as well as have antifungal properties in soil (Burelle, 2006). A study conducted by Walter and Rodriguez-Kabana (1992), revealed the nematicidal effect of furfural at low concentrations. This study also revealed the cost effectiveness and non-phytotocixity of the product. This indicates the potential of the product to control pests and diseases.

During this study, the non-target effect of furfural on other soil micro-flora was also assessed. Under greenhouse or controlled environments, furfural eradicated all fungi in the soil. In contrast the product only decreased the number of bacterial colonies without eradicating them. However, for the autoclaved soil, the bacterial colonies were eventually eradicated, because of the already low numbers.

Under natural field conditions, furfural decreased the number of fungi, without eradicating them. However the product eventually prompted the growth of yeasts in the soil. When the soil was inoculated with *G. citricarpa* (10^4 spores/ml), there was a decrease in the number of fungal colonies. This may be attributed to competition between the original and the introduced pathogen. Treatment with water has caused the fungi to flourish. On the other hand, furfural caused an increase in the numbers of bacterial colonies under natural conditions.

Overall, furfural does not have significant negative effect on microbial population dynamics in the soil micro-flora. Previous studies conducted by Rajendran *et al*. (2003) on the activities of furfural on soil flora and fauna have revealed that though the product controlled nematodes such as *Meloidogyne arenaria* and *Rotylenchulus reniformis*, there was no significant difference between free-living nematode populations in the treated and untreated soil. This implies that furfural does not have a harmful non- target effect on microbial ecosystems. This preliminary study provides some insight in the potential of a commercial product furfural and its potential use in the control of CBS. Yet, many questions still remain regarding the efficacy of the product over time, its fungicidal and fungistatic activity and the required concentration to sustain biofumigation activity.

4.2.4 CONCLUSIONS AND RECOMMENDATIONS

In conclusion, furfural presented potential as a fungicidal or fungistatic in controlling the citrus black spot pathogen *G. citricarpa*. The product can be applied through different modes of action such as contact or volatile. However further studies still need to be done to asess the certain aspects of furfural in relation to *G. citricarpa* to control the pathogen on citrus fruits and leaves

The amount of furfural in the soil need to be assessed over a period of time in order to determine the application rates when the product is used commercially for the control of *G. citricarpa* and other diseases. This is very crucial in determining the exact quantity of the product to be applied and the duration of its persistence in the soil. This in turn will assist in drafting application programmes (application intervals).

It has been thought about that the soil could be the source of inoculum for *G. citricarpa*. During this study, it was demonstrated that the pathogen can survive in the soil for a considerable period of time (four weeks) under natural conditions. However, the role of the soil in disease dissemination can not be conclusively stated, since the pathogen viability in the soil overtime remains unknown. It is therefore deemed necessary for further studies to determine the pathogenicity of *G. citricarpa* in the soil after a period of time, by re-isolating it from the soil.

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CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Citrus is a major foreign exchange generator for exporting countries like South Africa (SA). With globalisation and trade liberalisation among countries, it is important that international regulations and standards are enforced to control the movement of products in order to protect human, animal, plant and environmental health.

Citrus fruit and leaf pathogens such as *Guignardia citricarpa* Kiely are considered a major cause of phytosanitary risk (Kotze, 1981) for citrus exporting countries such as SA, Mozambique, Namibia and Swaziland. The pathogen causes Citrus Balck Spot (CBS) by attacking the fruits and causes blemishes.

Current management practice is mainly through the use of synthetic fungicides applied postharvestly. However, there is public concern over the potential negative impact of synthetic chemicals on the environment and on human health (Norman, 1988). This has led to the implementation of rigorous legislation regarding the amount of chemical residues acceptable on export produce in most European and American markets.

In order to manage the disease effectively, strategic plans of commercialisation is essential. However, this requires extensive knowledge regarding control strategies and the economic impact of the disease on growers and industry at large. The research presented in this thesis offers critical information regarding the potential impact of CBS on the future of the industry. Four countries with different economical and historical backgrounds were assessed in this study. Due to difficulties in accessing the information from growers, only six questionnaires were completed from SA, four from Namibia, nine from Mozambique (one commercial and eight subsistence farmers) and one from Swaziland. Generally there was poor response from the growers. However, some of the questionnaires were adequately completed which provided an overview of individual growers' status in terms of CBS.

Although inadequate management and a lack of infrastructures impede the control of CBS in developing countries, it is evident that most of the surveyed growers are aware of the importance of the disease. This is especially true for subsistence farmers in Mozambique, where CBS is not

managed. Citrus black spot is effectively managed in SA as compared to other surveyed countries in Southern African Development Community (SADC). In addition to chemicals and orchard sanitation, SA is the only surveyed country where growers make use of disease forecasting models to predict the time of spore release (critical time of infection). This time signals the beginning of the spraying program. The management of the disease in the other surveyed countries including Namibia, where CBS was never reported, mostly relies on the use of chemicals and general orchard sanitation. In Swaziland, growers mainly make use of chemicals to manage the disease. Mancozeb, benomyl, strobilurins, dithane, carbendazim, abamectin, derosal, copper hydroxide and oxychloride and mineral oil (as an additive) form part of the spraying programmes in the surveyed countries. Although the average annual rainfall measurements in the surveyed countries' regions fall in the same range (501-750mm), the low incidences (poorly reported) of CBS in Namibia, as compared to other countries, can be attributed to high evaporation rates (Anon, 2007).

Due to increasing public concern on the extensive use of chemicals, alternative control measures have been developed to manage pre and postharvest diseases (Janisiewicz & Korsten, 2002). In this study, the efficacy of furfural (a product from sugarcane bagasse) was assessed as a control agent of *G. citricarpa*. Furfural has been previously reported to have antifungal activities (Burelle, 2006), for being non-phytotoxicity (Walter & Rodriguez-Kabana, 1992) and is more environmentally appealing. The product demonstrated potential in controlling the pathogen through volatiles or direct contact with the pathogen on leaves, fruits and soil. It was further tested for its non-target effect on soil micro-flora, when used as a soil drench. The study also demonstrated that the soil microbial populations were not significantly affected under natural conditions, thus making it a potential alternative for the control of CBS. Attempts to continuously develop and test such a potential product are ongoing processes. Further experiments involving different application programs on trees and in soils need to be conducted in several locations in order to take the product into a commercial phase as a control agent for CBS. To date, there are no available references on the use of furfural to control CBS. However, this study has demonstrated that a formulation of 8% and 10% furfural can reduce CBS incidences with minimal non-target effect.

In conclusion, although only few questionnaires were returned, strategic planning processes should take into account the information eminating from this study. Surveys are time consuming and therefore face to face or direct interview provide the best alternative in studies like this. Furthermore, CBS is controlled to a certain extend in the region. However, there is further need for training and capacity building on the aspects of CBS to ensure the sustainability of the industry in the region. Chemicals are the major components of any plant disease control measures (Agrios,

1997). The accessibility and use of these should therefore be recommended for CBS for any citrus grower in integration with other management strategies such as sanitation and disease forecasting models. Even though furfural has demonstrated potential in controlling CBS with low non-target effect on soil micro-flora, the product is still an experimental component and may take many years before it is registered for commercial application.

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APPENDIX A

University of Pretoria
Universiteit van Pretoria

Interview Code / *Onderhoud Kode:* ……..

Interview Date / *Onderhoud Datum*: 2007/ …../……..

Research project: Impact assessment of Citrus Black Spot and the cost-benefit analysis of compliance to food safety systems/ standards

Navorsings Projek: *Beraming van die impak van Sitrus Swartvlek en kostevoordeel-analise vir die voldoening aan voedselveiligheidssisteme en -standaarde*

Period of Investigation: August 2005 to August 2006
Ondersoek period: *Augustus 2005 to August 2006*

Research Project Title: Impact assessment of Citrus Black Spot (CBS) in Southern Africa with emphasis on a) disease occurrence and prevalence and b) economic and social impact.
Titel van Navorsingsprojek: *Beraming van die impak van Sitrus Swartvlek (SSV) in Suidelike Afrika met die klem op a) siekte voorkoms en verspreiding b) ekonomiees en sosiale impak.*

Overall aim of the study: Survey of the occurrence of CBS in citrus areas of Southern Africa and quantification of economic and social impacts thereof.
Doel van die Navorsing: *Opname van die voorkoms van SSV in sitrusgebiede van Suidelike Afrika en bepaling van die mate van impak op ekonomiese en sosiale vlakke.*

Motivation of the study: The Department of Microbiology and Plant Pathology at the University of Pretoria is currently doing a project on the impact of CBS on trade and its effect on fruit exports from Southern Africa to the European Union. The citrus industry is of major social-economic importance in Southern Africa. However, production is faced with a major threat, the occurrence of CBS, which reduced yield and affect access to the international markets. The Project investigates all aspects of CBS, including its occurrence, spread, symptoms and control measures, and its socio-economic impact, to enable recommendations on how to improve the industry.
Motivering vir die Studie: *Die Departement Microbiology en Plantpatologie by die Universiteit van Pretoria doen tans navorsing oor die impak van SSV op die handel en die effek daarvan op die uitvoer van sitrus vrugte vanaf Suidelike Afrika na die Europese Unie. Die sitrus industrie is van groot sosio-ekonomiese belang in Suidelike Afrika. Sitrus produksie word egter bedreig deur die voorkoms van SSV, wat die opbrengs verminder en toegang tot internasionle markte belemmer. Hierdie projek ondersoek al die aspekte van SSV, insluitend die voorkoms daarvan, verspreiding, simptome en beheer maatreëls, en die sosio-ekonomiese implikasies, ten einde voorstelle ten opsigte van die verbetering van die industrie daar te stel.*

Project funding: National Research Foundation (NRF)
Projek befondsing: ***Nationale Naworsings Raad***

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SECTION A: CONTACT INFORMATION
AFDELING A: KONTAKINLIGTING

Name of farmer/ Naam van produsent***:*** ……

Tel. No / *Tel. No*:……Cell No / *Sel No*:……

Fax No / *Faks No*:……

Email address / *E-pos adres*:……

Postal address / *Posadres*:……

Region / *Streek*:……

Name of farm / *Naam van plaas*:……

PUC No:……

Name of packhouse (if on farm) / *Naam van pakhuis (indien op plaas)*:……

Name of packhouse manager and contact number/ *Naam van pakhuis-bestuurder en kontaknommers*:

……

Tel. No / *Tel No*:…… Cell No / *Sel No*:……

Name of packhouse (if not on farm) / *Naam van pakhuis (indien nie op plaas nie*):……

Name of packhouse manager and contact number / *Naam van pakhuis-bestuurder en kontaknommer*:

……

Tel No / *Tel No*:……...Cell No/ *Sel No*:……

SECTION B: BACKGROUND INFORMATION OF THE FARM
AFDELING B: AGTERGROND-INLIGTING VAN DIE PLAAS

1. Size of farm (ha) / *Grootte van plaas (ha)*:……
2. Kindly supply the information on the crops you grow on the farm, the area planted and the volume produced:
   Noem asb die gewasse wat op die plaas verbou word, die oppervlak daaronder geplant, en hoeveel jaarliks geproduseer word:

Citrus cultivars ***Tipe gewas***	**Area planted (ha)** ***Oppervlakte geplant (ha)***	**Annual tonnage** ***Jaarlikse opbrengs (ton)***	**Other crops** ***Ander gewasse***	**Area planted** ***Area aangeplant***
1				
2				
3				
4				
5				

3. Number of permanent workers / *Aantal permanente werkers*: ……
4. Number of part-time workers / *Aantal deeltydse werkers*:……

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SECTION C: CERTIFICATION INFORMATION
AFDELING C: SERTIFIKASIE-INLIGTING

1. Please complete the following table on the compliance with food safety systems

   Voltooi asb. die volgende tabel aangaande die voldoening aan voedselveiligheidsisteme:

FOOD SAFETY SYSTEMS ***VOEDSELVEILIGHEID-SISTEEM***	**SPECIFIC COST (in R)** ***SPESIFIEKE KOSTE (in R)***						**PART OF FARMING COSTS** ***DEEL VAN BOERDERY -KOSTE***
	Globalgap	**BRC**	**NATURES CHOICE**	**OTHER (SPECIFY)** ***ANDER (SPESIFISEER)***	**OTHER (SPECIFY)** ***ANDER (SPESIFISEER)***	**OTHER (SPECIFY)** ***ANDER (SPESIFISEER)***	
1. Traceability ***Opspoorbaarheid***							
Once off purchase of sign posts *Eenmalige aankoop van uithangborde*							
Continuous – Labels and Stickers *Deurlopend – Etikette en plakkers*							
Once off – Bar code system *Eenmalig – Strepieskode sisteem*							
2. Record Keeping ***Dokumentasie***							
Continues cost – Stationary *Deurlopende kostes - skryfbehoeftes*							
Once off – Purchase visitors book *Eenmalig – Aankoop van besoekersboek*							
Once off – Computer/s (hardware and software) upgrade every 5 years *Eenmalig – Rekenaar/s (apparatuur, programmatuur); opgradering elke 5 jaar*							

3. Internal Audit ***Interne Oudit***							
Initial training cost of staff / quality manager or consultant annual cost *Aanvanklike opleidingskoste vir personeel / kwaliteits-bestuurder of jaarliks vir raadgewer*							
4. Physical facilities ***Fisiese fasiliteite***							
Administration offices *Administratiewe kantore*							
Fertiliser storage *Kunsmis berging*							
Crop protection products storage facility *Bergingsfasiliteit vir gewasbeskermingsprodukte*							
Worker canteen *Kantien vir werkers*							
Toilets according to Act (1 per 8 workers) *Toilette volgens wet (1 per 8 werkers)*							
Pesticide filling station *Plaagdoder-vulstasie*							
5. Consultant Services ***Raadgewersdienste***							
Setup of traceability system *Opstel van opspoorbaarheid-sisteem*							
To establish record keeping system *Vestiging van dokumentasie-sisteem*							
6. Accredited Laboratories ***Geakkrediteerde Laboratoriums***							
Annual pesticide residue testing *Jaarlikse toets van plaagdoder-residue*							
Check maximum levels for heavy metals according to Codex Alimentarius							

Toetsing van maksimum swaarmetaal-residue in ooreenstemming met Codex Alimentarius							
Analyse of irrigation water (at least once a year) by a suitable laboratory *Analise van besproeiings-water (minstens 1x per jaar) deur geskikte laboratorium*							
Annual analysis of water for post harvest washing *Jaarlikse analise van water gebruik vir na-oes wasproses*							
7. Planting Material ***Plantmateriaal***							
Certified nursery *Erkende kwekery*							
If own nursery: upgrading to obtain certification *Indien u eie kwekery: opgradering om erkenning te verkry*							
8. Additional service providers ***Bykomstige Diensverskaffers***							
Calibration of scales *Kalibreer van weegskale*							
Rodent traps around buildings, pack stores, pesticide stores etc. *Lokvalle vir knaagdiere rondom geboue, bergplekke, store ens.*							
Maintain IPM system *Instandhouding van geïntegreerde plaagbeheer-sisteem*							
Computer programs *Rekenaarprogramme*							
9. Certification ***Sertifisering***							
Application costs *Toepassingskostes*							
Pre-assessment costs							

Voor-ramingskostes							
Certification costs *Sertifiseringskostes*							
Annual fees *Jaarlikse fooie*							
Date of certification *Datum gesertifiseer*							
Certificate number *Sertifikaatnommer*							
Certification body *Sertifiseringsliggaam*							

2. Additional certification / *Bykomstige sertifisering*

2.1 Do you plan any additional certification / *Beplan u enige addisionele sertifiserings?*

Yes / *Ja*	No / *Nee*

2.2 If the answer to the previous question is yes, please list the other certificationss/ *Indien ja, lys asb. die ander sertifiserings*

……...

……...

2.3. When do you plan to go for these certifications?

Wanneer beplan u om hierdie sertifisering te laat doen?

……..……...

……..……..

Screening questions / *Siftingsvrae*:

Mark the applicable block with a cross / *Merk die toepaslike blokkie met 'n kruis:*

1. Are you the/ *Is u die*:

Owner *Eienaar*	Farm Manager *Plaas bestuurder*	Other (Please specify) *Ander (Spesifiseer asb)*	

2. Are you familiar with Citrus Black Spot? / *Is u bekend met Sitrus Swartvlek?*

Yes *Ja*	No *Nee*	

3. Are you familiar with Citrus Black Spot symptoms? / *Is u bekend met die simptome van Sitrus Swartvlek?*

Yes *Ja*	No *Nee*	

4. Do you currently have Citrus Black Spot on your farm? / *Is daar huidiglik Sitrus Swartvlek op u plaas?*

Yes *Ja*	No *Nee*	

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SECTION D: CLIMATIC CONDITIONS
AFDELING D: KLIMAATSTOESTANDE

1. Do you have access to data from a weather station? Yes/*Ja* | No/*Nee*
   Het u toegang tot data vanaf 'n weerstasie?

2. If yes, what is the name and telephone number of the station? / *Indien wel, wat is die naam en telefoonnommer van die weerstasie?*
Name / *Naam:* ……Tel No / *Tel No:*…….....

3. What is the distance between the weather station and the farm?

Wat is die afstand tussen die weerstasie en die plaas?……..……...

4. What is the lowest and highest average summer day-and-night temperature? [MARK with 'H' for Highest and 'L' for Lowest]
   Wat is die laagste en hoogste gemiddelde somer dag-nag temperatuur [MERK met 'n 'H' vir Hoogste en 'L' vir Laagste]

   Summer (day) / *Somer (dag)*:

15 - 20ºC	21 - 25ºC	26 - 30ºC	> 30ºC	

   Summer (night) / *Somer (nag)*:

<10ºC	10 - 15ºC	16 - 20ºC	21 - 25ºC	

5. What is the average winter day-and-night temperature? [MARK with an X] / *Wat is die gemiddelde dag- en nagtemperature vir die Winter? [MERK met 'n X]*

   Winter (day) / *Winter (dag)*:

<10ºC	10 - 15ºC	16 - 20ºC	21 - 25ºC	> 25ºC	

   Winter (night) / *Winter (nag)*:

<10ºC	10 - 15ºC	16 - 20ºC	> 20ºC	

6. What is the average rainfall per year? [MARK with an X] / *Wat is die gemiddelde reënval per jaar?[MERK met 'n X]*

< 250 mm	250-500 mm	501-750mm	751-1000mm	>1001mm	

7. What is the approximate altitude of your farm in meters above sea level? [MARK with an X] / *Wat is die geraamde hoogte bo seespieël (in meter) van u plaas?[MERK met 'n X]*

0-300	301-600	601-900	901-1200	1201-1500	1501-1800	>1800	

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SECTION E: CITRUS BLACK SPOT BACKGROUND
AFDELING E: SITRUS SWARTVLEK AGTERGROND

1. When did you first encounter Citrus Black Spot on your farm and how severe was it? / *Wanneer het u vir die eerste keer Sitrus Swartvlek op u plaas opgemerk en hoe ernstig was dit?*

……...
……...
……

2. Please complete the following table, in terms of the incidence and severity of Citrus Black Spot on your farm

(If possible, please include all the information from the first incidence on your farm up to the last production season)

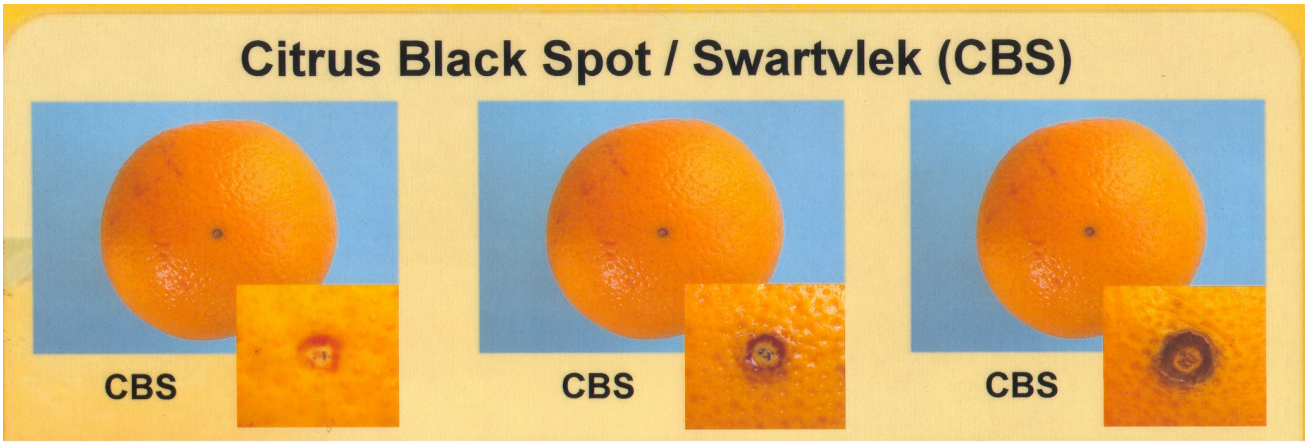
Voltooi asb die volgende tabel in terme van insidensie en felheid van Sirtus Swartvlek op u plaas

(Sluit asb al die inligting in vanaf die eerste insident tot en met die laaste produksie seisoen, indien moontlik)

Production season ***Produksie seisoen***	**Citrus cultivar** ***Sitrus Kultivar***	**Preharvest yield loss (in cartons)** ***Voor-oes verlies (in kartonne)***	**Percentage preharvest loss due to CBS** ***Persentasie voor-oes verlies a.g.v. SSV***	**Postharvest yield loss (in cartons)** ***Na oes verlies (in kartonne)***	**Percentage postharvest loss due to CBS** ***Persentasie na-oes verlies a.g.v. SSV***	**Average price received per carton (R)** ***Gemiddelde prys ontvang per ton (R)***

3. Can you describe the symptoms that you normally see on the fruit in detail (**with the aid of the pictures below**)? / *Beskryf die simptome wat u normaalweg op die vrugte sien breedvoerig (**gebruik die hulp van fotos**)*

……
……
……



4. How do you manage Citrus Black Spot, briefly describe your management approach / *Hoe bestuur u Sitrus Swartvlek beskryf kortliks u bestuursprogram?*

……
……
……

5. Are you using a consultant to predict spore release? / *Gebruik u 'n konsultant om spoor vrystelling te voorspel?*

Yes *Ja*	No *Nee*

6. If yes, who is the consultant (name, surname and address) / *Indien wel, wie is die konsultant (naam, van, adres):*

Name/*Naam*:……
Address/*Adres*:……
Tel:……..Cell/*Sel*:……

7. Do you spray fungicides according to the prediction modeling? / *Spuit u swamdoders volgens die vooruitskattingsmodel?*

Yes *Ja*	Sometimes *Soms*	No *Nee*

8. If yes or sometimes, how effective is it in better managing Citrus Black Spot?
Indien 'Ja' of 'Soms', hoe effektief verbeter dit die bestuur van Sitrus Swartvlek?

……
……

9. Which fungicides do you use to control Citrus Black Spot and when do you spray them?
Watter swamdoders gebruik u om Sitrus Swartvlek te beheer en wat is die spuitprogram?

Fungicides *Swamdoders*	***Fungicides application dates* / Swamdoder toedieningsdatums**			
	First application date *Eerste toedieningsdatum*	Second application date *Tweede toedieningsdatum*	Third application date *Derde Toedieningsdatum*	Other application dates *Ander toedieningsdatums*
Azoxystrobin				
Benomyl				
Carbendazim				
Copper hydroxide *Koperhidroksied*				
Copper oxychloride *Koperoksichloried*				
Fosetyl-Al				
Mancozeb				
Pyraclostrobin				
Trifloxytrobin				
Zinc oxide *Sinkoksied*				
Zineb				
Others (specify) *Ander (spesifiseer)*				

10. Which orchard sanitations are in place for effective control of CBS in your farm?

Watter sanitasiemiddels of –prosesse is tans aangewend vir effektiewe beheer van SSV op u plaas?

……
……
……

11. Have the graders in your packhouse been trained to identify CBS?

Is die gradeerders in u pakhuis opgelei om SSV te identifiseer?

Yes *Ja*	No *Nee*

12. Do you monitor packing lines and packed cartons to ensure that CBS has been effectively eliminated?

Monitor u die paklyne en verpakte kartonne om te verseker dat SSV effektief uitgeskakel is ?

Yes *Ja*	No *Nee*

13. How did Citrus Black Spot affect the total citrus yield in the past four years (loss in the orchard and packhouse)? ***Hoe het Sitrus Swartvlek die totale sitrus opbrengs orr die laaste vier jaar beinvloed (verlies in boord en pakhuis)?***

(a) When not sprayed / *Nie gespuit nie:*

Year ***Jaar***	**Loss in yield (in cartons) / *Verlies in opbrengs (ton)***	
	Expected number of cartons *Verwagte aantal kartonne*	Actual number of cartons packed *Werklike aantal kartonne verpak*
2002		
2003		
2004		
2005		

(b) When sprayed / *Wanneer bome gespuit:*

Year ***Jaar***	**Loss in yield (in cartons) /** ***Verlies in opbrengs (ton)***	
	Expected number of cartons *Verwagte aantal kartonne*	Actual number of cartons packed *Werklike aantal kartonne verpak*
2002		
2003		
2004		
2005		

SECTION F: COST IMPLICATIONS OF CITRUS BLACK SPOT
AFDELING F: KOSTE-IMPLIKASIES VAN SITRUS SWARTVLEK

1. What was the approximate expenditure incurred in managing CBS for the last four production season? / *Wat was u beraamde kostes vir die bestuur van Sitrus Swartvlek oor die laaste vier produksieseisoene?*

Chemical treatment / *Chemikalie-behandeling*	**Expenditure per season (R)** *Uitgawes per seisoen (R)*				**Treatment scope / Hectares treated** *Omvang van Behandeling / Hektaar behandel*			
	2002	**2003**	**2004**	**2005**	**2002**	**2003**	**2004**	**2005**
1.Azoxystrobin								
2.Benomyl								
3. Carbendazim								
4. Copper hydroxide / *Koperhidroksied*								
5. Copper oxychloride / *Koperoksichloried*								
6. Fosetyl-Al								
7.Mancozeb								
8.Pyraclostrobin								
9.Trifloxytrobin								
10.Zinc oxide / *Sink oksied*								
11.Zineb								
12. Others / *Ander*								
13. Tractor (cost, fuel, service, etc.) *Trekker (kostes, brandstof, dienste, ens.)*								
14.Labour – Permanent: Seasonal: *Werkers – Permanent: Seisoenaal:*								
15. Consultation / *Konsultasies*								
16. Training of employees *Opleiding van werkers*								
17. Packhouse maintenance *Instandhouding van pakhuis*								
18. Food safety compliance (specifically for CBS) *Voldoening aan voedselveiligheid-standaarde (spesifiek vir SSV)*								
19.Laboratory tests (specifically for CBS) *Laboratoriumtoetse (spesifiek vir SSV)*								
TOTAL /TOTAAL								

2. What cost effects does CBS have in the exporting of Citrus to others countries?
Watter koste-implikasies het SSV in die uitvoer van sitrus na ander lande?

……..
……..
……..

3. What are the common defects in the packhouse, which lead to rejection for export?
Watter algemene defekte in die pakhuis aangetref, lei tot afkeuring vir uitvoer?

……..
……..
……..

4. Please complete the following table with regard to effects of CBS on export and the local markets:
Voltooi asb. die volgende tabel rakende die effek van SSV op uitvoer en die plaaslike markte:

Production year ***Produksie-jaar***	**Total volume packed for:** ***Totale volume gepak vir:***				**Total volume rejected for export** ***Totale volume afgekeur vir uitvoer***	**Total income received (R)** ***Totale inkomste ontvang (R)***	**Total expenditure (R)** ***Totale uitgawes (R)***
	Export ***Uitvoer***	**CBS sensitive market** ***SSV sensitiewe mark***	**CBS non-sensitive market** ***SSV nie-sensitiewe mark***	**Local market** ***Plaaslike mark***			
2001							
2002							
2003							
2004							
2005							

Thank you very much for your time and assistance!

Baie dankie vir u tyd en samewerking!

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APPENDIX B

Código Da Entrevista: .....

Data Da Entrevista: 2006/...../.....

University of Pretoria

Universiteit van Pretoria

Projeto De Pesquisa: Avaliação do impacto do mancha preto do citrino

Período da investigação: Agosto ate Maio 2006

Título De Projeto Da Pesquisa: Impacte a avaliação do mancha preto do citrino em África do sul com ênfase

a) na ocorrência da doença e o prevalencia e

b) impactos econômicos e sociais

Objectivo total do estudo: Avaliacao da ocorrência do "Mancha Preto do Citrino" em áreas do citrino de África do sul e quantificação de impactos econômicos e sociais disso.

Motivacao para o estudo: O Departamento de Microbiologia e do Patologia de planta na universidade de Pretoria está fazendo atualmente um projeto no impacto do ponto preto do citrino no comércio e seu efeito em exportações da fruta de África do sul à união européia. A indústria do citrino é de importância socio-economic principal em África do sul. Entretanto, a produção é enfrentada com uma ameaça principal, a ocorrência do ponto preto, que reduz o rendimento e afeta o acesso ao mercado internacional. O projeto investiga todos os aspectos do ponto preto do citrino, incluindo sua ocorrência, propagação, sintomas e medidas de controle e suas implicações socio-economic, permitir recomendações em como melhorar a indústria.

Projeto e financiado: Pela fundação nacional da pesquisa

Equipe de pesquisa: Prof Lise Korsten, Departamento de Microbiologia e Pathologia De Planta, Universidade de Pretoria

Prof Johan Kirsten, Departamento de Economia Agricultur e Desenvolvimento Rural, Universidade de Pretoria

Ms Lorna Halueendo, Departamento de Microbiologia e Pathologia De Planta, Universidade de Pretoria

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Fax. 0027-12-420 4588

Estudo conduzido por: Ms Lorna Halueendo
Celular. 0824317556

Cláusula de confidencia: Toda a informação será mantida estritamente confidencial e não feita público. Nenhuma publicação ou relatório que emanar deste trabalho refletirã o nome de companhia

SECIONE A: CONTATE A INFORMAÇÃO

Nome do fazendeiro:.....

Do Tel. :..... Celular:.....

Fax. no:.....

Email:.....

.

Endereço postal:.....

Região:.....

Nome da fazenda:.....

PUC:.....

Nome de empacotador (se estiver) na fazenda:.....

Nome do gerente do sector de empacotameto nommer de kontacto:

Nome:.....

Telefone:.....Celular:.....

Nome de empactador (se não for) na fazenda:.....

Nome do gerente do sector de empacotameto nommer de kontacto:

Nome:.....

Nenhum do telefone:.....Celular.....

SEÇÃO B: INFORMAÇÃO DE FUNDO DA FAZENDA

1. Tamanho de fazenda (ha):..... .....

2. Fornece amavelmente a informação sobre culturas que você produz na fazenda, área plantada e volume da producao obtida

Tipo de cultura	A área plantada (ha)	Producao anual (ton)

3. Número dos trabalhadores permanentes:.....

4. Número de trabalhadores contratados:.....

SEÇÃO C: INFORMAÇÃO DA CERTIFICAÇÃO

A fazenda esta certificado pela **Globalgap**? Sim/ Não

Data da primeira certificação: .....

Certificado da **Globalgap** No: .....

Organismo da certificação: .....

Empacotamento e certificado pela **HACCP** ? Sim / Não

Data da primeira certificação :.....

No. do certificado de **HACCP**:.....

Organismo da certificação:.....

Empacotamento e certificado pela **BRC**? Sim / Não

Data da primeira certificação:.....

No do certificado de **BRC**:.....

Organismo da certificação:.....

Émpacotamento **escolha natural** e certificada? Sim / Não

Data da primeira certificação :.....

No. do certificado da escolha naturezas:.....

Organismo da certificação :……

Outras certificações, p.ex é Orgânico, justo, fazenda a bifurcar-se etc.:.....

Data da primeira certificação :.....

Organismo da certificação:.....

Algumas outras certificações, p.ex é comércio orgânico, justo, fazenda a bifurcar-se etc. :.....

Data da primeira certificação :.....

Corpo da certificação :.....

Algumas outras certificações, isto é comércio orgânico, justo, fazenda a bifurcar-se etc. :.....

Data da primeira certificação :.....

Organismos da certificação :.....

Perguntas da seleção

Marque o bloco aplicável com uma cruz

1. Você é o:

Dono	Gerente	Outro (especifique por favor)

2. Estas familiarizado com a mancha preta do citrino?

Sim	Não

3. Familiar os sintomas do mancha preto do citrino?

Sim	Maisamemos	Não

4. Existe atualmente o mancha preto do citrino em sua fazenda?

Sim	Não

SEÇÃO D: CONDIÇÕES DE CIMATIC

1. Você tem acesso aos dados d tempo de uma estação de meteorologica?

Sim	Não

2. Se sim, qual é o nome e o número de telefone da estação?

Nome:.....

No.do telefone:.....

3. Qual é a uma distância entre a estação de tempo e a fazenda?

.....

.....

.....

4. Qual é a temperatura média mais baixa e mais alta da dia-e-noite do verão? [MARCA A RESPOSTA COM ` H ' para o mais alta e ` L ' para o mais baixo]

Verão (dia):

15 - 20ºC	21 - 25ºC	26 - 30ºC	> 30ºC	V1	06

Verão (noite):

<10ºC	10 - 15ºC	16 - 20ºC	21 - 25ºC	V2	07

5. Qual é a temperatura média da dia-e-noite do inverno? [MARCA RESPOSTA COM X]

Inverno (dia):

<10ºC	10 - 15ºC	16 - 20ºC	21 - 25ºC	> 25ºC	V3	08

Inverno (noite):

<10ºC	10 - 15ºC	16 - 20ºC	> 20ºC	V4	09

6. Qual é a precipitacao media anual? [MARCA RESPOSTA COM X]

< 250 mm	250-500 mm	501-750mm	751-1000mm	>1001mm	V5	10

7. Qual é a altitude aproximada de sua fazenda em metros acima do nível do mar?
[MARCA RESPOSTA COM X]

0-300	301-600	601-900	901-1200	1201-1500	1501-1800	>1800	V6	11

SEÇÃO E: CULTIVARS DO CITRINO

1. A tabela abaixo inclui cultivars do citrino, número das árvores e anos / Periodo de crescimento

1. POMELOS. Indique por favor o cultivar do pomelo /toranja produzido na fazenda. [MARCA CÓDIGO COM X NA COLUNA 1]Approximadamente quantas árvores de cada cultivar tem na fazenda? [MARCA CÓDIGO COM X Na COLUNA 2]. Em que ano foram plantados? [MARCA CÓDIGO COM X Na COLUNA 3]. Estas árvores foram compradas em viveiro certificado? [MARCA CÓDIGO COM X Na COLUNA 4]

COLUNA 1		COLUNA 2					COLUNA 3					COLUNA 4	
Cultivar		Número das árvores					O ano de plantio					Produtor certificado	
1.Pomelo		<10 000	10 001-100 000	100 001 – 250 000	250 001 – 500 000	>500 000	Antes de 1964	1965 – 1974	1975 – 1984	1985 – 1994	1995-2004	Sim	Não
													2
Marsh	V7	1	2	3	4	5	1	2	3	4	5	1	2
Marsh/Nartia	V8	1	2	3	4	5	1	2	3	4	5	1	2
Redblush	V9	1	2	3	4	5	1	2	3	4	5	1	2
Star Ruby	V10	1	2	3	4	5	1	2	3	4	5	1	2
Nelruby	V11	1	2	3	4	5	1	2	3	4	5	1	2
Ray Ruby	V12	1	2	3	4	5	1	2	3	4	5	1	2
Rio Red	V13	1	2	3	4	5	1	2	3	4	5	1	2
Java	V14	1	2	3	4	5	1	2	3	4	5	1	2
Pomelit (X202)	V15	1	2	3	4	5	1	2	3	4	5	1	2

2. VALENCIAS. Indique por favor o cultivar do Valença produzido na fazenda. [MARCA CÓDIGO COM X Na COLUNA 1]. Quantas árvores de cada cultivar são cultivads aproximadamente na fazenda? [MARCA CÓDIGO COM X Na COLUNA 2s]. Em que ano estas árvores foram plantadas? [MARCA CÓDIGO RESPOSTA COM X Na COLUNA 3]. Estas árvores foram compradas de um produtor certificado? [MARCA O CÓDIGO COM X Na COLUNA 4].

COLUNA 1		COLUNA 2					COLUNA 3					COLUNA 4	
Cultivar		Número das árvores					O ano de plantio					Produtor certificado	
		<10 000	10 001-100 000	100 001 – 250 000	250 001 – 500 000	>500 000	Antes de 1964	1965 – 1974	1975 – 1984	1985 – 1994	1995-2004	Sim	Não
Amanzi	V16	1	2	3	4	5	1	2	3	4	5	1	2
Delta	V17	1	2	3	4	5	1	2	3	4	5	1	2
Du Roi	V18	1	2	3	4	5	1	2	3	4	5	1	2
Excelsior	V19	1	2	3	4	5	1	2	3	4	5	1	2
Margaret	V20	1	2	3	4	5	1	2	3	4	5	1	2
McLean	V21	1	2	3	4	5	1	2	3	4	5	1	2
Midnight	V22	1	2	3	4	5	1	2	3	4	5	1	2
Olinda	V23	1	2	3	4	5	1	2	3	4	5	1	2
Valencia Late	V24	1	2	3	4	5	1	2	3	4	5	1	2
Valentine	V25	1	2	3	4	5	1	2	3	4	5	1	2

3. CITRINO MACIO. Indique por favor o cultivar do citrino macio produzido na fazenda. [MARCA CÓDIGO COM X Na COLUNA 1]. Quantas árvores de cada cultivar existe aproximadamente na fazenda? [MARCA CÓDIGO COM X Na COLUNA 2]. Em que ano estas árvores foram plantadas? [MARCA CÓDIGO COM X Na COLUNA 3]. Estas árvores foram compradas de um productor certificado? [MARCA CÓDIGO COM X Na COLUNA 4].

COLUNA 1		COLUNA 2					COLUNA 3					COLUNA 4	
Cultivar		Número das árvores					O ano de plantio					Produtor certificado	
		<10 000	10 001-100 000	100 001 – 250 000	250 001 – 500 000	>500 000	Antes de 1964	1965 – 1974	1975 – 1984	1985 – 1994	1995-2004	Sim	Não
Clem Late	V26	1	2	3	4	5	1	2	3	4	5	1	2
Ellendale	V27	1	2	3	4	5	1	2	3	4	5	1	2
Fairchild	V28	1	2	3	4	5	1	2	3	4	5	1	2
Imamura	V29	1	2	3	4	5	1	2	3	4	5	1	2
Kuno	V30	1	2	3	4	5	1	2	3	4	5	1	2
Miho Wase	V31	1	2	3	4	5	1	2	3	4	5	1	2
Minneola	V32	1	2	3	4	5	1	2	3	4	5	1	2
Nouvelle	V33	1	2	3	4	5	1	2	3	4	5	1	2
Nova	V34	1	2	3	4	5	1	2	3	4	5	1	2
Nules	V35	1	2	3	4	5	1	2	3	4	5	1	2
Oroval	V36	1	2	3	4	5	1	2	3	4	5	1	2
Owari	V37	1	2	3	4	5	1	2	3	4	5	1	2
Robin	V38	1	2	3	4	5	1	2	3	4	5	1	2
SRA 63	V39	1	2	3	4	5	1	2	3	4	5	1	2
SRA 70	V40	1	2	3	4	5	1	2	3	4	5	1	2
SRA’s (84-92)	V41	1	2	3	4	5	1	2	3	4	5	1	2
Thoro Temple	V42	1	2	3	4	5	1	2	3	4	5	1	2
Nardocott	V43	1	2	3	4	5	1	2	3	4	5	1	2
Mor	V44	1	2	3	4	5	1	2	3	4	5	1	2
Orr	V45	1	2	3	4	5	1	2	3	4	5	1	2

4. LIMÕES. Indique por favor o cultivar de limões produzido na fazenda. [MARCA CÓDIGO COM X Na COLUNA 1]. Quantas árvores de cada cultivar tem aproximadamente na fazenda? [MARCA CÓDIGO COM X Na COLUNA 2]. Em que ano estas árvores foram plantadas? [MARCA CÓDIGO COM X Na COLUNA 3]. Estas árvores foram compradas de um produtor certificado? [MARCA CÓDIGO COM X Na COLUNA 4].

COLUNA 1		COLUNA 2					COLUNA 3					COLUNA 4	
Cultivar		Número das árvores					O ano de plantio					Produtor certificado	
		<10 000	10 001-100 000	100 001 – 250 000	250 001 – 500 000	>500 000	Antes de 1964	1965 – 1974	1975 – 1984	1985 – 1994	1995-2004	Sim	Não
Lemons													
Eureka	V46	1	2	3	4	5	1	2	3	4	5	1	2
Fino	V47	1	2	3	4	5	1	2	3	4	5	1	2
Lisbon	V48	1	2	3	4	5	1	2	3	4	5	1	2
Verna	V49												

5. OUTROS CITRINOS. Há algum outro cultivars que é produzido e que não foi mencionado? Se assim, indique por favor o cultivar do outro citrino na fazenda [ESCREVA A RESPOSTA SOB o ` OUTROS']. Approximadamente quantas árvores deste cultivar tem na fazenda? [MARCA CÓDIGO DA RESPOSTA COM X NA COLUNA 2] em que ano estas árvores foram plantadas? [MARCA CÓDIGO Da RESPOSTA COM X Na COLUNA 3]. Estas árvores foram compradas de um produtor certificado? [CÓDIGO Da MARCA CÓDIGO Da RESPOSTA COM X Na COLUNA 4].

COLUNA 1		COLUNA 2					COLUNA 3					COLUNA 4	
Cultivar		Número das árvores					O ano de plantio					Produtor certificado	
		<10 000	10 001-100 000	100 001 – 250 000	250 001 – 500 000	>500 000	Antes de 1964	1965 – 1974	1975 – 1984	1985 – 1994	1995-2004	Sim	Não
Outro													
	V50	1	2	3	4	5	1	2	3	4	5	1	2
	V51	1	2	3	4	5	1	2	3	4	5	1	2
	V52	1	2	3	4	5	1	2	3	4	5	1	2
	V52	1	2	3	4	5	1	2	3	4	5	1	2

SEÇÃO F: INFORMACAO SOBRE MANCHA PRETA DO CITRINO

1. Quando é que você encontrou primeiramente o mancha preto do citrino em sua fazenda e qual era a severidade?

……
……
……
……

2. Completo por favor a seguinte tabela, em termos da incidência e da severidade domancha preto do citrino em sua fazenda **(se possível, inclua por favor toda a informação desde a primeira incidência em sua fazenda até a última estação da produção)**

Estação da produção	**Cultivar do citrino**	**Perda amtes da colheita do rendimento (tonelada)**	**Perda postcolheita do rendimento (tonelada)**	**Rendimento total (tonelada) excluindo perdas do CBS**	**Preço médio recebido por tonelada (R)**

3. Pode descrever os sintomas que você vê normalmente na fruta em detalhes ? (**com ajuda de images**)

……
……
……
……..

4. Como você controla amancha preto do citrino?

……
……
……
……

5. Você está usando um consultor para predizer a libertação de esporos?

Sim	Não

6. Se sim, que é o consultor (nome, apelido e endereço)

Nome:……

Endereço:……..

Tel:……..Cell:……..

7. Você pulveriza fungicides de acordo com modela / recomendacao prevista ?

Sim	Às vezes	Não

8. Se sim ou às vezes, qual é a eficacia deste no controlo da mancha preta do citrino?

……
……
……
……
……

9. Que fungicidos você usa pona controlar o mancha preto do citrino e como é o programa de pulverizacao?

Fungicidos	**Datas da aplicação dos fungicides**			
	Primeira data da aplicação	Segunda data da aplicação	Terceira data da aplicação	Outras datas da aplicação

10. Como o ponto preto do citrino afetou o rendimento total do citrino nos quatro anos passados (perda no pomar e no empacotamento)?

(a) Quando não pulverizado

Ano	**Perda no rendimento (em toneladas)**	
	Perda nos pomares	**Perda no empacotamento e ao longo da corrente de fornecimento**
2002		
2003		
2004		
2005		

(b) Quando pulverizado

Ano	**Perda no rendimento (em toneladas)**	
	Perda nos pomares	**Perda no empacotamento e ao longo da corrente de fornecimento**
2002		
2003		
2004		
2005		

11. Qual roi a despesa aproximada contraida no controlo do "Mancha Preta do Citrino" para as últimas quatro campanhas da produção?

Produto químico/Tratamento	Despesa por a estação / Campanha (Mt)				Nivel do tratamento/hectares do citrino tratados			
	2002	2003	2004	2005	2002	2003	2004	2005
1.Azoxystrobin								
2.Benomyl								
3. Carbendazim								
4. Copper hydroxide								
5. Copper oxychloride								
6. Fosetyl-Al								
7.Mancozeb								
8.Pyraclostrobin								
9.Trifloxytrobin								
10.Zinc oxide								
11.Zineb								
Trator								
Implemento de pulverizacao								
Mao- de- obre								
Consultores								
TOTAL								

MUITO MUITO OBRIGADO PELO SEU TEMPO E AUXÍLIO