Chapter 3 — A review of Citrus Black Spot

3.1 Abstract

Citrus Black Spot (CBS) is an important fruit disease of Citrus species in many parts of the world. It was first recorded in Australia and today it is prevalent in many of the major citrus producing countries, including Brazil, China and India. The pathogen causes mainly fruit spots that render fruit unacceptable to the export market and in humid areas fruit loss is a major concern. Of more importance is the potential phytosanitary risk and resultant barrier to trade associated with the disease. Recent renewed interest in CBS has been brought about by the need to provide scientific justification for the phytosanitary barriers to trade, since countries may not pose unnecessary or disguised restrictions on international trade unless based on scientific justifiable principles. Within this chapter the current literature regarding the pathogen, disease, etiology and epidemiology are reviewed.

3.2 Origin, history and distribution

The origin of Citrus Black Spot (CBS) is not certain, but it is speculated that CBS originated in Asia which is also the centre of origin of the citrus host (Reuther et al., 1967). In English literature this disease was first described by Benson (1895) and subsequently recorded by Cobb (1897) from diseased fruit in Australia. The first record of CBS in South Africa (SA) was in 1929 (Doidge, 1929). The disease is widespread and has been recorded in Argentina (Garran, 1996), Bhutan (European Union, 1998), Brazil (European Union, 2000a), China (European Union, 1998), Ghana (Timmer, 2005, Personal Communication), India (Brodrick, 1969), Indonesia, Kenya, Mozambique (European Union, 1998), Nigeria (Baayen et al., 2002), Philippines, Swaziland, Taiwan, Uruguay (Kotzé, 2000) West Indies (Calavan, 1960), Zambia and Zimbabwe (European Union, 1998).

In some countries where CBS occurs, certain production areas are known to be free of the disease. In South Africa, these disease-free areas include all the citrus production regions within the Western Cape (European Union, 1998; Mabiletsa, 2003) and the Northern Cape (le Roux, 2004, Personal Communication; Mabiletsa, 2003; USDA/APHIS, 2002). Citrus Black Spot has not been reported in some of the citrus producing areas of Australia (Barkley, 2003, Personal Communication; European Union, 1998; Kiely, 1970), Brazil (European Union, 2000a) and China (European Union, 1998).

CBS has also not been recorded in any part of the Mediterranean or Europe (European Union, 1998; Baayen et al., 2002; Kotzé, 2000) and is absent from Chile and the citrus growing areas of the United States of America (USA)(Figure 3.1)(Baayen et al., 2002; Cook, 1975; European Union, 2000b; Kotzé, 1981). In some countries, such as Japan and New Zealand, the status of CBS is uncertain and reports on the occurrence of the disease in these countries are conflicting (CABI/EPPO, 1998; Sutton & Waterson, 1966).

The profile of global CBS distribution appears to reflect the historic introduction and distribution of citrus with the exception that it is restricted to areas where climate favours the occurrence of the pathogen. The disease is known to have spread to areas where the climate is conducive for the persistence of the species (Wager, 1952). The risk of CBS spreading to areas where it does not currently occur appears to depend on climate.

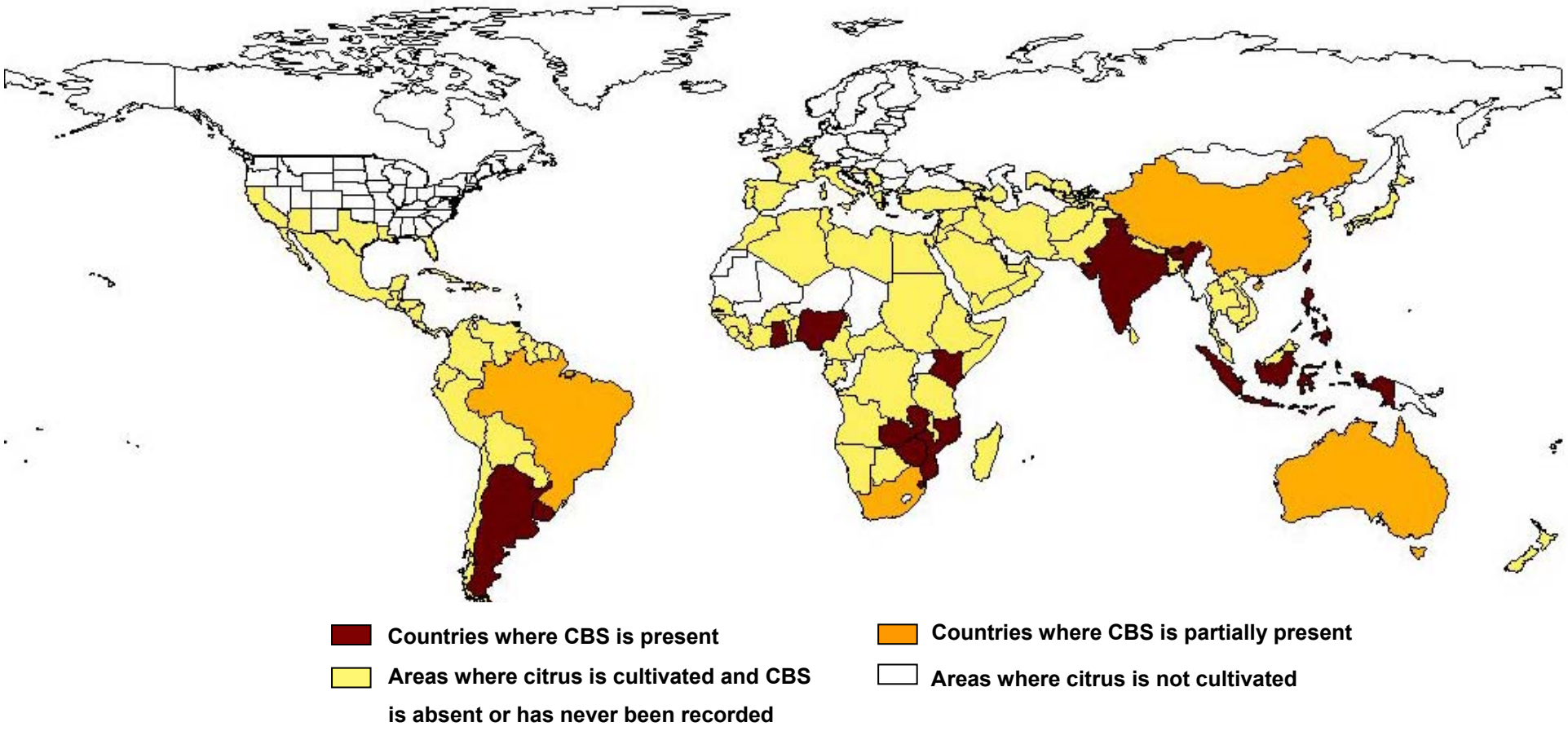

Figure 3.1 — The global distribution of Citrus Black Spot (CBS) as officially recorded.

3.3 Symptoms

The CBS pathogen mainly causes symptoms on fruit and to a lesser extent on leaves. It is thought that the pathogen may also cause some damage to twigs, but this has not been unequivocally confirmed (Calavan, 1960; Sutton & Waterson, 1966).

3.3.1 Leaf symptoms

Symptoms occur more frequently on the leaves of lemon trees than on those of oranges. Leaf infection within a tree varies considerably, and the number of lesions per leaf may be a few or numerous (Wager, 1952). On immature leaves symptoms are not prevalent (Kiely, 1949). Symptoms first start to appear three to ten months after initial infection (Wager, 1952). Small pin-point sunken lesions are visible on both sides of the leaf (Kiely, 1948b; Wager, 1952). These lesions are perfectly round, have a grey or light brown centre, a black to reddish circumference and are surrounded by a yellow halo. Sometimes pycnidia can be seen in the centre of the lesion on the upper side of the leaf (Wager, 1952). Further colonisation of the leaf only happens after leaf drop. The fungus eventually produces perithecia and pycnidia over the surface of the dried leaf within the leaf litter (Kotzé, 1996).

3.3.2 Fruit symptoms

Disease symptoms are most noticeable on mature fruit (Kiely, 1969), although symptoms may appear on immature fruit (Whiteside, 1965), especially lemons (Wager, 1952). Symptoms are confined to the surface of the fruit (Wager, 1952). Lesions may appear as a single spot or up to a thousand spots per fruit (Calavan, 1960). Even though the rind of infected fruit may become severely necrotic, the disease rarely causes post-harvest decay (Kotzé, 1981), but may cause premature fruit drop (Wager, 1952). Three kinds of symptoms are widely recognised: hard spot, first described by Cobb (1897); freckle spot; and virulent spot, both first described by Kiely (1948b). Lesions are well defined and occur at various stages of rind maturity (Kiely, 1948b). Two other symptoms, speckled blotch and cracked spot are not as widely recognised and occur predominantly in South Africa (McOnie, 1965b) and Brazil (De Goes et al., 2000) respectively.

CBS symptoms are variable in appearance and can easily be confused with symptoms caused by other citrus pathogens, especially freckle spot and speckled blotch (Bonants et al., 2003). Since the disease influences citrus trade world-wide, recent research has focused on developing fast and reliable methods for the detection of CBS from lesions on fruit (Baayen et al., 2002; Bonants et al., 2003; Meyer et al., 2001).

3.3.2.1 Hard spot

Hard spot consists of circular brown lesions originating from an initial slight depression. These lesions tend not to increase in diameter, but sink in the centre and form a crater-like depression. The tissue in the centre turns grey-white and pycnidia may develop therein (Korf, 1998). Perithecia never develop within hard spot lesions (Kotzé, 1981). The rim of these lesions is

typically black (Korf, 1998). Generally hard spot lesions are few in number per fruit, but more than 50 lesions per fruit have been observed (Kiely, 1948b). These lesions appear when fruit start maturing and may even appear before the colour has changed from green to orange (Kotzé, 1981).

Figure 3.2 — Hard spot lesions on a mature Valencia orange. Photograph courtesy of Hennie Korf.

3.3.2.2 Freckle spot

Multiple (up to several hundred), separate, deep orange to brick red lesions may appear simultaneously on a portion of the fruit surface, usually the side that is more exposed to the sun (Kiely, 1948b). Fruit become affected while still on the tree and lesions are at first about a millimetre in diameter and slightly depressed at the centre. Lesions grow fast and reach two to three millimetres in diameter before turning brown and ceasing growth. The depth of the lesion might increase, depending on the thickness of the rind. These symptoms are generally devoid of pycnidia (Bonants et al., 2003). Fruit with freckle spot are usually more unsightly than those with only hard spot, and this symptom severely influences the marketability of fruit (Kiely, 1948b). Individual lesions may coalesce or form a tearstain lesion. Coalesced lesions could turn into virulent spot. This symptom mostly appears after the fruit have changed colour from green to orange (Kotzé, 1981).

3.3.2.3 Virulent spot

Virulent spot appears on unblemished and blemished fruit (Kiely, 1948b). On blemished fruit, freckle spot lesions coalesce to form virulent spot. Infection centres develop rapidly and black pycnidia may develop inside these centres (Calavan, 1960; Kiely, 1948b). Lesions on unblemished fruit may originate as small sunken red to brown spots or as irregularly depressed centres approximately 6 mm in diameter showing no colour change (Calavan, 1960). Lesions assume irregular shapes and develop late in the season on fully mature fruit. These lesions could be surrounded by brown necrotic tissue and cause post-harvest losses (Kiely, 1948b; Kotzé, 1981).

3.3.2.4 Speckled blotch

Another symptom, known as speckled blotch, occurs infrequently on fruit. It was first thought to be Melanose [*Diaporthe citri* (Faw.) Wolf], but later it was concluded that the causal organism was *Guignardia citricarpa* (Kiely) (McOnie, 1965b). Blotching consists of separate, roughly circular spots, 1–2 mm in diameter, either depressed or slightly raised. At first appearance the spots are brick red but turn dark brown in colour over a period of two weeks (Kiely, 1960). Speckled blotch may develop into hard spot as the season progresses (Kotzé, 1981). These lesions are usually devoid of pycnidia (Bonants et al., 2003). In South Africa, all of the above-mentioned symptoms have been reported (Wager, 1952).

3.3.2.5 Cracked spot

In Brazil, a new symptom has recently been associated with CBS. This symptom appears in fruit older than 6 months and is characterized by the presence of superficial lesions which are variable in size and appear cracked. The symptoms are slightly salient, can occur individually or in groups and do not contain any pycnidia. Cracked spot has been proposed as the name for this new type of symptom (De Goes et al., 2000).

3.4 Factors that influence symptom development and severity on fruit

Symptoms may first be expressed during fruit development on the tree or after harvesting. Expression is generally promoted by relatively high temperatures and high light intensities (Brodrick & Rabie, 1970; Kellerman, 1976; Kellerman & Kotzé, 1977; Kiely, 1969; Kotzé, 1961; Kotzé, 1963, 1971; Whiteside, 1967). Low temperatures reduces fruit symptom development (Brodrick, 1969).

Pre-harvest symptom development on fruit is dependent on weather conditions, and on the age and condition of the host tree (Kiely, 1969; Kotzé, 1996). Consequently, older trees (Kotzé, 2000); trees suffering from root rot (Whiteside, 1965), wilting, or element deficiencies (Kotzé, 1961); and trees affected by drought (Kiely, 1969) or hail damage (Kellerman, 1975) are more susceptible to CBS. Symptoms also develop more rapidly as the rind matures. Thus, factors that influence rind maturation, such as soil moisture, can also influence the occurrence of symptoms (Kiely, 1969).

Fruit can also develop symptoms while in transit to export markets (Brodrick, 1969; Kiely, 1948b; Loest, 1958; Smith, 1962), particularly if fruit are not moved rapidly into the cold chain. Fruit exported from South Africa are shipped in chilled containers, however transport from farms to ports is not usually part of the cold chain (Mather, 1999) and fruit sometimes develop symptoms during this period.

3.5 Nature of the causal organism and biotypes

McAlpine gave the first detailed descriptions of the anamorphic stage of the causal organism in 1899. He assigned the pathogenic organism to the genus *Phoma* and described it as a new species, *Phoma citricarpa* McAlpine (Kiely, 1948b). In 1953, the name was changed to *Phyllostictina citricarpa* (McAlp.) Petrak (Hudson, 1962) and in 1973 van der Aa renamed the anamorph *Phyllosticta citricarpa*, by which name it is still recognised today (Kotzé, 1996). The teleomorphic stage was described by Kiely as *Guignardia citricarpa* (Kiely, 1948b). The spermatial state is in *Leptodothiorella* and the synanamorph has not formally been described (Baayen et al., 2002).

Guignardia citricarpa is an endophyte of citrus and has been isolated extensively from healthy citrus tissue (Araujo et al., 2001; Azevedo et al., 2000; Glienke-Blanko et al., 2002). The term endophyte means that the organism colonises internal plant tissues of the host and inhabits the plant organs for a part of its life cycle without causing apparent harm (Petrini, 1991).

The endophytic nature of species belonging to the Genus *Guignardia* caused confusion in the past, since all isolates of *Guignardia* obtained from citrus plant material were previously considered to be the citrus pathogen. Sueda (1941) indicated that the pathogen could be found in healthy citrus plants of all ages and varieties. Similarly, Wager (1952) and Kiely (1950) showed that *G. citricarpa* occurred in disease-free regions and that it could be isolated from wild plants. However in 1964, McOnie described a non-pathogenic form of *G. citricarpa* that was similarly isolated from symptomless citrus and 14 other wild plants from South Africa. At the time, this non-pathogenic form of *Guignardia* was distinguished in culture from the pathogenic type by its darker colour, faster growth, and production of perithecia containing ascospores.

The geographical distribution of the non-pathogenic form is much wider than that of the pathogen. Furthermore, non-pathogenic *Guignardia* occurs in countries without CBS such as Spain, Sicily and Israel (Baayen et al., 2002). Although the pathogen may also be isolated from symptomless citrus, in culture it produces pycnidia rather than perithecia (Sutton & Waterson, 1966).

Finally, in 2001, Meyer et al. (2001) distinguished the pathogenic form from the non-pathogenic form of *Guignardia*. They used restriction enzyme digestion fingerprints of the Polymerase Chain Reaction (PCR) product of a portion of the internal spacer region (ITS) of the ribosomal DNA operon. Baayen et al. (2002) confirmed that there are two distinct species of *Guignardia* through analyses of the sequences of the ITS region. They distinguished ITS groups I and II of *Guignardia citricarpa*, *senso lato*. Group I consisted of all isolates from CBS infected fruit and had similar growth rates and morphology to the pathogen as described by McOnie (1965a). Group II grew rapidly, was not associated with CBS symptoms, and originated from a range of host species. Baayen and co-workers (2002) proposed that isolates with the morphology and ITS sequence of group II should be designated *Guignardia mangiferae* A.J. Roy. This fungus is

a cosmopolitan endophyte of woody plants. It can be pathogenic in some instances and is known to cause minor fruit spot of guava (Baayen et al., 2002), but is not pathogenic on citrus.

As the identity of the pathogen has been ambiguous for so many years, much of the past research on *G. citricarpa* is questionable. For instance, Frean (1964) and Brodrick (1969) conducted physiological studies on what they believed to be *G. citricarpa*. Unfortunately, there is evidence to suggest that some of the isolates used during these studies were the non-pathogenic type of *Guignardia* (Baayen et al., 2002; Meyer et al., 2001). Their results, therefore, may not be entirely representative of *G. citricarpa*. The confusion surrounding the identity of the CBS pathogen has also caused inaccurate reports on the distribution and occurrence of the disease (McOnie, 1964c).

3.6 Epidemiology

3.6.1 Type of inoculum

Two kinds of spores, ascospores and pycnidiospores, may cause infection of citrus (Kotzé, 1996).

3.6.1.1 Ascospores

Windborne ascospores are seen as the primary source of inoculum (Kiely, 1948b; Kotzé, 1963; Sutton & Waterson, 1966). They are found abundantly within perithecia on the leaf litter. These fruiting bodies may also occur on dead twigs on the orchard floor but are never found on fruit or attached leaves (McOnie, 1965a).

Mature ascospores are forcefully discharged from mature perithecia. Perithecia maturation is not seasonal and mature ascospores may be found within leaf litter on the orchard floor all year round (Kiely, 1948b). In South Africa, perithecia ripen slower in winter than in summer and large numbers of ascospores may be trapped during summer (Kotzé, 1963). Alternate wetting and drying of the fallen leaves and variations in temperature provide optimal conditions for ascospore formation and maturation (Kiely, 1948a, 1948b). Perithecia will not mature in areas where the leaf litter is either constantly dry or constantly wet (Wager, 1949). In constantly wet weather conditions the leaf litter decomposes and the ascospore inoculum may be eliminated (Kiely, 1948b). This is thought to be a possible reason for the absence of CBS in certain parts of South Africa (Wager, 1949).

Ascospores are windborne, but their ejection from the mature perithecia is dependent on wetting; no spores are ejected if the perithecia are not wetted. The onset of rain, ascospore discharge and infection period are, therefore, closely related (Kotzé, 1963; McOnie, 1964b). However, heavy dews may also sometimes be sufficient to secure ejection of ascospores (Kiely, 1948a). Low temperatures do not influence the release of ascospores (Kotzé, 1963).

3.6.1.2 Pycnidiospores

In addition to perithecia, pycnidia containing pycnidiospores are abundant on dead leaves beneath trees (Kiely, 1948b). Pycnidia may occur in fruit lesions, on dead twigs, and sparsely within lesions on attached leaves or on fruit stalks.

In wet weather mature pycnidiospores ooze as a gelatinous mass from pycnidia contained in lesions on the rind of infected mature fruit hanging on the tree. These spores require water for dispersal (Sutton & Waterson, 1966; Whiteside, 1967). Similarly masses of gelatinous pycnidiospores are produced from pycnidia on fallen leaves. These pycnidiospores do not have any mechanisms of dispersal (Kotzé, 1996), nor do pycnidiospores originating from lesions on infected fruit which have fallen from the tree (McOnie, 1964b). What happens to these spores after release from the pycnidia is not known; it is suggested that some of these spores might reach the tree canopy by the splashing of raindrops (Kotzé, 1981) but presumably the majority of spores are washed into the soil when it rains.

3.6.2 Other sources of inoculum

Mycelium latently present in citrus trees may be a source of inoculum (Kiely, 1949). If the CBS pathogen in such trees is introduced to new, uninfected citrus production areas CBS might successfully establish in the new area (Calavan, 1960). In the past, CBS have been transmitted to uninfected areas via this source of inoculum (Kiely, 1949; Wager, 1952).

3.6.3 Infection of fruit

Young fruit are highly susceptible to infection. The period of susceptibility extends from blossoming (anthesis) until about five months later. Thereafter, infections no longer take place regardless of the prevailing weather conditions (Kotzé, 2000). This is as a result of an increase in fruit resistance, rather than a decrease in inoculum (Whiteside, 1965).

3.6.3.1 Infection by ascospores

Infection takes place when the thick walled apresoria of a germinating fungous spore penetrates the rind of the fruit. After penetrating the rind, the fungus forms a resting body within the rind tissue. This resting body remains dormant until rind maturity when conditions are conducive for growth (Kiely, 1948b, 1970; Kotzé, 1963). This kind of infection is known as a latent or quiescent infection (Kiely, 1969). The latent period may last several months (Cook, 1975; Kotzé, 1963). Consequently, *G. citricarpa* may be isolated from apparently healthy citrus fruit tissues (Yin et al., 1981). Ascospores cannot infect healthy mature fruit (Wager, 1949).

3.6.3.2 Infection by pycnidiospores

Infection by pycnidiospores happens when spores from late-hanging, infected, mature fruit are washed down to young susceptible leaves and fruit (Sutton & Waterson, 1966; Whiteside, 1965, 1967). Similar to ascospore infections, pycnidiospores from lesions on mature infected fruit are unable to infect other healthy mature fruit (Calavan, 1960; Korf, 1998; Wager, 1952).

Pycnidiospores from fallen leaves and fruit are not thought to readilly cause infection of fruit, since their dispersal to fruit hanging on the trees, unless splashed by raindrops, seems unlikely (Kotzé, 1996; Kotzé, 1981; Kotzé, 2004, Personal Communication; McOnie, 1964b). Since pycnidiospores are not seen as a major source of inoculum, the exact mechanisms of infection have not been investigated.

3.6.4 Infection of leaves

Only young leaves are susceptible to infection (Kotzé, 1996) and any new leaf flushes that coincide with wet weather may become infected (Whiteside, 1965). Leaves remain susceptible for up to nine months and the pathogen can readily be re-isolated from previously inoculated material (Labuschagne, 2003, Personal Communication). Leaf infections remain predominantly latent until leaf drop and desiccation, although lesions may appear on mature attached leaves (Whiteside, 1965).

3.6.4.1 Infection by ascospores

Infected leaves fall to the ground a year or longer after infection and eventually produce mature ascospores, which are forcefully released from perithecia and may infect young fruit and leaves and so complete the infection cycle (Whiteside, 1965). Specific mechanisms of ascospore infection of leaves have not been studied.

3.6.4.2 Infection by pycnidiospores

When a pycnidiospore reaches a susceptible leaf, the spore will germinate and form an appresorium which gives rise to infection pegs that penetrates the leaf. Pycnidiospores can only be dispersed by water and it seems unlikely that these spores will move from the leaf litter to infect the leaves still hanging on the tree (Kotzé, 1996). However, it is thought that water splashing up from the ground when it rains can carry pycnidiospores which might infect low hanging leaves (Kotzé, 1981; Kotzé, 2004, Personal Communication).

3.6.5 Factors that influence the infection of fruit and leaves by ascospores

Germinating spores require special climatic conditions before they can penetrate fruit or leaves. Ascospore infection frequency is determined by the rainfall pattern and climatic conditions greatly influence the intensity of infection (Wager, 1952; Whiteside, 1967). If conditions are not favourable for the development and maturation of the pathogen's fruiting bodies, citrus fruit and leaves may escape ascospore infection (Whiteside, 1967). Additionally, availability of spore inoculum during the time when young fruit and leaves are susceptible has an important influence on the rate of infections and disease severity (Whiteside, 1965, 1967).

3.6.6 Importance of ascospores and pycnidiospores in infections

It is widely accepted that ascospores are the major source of inoculum in South Africa and Australia where predominantly only one cycle of disease infection occurs annually. The critical period for ascospore infection is approximately within a single five-month window period when

fruit set coincides with rainfall. Late-hanging infected mature fruit are removed from trees a month before the new season's fruit sets (Kiely, 1948b, 1970; Kotzé, 1963, 1996; McOnie, 1965a). Therefore, pycnidiospores cannot be a major source of inoculum as mature CBS infected fruit and susceptible young fruit never occur simultaneously on the same trees. However, this is not true for citrus produced in Brazil where rain is not so confined to a single season and flowering may occur more than twice a year. Therefore, the epidemiology of CBS in Brazil is different to that found in other parts of the world and the role of pycnidiospores is as important as that of ascospores (Sposito et al., 2001). In Zimbabwe, where the disease is rare and localized, it was found that waterborne pycnidiospores, mostly seen as secondary inoculum, was the most important source of inoculum (Whiteside, 1967).

3.6.7 Recommendations for future research

Although in-depth research has been conducted into the nature of the CBS pathogen and its etiology, there are still major gaps in the knowledge base of this complex disease. For instance, all potential sources of inoculum, such as the latent mycelium found in leaf and fruit tissues, and the perithecia on dead twigs, have not been investigated. The survival of spores in soil and the potential role of soil in the disease cycle was only recently studied for the first time (Lise Korsten, 2004, Personal Communication), but has not as yet been elucidated. Similarly, little is known on the exact role of insects in the dissemination of CBS.

To a certain extent the confusion between the non-pathogenic and pathogenic types of *Guignardia* that existed in the past hampered attempts to fully understand the epidemiology of the disease. However, as molecular techniques to reliably distinguish between the pathogen and the non-pathogen now exist, it is recommended that aspects of the epidemiology of CBS be revisited.

3.7 Host plants

Almost all commercial citrus species are susceptible to CBS but lemons are the most susceptible. When CBS is found in a new area, it will probably first be seen on lemons before other citrus is affected (Kiely, 1948b; Kotzé, 2000). Persian limes (Timmer, 2005, Personal Communication) and sour orange and its hybrids are not susceptible (Kotzé, 1981), and rough lemons are thought to be tolerant (Wager, 1952).

Various non-citrus species native to Australia and South Africa were reported to carry latent infections of *G. citricarpa* that could act as a source of inoculum (Kiely, 1948a, 1948b; Wager, 1952). However, *Guignardia* isolates from non-citrus hosts were identified as *G. mangiferae* by McOnie (1964c), a result later confirmed by Meyer et al. (2001) and Baayen et al. (2002), using molecular techniques.

3.8 Control

CBS control is primarily chemical and reliant on effective disease forecasting models. However, the most important non-chemical approach in CBS control is to use cultural techniques to reduce transmission. Efforts to breed resistant varieties have not been successful (Calavan, 1960) and as far as can be determined, no major breeding program is being investigated.

3.8.1 Preventing spread

As trees that are in a poor condition are more susceptible to CBS, maintaining tree vigour can reduce the incidence of CBS (Calavan, 1960; Kellerman, 1975; Kiely, 1971; Kotzé, 1961; Loest, 1968). Sources of pycnidiospore inoculum may be removed by removal of diseased mature, late-hanging fruit before the new crop sets (Calavan, 1960; Kiely, 1969; Kiely, 1970; Kotzé, 1996). Similarly, ascospore inoculum can be removed by the removal of leaf litter from the orchard floor (Kotzé, 2002, Personal Communication).

3.8.2 Pre-harvest control

Citrus Black Spot can be controlled by the timely application of appropriate fungicides either to protect fruit, or to eradicate infections and prevent symptom development (Kellerman, 1976; Kellerman & Kotzé, 1977). The effectiveness of fungicide applications is particularly reliant on the number and timing of applications (Kellerman, 1976). In South Africa, control of CBS has mostly relied on continuous protection of young citrus fruit during the potential infection period when the host is most susceptible and pathogenic spores are present (McOnie & Smith, 1964).

The earliest method of controlling CBS was by applying a Bordeaux mixture (as preventative measure)(Benson, 1895; Cobb, 1897; Kiely, 1948b), which was later found to result in copper toxicity (Kotzé, 1964). In 1964, dithiocarbamates were introduced as preventative control measures by first applying Zineb (active ingredient zinc ethylene bisdithio-carbamate) and later Mancozeb (active ingredient manganese ethylene bisdithio-carbamate)(Kotzé, 1964). These proved superior to copper based products (Kellerman, 1976; Kellerman & Kotzé, 1977), as they did not retard fruit coloration or result in dark rind injuries (McOnie & Smith, 1964). This group of chemicals were replaced by Benomyl [active ingredient methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate] — a preventative and curative approach (Kellerman & Kotzé, 1973, 1977) — in 1971 (Kiely, 1971). However, by 1984 the CBS fungus had developed resistance to the frequent spraying of Benomyl (Herbert & Grech, 1985). Current research suggests that strobilurins hold some potential for the control of CBS (Kotzé, 1996; Miles et al., 2004; Schutte et al., 1996; Tollig et al., 1996).

3.8.3 Post-harvest control

A water-wax emulsion can be applied to harvested fruit to reduce the development of CBS during storage at 16-27°C (Seberry et al., 1967). Since light and temperature affect the development of symptoms on fruit (Smith, 1962), it may also be transported in dark coloured

wrappers to reduce light intensity (Brodrick, 1969). Furthermore, low temperature storage and shipment of fruit will inhibit symptom development in latently infected fruit (Calavan, 1960; Kiely, 1970).

3.9 Economic importance

Before the implementation of phytosanitary barriers against the import of citrus fruit from areas where the disease occurs to disease-free regions (European Union, 1977), all losses as a result of CBS were attributed to injured fruit not fit for marketing. The CBS lesions on fruit significantly lower the market value of fruit and result in the product being re-directed for processing (Calavan, 1960; Cobb, 1897; Kellerman & Kotzé, 1977; Wager, 1945).

Already in 1895, CBS caused significant losses throughout Australia (Benson, 1895) and in 1939, the industry suffered a major economic setback due to a severe CBS epidemic in New South Wales (NSW). This resulted in an oversupply of unwanted CBS infected fruit on the local market. In 1945, 90% of citrus fruit produced from unsprayed orchards in the northern provinces of SA (today Limpopo and Mpumalanga), were rendered unfit for export (Sutton & Waterson, 1966). During the 1960s, numerous citrus growing areas in SA reported similar major losses in production, as up to 60% of fruit intended for the export market was unsuitable for export (Brodrick, 1969). Within the 1960s it was speculated that world-wide losses due to this disease amounted to millions of dollars (Calavan, 1960). By 1970, losses due to CBS were so severe in NSW that it was seen as the most serious disease affecting citrus production in Australia (Kiely, 1970).

Pre-harvest CBS losses arise when severely affected fruit drop prematurely in the orchard and go to waste (Wager, 1945; Wager 1952; Kotzé, 2000). Post-harvest CBS losses are not always apparent as latent, asymptomatic export fruit may develop CBS symptoms while in transit to the harbour (Brodrick, 1969; Kiely, 1948b; Loest, 1958; Smith, 1962). Furthermore, CBS control programmes are costly (Cobb, 1897; Kotzé, 1961). However, if not controlled CBS may cause total loss of the crop (Seberry et al., 1967) and therefore in some areas, citrus production will be impossible without effective CBS control programs (Smith, 1996). Finally, CBS affects international trade in citrus. The EU and the USA reject consignments of fruit containing CBS infected fruit, with economic implications to the citrus industry of the country of origin.

3.10 Phytosanitary barriers to trade & inspection and detection methods

Currently the EU and the USA enforce phytosanitary regulations that restrict the import of citrus from CBS infected areas (Anonymous, 1986; Baayen et al., 2002). These regulations aim to prevent CBS entering and establishing in the EU and the USA (European Union, 1998, 2000c). Imports of citrus from CBS affected regions are not entirely prohibited, but fruit may only be imported if evidence can be provided that effective pre-harvest spray programs exist and no disease symptoms were observed in official inspections of export consignments at packinghouses and at ports (European Union, 2000c). Shipments of imported fresh citrus fruits

are also inspected at the ports of entry into the EU (Bonants et al., 2003) and USA (USDA/APHIS, 2002) by phytosanitary services of the importing countries. Any consignments that do not meet requirements may be refused, and they will also be rejected if they are found to contain CBS infected fruit (Bonants et al., 2003).

Methods for the pre-harvest and post-harvest detection of CBS include visual inspection of fruit and the isolation of the pathogenic strain from fruit lesions (Fogliata, 2000; Whiteside, 1967). Citrus Black Spot may be diagnosed visually by the readily recognisable CBS fruit symptoms, such as hard spot. However, speckled blotch and freckle spot may be confused with symptoms of other citrus diseases such as true melanose (*D. citri*) and greasy spot (*Mycosphaerella citri* Whiteside) (Baayen et al., 2002). In such cases, the fungus is cultured by removing lesions from the peel and incubating it for five days (Bonants et al., 2003). However, the two biotypes of *Guignardia* are morphologically similar and this test may be an unreliable method of detecting the pathogen. In addition, *Guignardia* may take up to 14 days before forming mature pycnidia in culture, during which time the value of a consignment will decrease significantly (Baayen et al., 2002). To speed up the process, Bonants et al., (2003) developed a rapid PCR detection method for the diagnosis of CBS lesions originating from speckled blotch or freckle spot symptoms. The primer set of the PCR-detection method (GCF3/GCR7) is selective for *G. citricarpa* and does not amplify any other fungi present within citrus peel (Bonants et al., 2003).

Spread of CBS to countries that do not have the disease can be prevented by appropriate quarantine measures. The potential future spread of the disease will rely on the effective application of these measures (Kotzé, 1981; Kotzé, 1996). However, the pathogen has not established in some areas despite repeated introductions of suitably infectious material. Areas where CBS has not established include the inland citrus growing areas of NSW, Australia (Barkley, 2003, Personal Communication; Whiteside, 1967), and the Western Cape region in South Africa (Mabiletsa, 2003; Smith, 1962). Therefore, the introduction of diseased material does not necessarily imply that it will eventually cause a CBS disease epidemic, especially if climatic conditions are not suitable for disease development. This should be taken into consideration when enforcing phytosanitary barriers to trade.

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Chapter 4 — Analysis of the suitability of European climate for establishment of Citrus Black Spot disease caused by *Guignardia citricarpa* Kiely

4.1 Abstract

Citrus Black Spot (CBS), caused by *Guignardia citricarpa* Kiely, is an economically important fungal disease of citrus. The disease is widespread in some citrus producing countries, occurring in Southern Africa and Australia but not in Europe. To prevent the disease from spreading to Europe, the EU has placed phytosanitary barriers on the importation of fruit from areas infected with CBS. The aim of this study is to evaluate whether the European climate is conducive to the development of the disease. This is done using the Match Climates function of the software package CLIMEX. The climate of 16 locations in South Africa with known CBS presence are compared with the climate of other locations in South Africa, Europe and Australia. The model successfully predict areas of CBS presence and absence in South Africa and Australia, and results suggest that the climate in Europe is not suitable for the establishment of the CBS pathogen.

4.2 Introduction

Citrus Black Spot (CBS) caused by *Guignardia citricarpa* Kiely, is a foliar and fruit disease of citrus. It occurs, amongst other countries, in parts of South Africa (SA) and Australia (Sutton & Waterson, 1966; European Union, 1998; Kotzé, 2000; Baayen et al., 2002), but it has not been reported in European countries (European Union, 1998). The disease affects the rind of the fruit, causing superficial lesions (Kotzé, 1981; Snowdon, 1990). Most commercial citrus cultivars are susceptible, especially lemons.

South Africa is the third largest exporter of fresh citrus fruit after Spain and the United States of America (FAO, 2002). The South African Citrus Industry is dependent on these exports, particularly to European countries (Mabiletsa, 2003). However, European Union (EU) quarantine regulations restrict the import of fruit from areas where CBS occurs (European Union, 1998, 2000), the implication being that there may be a risk of CBS establishing in Europe.

As for all plant pathogens, an outbreak of CBS requires at least the presence of an active strain of the pathogen, a susceptible host, and favourable climatic conditions (Booth et al., 2000). The inability of a pathogenic species to establish in an area where both the species and susceptible host are present may usually be attributed to unfavourable climatic conditions.

Robust examples where the effect of climate on the risk of establishment of potentially invasive plant pathogenic species has been estimated include rice blast disease, hop powdery mildew and hop downy mildew in Australia [caused by *Magnaporthe grisea* (Hebert) Barr, *Podosphaera macularis* Braun, and *Pseudoperonospora humuli* (Miyabe et Takahashi) respectively] (Lanoiselet et al., 2002; Pethybridge et al., 2003).

These studies were done using the Match Climates Function of CLIMEX (Hearne Scientific, Melbourne, Australia). CLIMEX is a dynamic simulation model that allows researchers to estimate the geographic distribution of a species as determined by climate (Sutherst et al., 2003). The Match Climates function enables the user to compare meteorological data from different places. It therefore allows an initial rough assessment of the likelihood of a species establishing in a new area based solely on the similarity of climate between the current range and the potential range (Sutherst et al., 2003).

The objective of this study is to estimate the potential risk of the disease occurring in European climates - the premise being that the pathogen can only persist in climates similar to those where it is currently found. If climatic conditions are unfavourable for the growth and survival of the pathogen, then it may be unable to establish in Europe. In this case, current phytosanitary barriers should be reconsidered.

4.3 Methodology

4.3.1 Data on the geographical distribution of Citrus Black Spot

Six field specialists with extensive knowledge of CBS carefully mapped areas of CBS presence and absence onto a map of South Africa (1:1 000 000, 2 x 2 m, Department of Geography, University of Pretoria). All areas in South Africa where *G. citricarpa* was either known to have been isolated from fruit or leaf surfaces or where symptoms of the disease have been observed by the field specialists, were indicated as areas with CBS presence. In the same manner, areas where no symptoms of the disease have ever been observed and no isolations of *G. citricarpa* were known to have been made from fruit or leaves were indicated as areas with CBS absence. Information obtained from recent surveys in which farms were investigated for CBS presence was also included. The areas of CBS presence or absence included commercial citrus production areas and areas of "garden" citrus, for instance scattered citrus trees on the experimental farm of the University of Pretoria. Some, but not all, areas were confirmed from literature (Wager, 1949, 1952).

Polygons, representing either CBS presence or absence as drawn on the map, were then transcribed by hand onto a smaller map (A3 –29.7 x 42 cm in size). The smaller map was scanned into a computer and information on the geographical occurrence of CBS was digitized using ArcView GIS 3.3 (Environmental Systems Research Institute)(Figure 4.1).

Copies of this map were made available to participants at the Citrus Growers' Association biennial Symposium (July 2002, Stellenbosch, South Africa). At the meeting, about 200 citrus growers and researchers from all over the country had the opportunity to confirm areas of presence and absence. Therefore, this distribution data was considered to be reliable, and of a resolution suitable for input into CLIMEX.

4.3.2 Outline of the CLIMEX model

CLIMEX provides three major functions:

1. Compare Locations — used to predict the potential geographic distribution of a species based on its climatic requirements.
2. Compare Years — used to examine the effect of climatic variation on the potential abundance of a species over consecutive years at the same location.
3. Match Climates — used to compare climates at different locations.

The present study focussed on Match Climates. CLIMEX for windows Version 1.1 was used throughout.

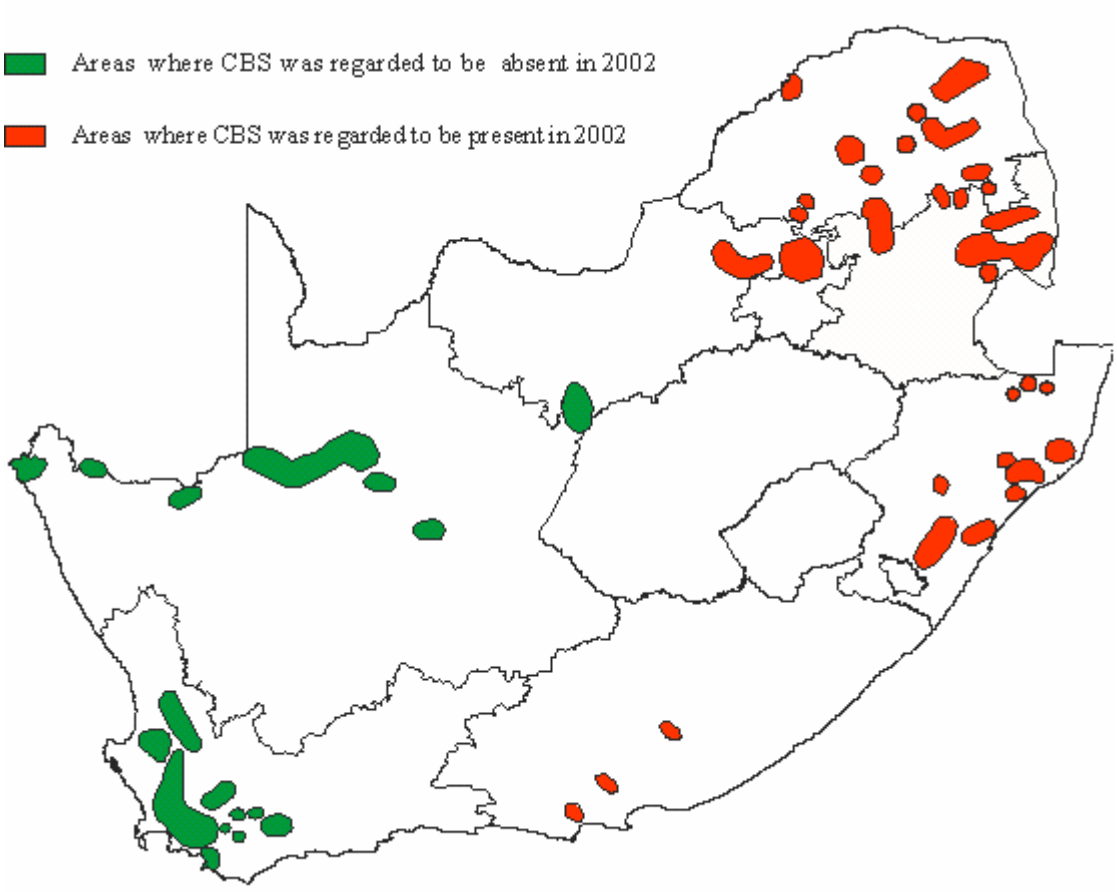

Figure 4.1 — The occurrence of Citrus Black Spot in South Africa in 2002 as determined by visual confirmation of symptoms and/or positive identification of the pathogen *Guignardia citricarpa*.

The Match Climates function in CLIMEX allows the prediction of the climatic similarity of a target location to another specified location by exploring long-term meteorological data for locations with climates similar to those of a chosen location (Sutherst et al., 1999). If locations in different hemispheres are being compared, CLIMEX shifts the data of the target location by six months to match that of the location selected by the user.

A similarity index lists the percentage similarity of the target locations to the nominated site. The index is a product of five parameters, which indicate the similarity for average monthly maximum daily temperature, average monthly minimum daily temperature, average monthly rainfall, rainfall pattern and relative humidity values at 9 a.m. and 3 p.m. These variables were selected because they have been shown to be the most meaningful when dealing with biological questions. Minimum and maximum temperature allow the user to identify extremes, while information about rainfall, relative humidity and rainfall pattern identifies differences in availability of moisture and assists in matching zones with similar seasonal rainfall (Sutherst, 2002, Personal Communication).

The user may decide how closely each of these parameters should be matched by assigning to each parameter a weighting between zero and one. If this value is set at one it will indicate, for

example, that the average monthly rainfall value has to be the same at both locations for a similarity of 100%. If the weighting is set to 0.8, then the rainfall can be slightly different at the two locations but the similarity index will be close to 100%. CLIMEX also allows the user to set an importance level for the climate stations. Choosing an importance level of five will include all the climate stations and provide a highly detailed result. Setting the importance level lower will result in the selection of only the most important locations, resulting in a less specific result (Sutherst et al., 1999).

In the present study, the weighting for all parameters was set to one. Moreover, so as to obtain the most detailed results possible, all climate stations in South Africa, Europe and Australia within the CLIMEX meteorological database were included.

To estimate the similarity between European climates and those where CBS occurs, an index of similarity sufficient for disease establishment was determined based on CBS distributions in South Africa. This similarity index was tested against the known distribution of CBS in Australia, and was subsequently applied to determine the likelihood of establishment in European countries.

4.3.3 Determination of an index of similarity sufficient for disease establishment

CLIMEX has a database of climates from more than 2 400 meteorological stations world-wide, 127 of which are in South Africa (Figure 4.2). For this study, 16 meteorological locations that occurred within, or very close to, the range of CBS presence in South Africa were selected (Figure 4.3). For each of the 127 locations in South Africa, the climate was compared to that of each of these 16 locations. From this, the maximum similarity between each of the 127 locations and a CBS locality was calculated. All CBS localities had maximum similarities of 100%, as they were compared to themselves, thus, CBS localities were only compared to each of the other 15 CBS localities [e.g. the climate of Johannesburg was allocated a maximum similarity index of 81.7% (to Pretoria), and not 100% (to Johannesburg)]. Matching CBS localities in this manner provides an upper limit on the maximum similarity required for the establishment of CBS.

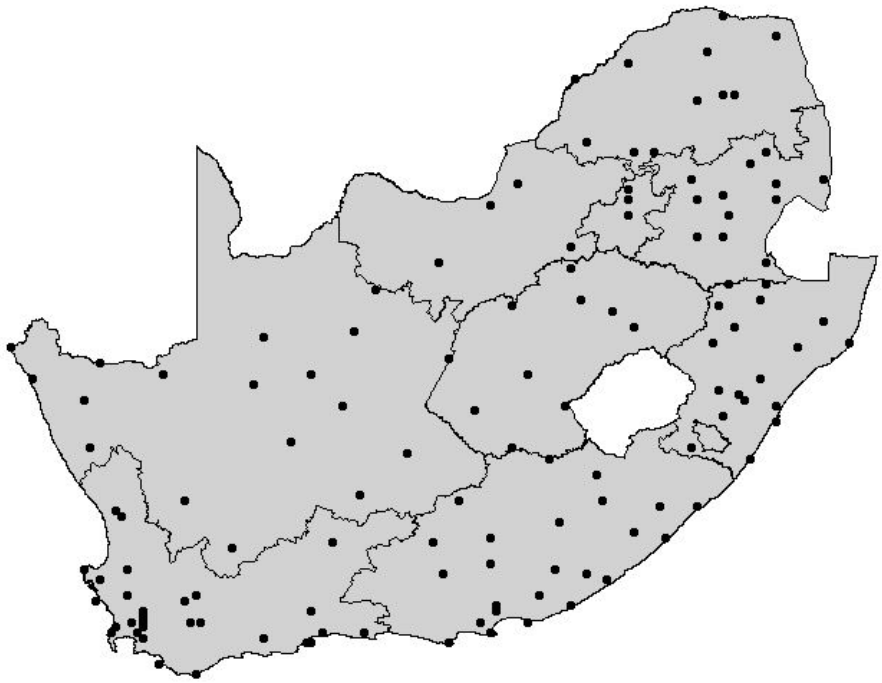

Figure 4.2 — The location (•) of the 127 climate stations in South Africa.

Figure 4.3 — Climate data locations in South Africa falling within the ranges of confirmed Citrus Black Spot presence. The spelling of locations used here is as in CLIMEX.

Prior to the implementation of the restriction on the movement of citrus propagation material by the Agricultural Pest Act of 1983 (Act no 36), CBS infected citrus fruit were moved freely to Western Cape harbours (Loest, 1958) and nursery trees originating from diseased areas (potentially carrying latent infections) were transported to, and planted in, the citrus growing areas of the Western Cape. Additionally, citrus fruit is presently marketed throughout the country irrespective of its source, and there are no restrictions on the movement of citrus fruit by the public (Kotzé, 2002, Personal Communication). Thus, sources of inoculum of *G. citricarpa* have been repeatedly introduced into the Western Cape.

Venter et al. (1995) conducted a survey of citrus producing regions of the Western Cape for the presence of *G. citricarpa*. A total of 17,200 microbial isolations were made from citrus fruits and leaves from the magisterial districts of Bredasdorp, Clanwilliam, Caledon, Heidelberg, Hermanus, Ladysmith, Montagu, Paarl, Piketberg, Robertson, Somerset West, Stellenbosch, Swellendam, Strand, Wellington, and Worcester. Results indicated that the citrus cultivation areas within these magisterial districts were all free of CBS.

As the absence of the disease in the Western Cape cannot be attributed to the absence of the host or pathogenic organism, it may be ascribed to the specific climate of this region. Furthermore, the disease-free status of the Western Cape region is currently recognised by the EU (European Union, 1998), and the United States Department of Agriculture (USDA) (USDA/APHIS, 2002).

Guignardia citricarpa has also not been detected in the Vaalharts district of the Northern Cape Province of South Africa (Anonymous, no date). Additionally, in an exercise to map the distribution of CBS in South Africa (section 4.3.1, page 69), all the citrus production regions in the Northern Cape were mapped CBS free. However, this disease-free status has not been recognised by the European nor North American authorities (at the time of assessment).

A disease matrix was subsequently drawn up that grouped all provinces in South Africa as potentially positive to CBS, except for the Northern Cape Province (CBS not detected) and the Western Cape (official disease-free status) (Figure 4.4).

In order to determine a maximum similarity index sufficient for disease establishment, it was assumed that the climate should match all those localities where CBS occurs. Conversely, a maximum similarity index that fails to match all the localities that are in the proximity of diseased areas would not be suitable for determining disease establishment in other parts of the world.

In New South Wales (NSW), Australia, CBS remains restricted to coastal citrus production regions (Barkley, 2003, Personal Communication; Whiteside, 1965), where it was first described in 1895 (Benson, 1895). Fruit and nursery trees infected with CBS have been introduced to the inland citrus production areas of NSW (e.g. Riverina in the south west, and Bourke and Narromine in the north west), but it has not become established (Kiely, 1970). However, the climate of this region is very dry. This again suggests that climate is responsible for limiting the distribution of CBS.

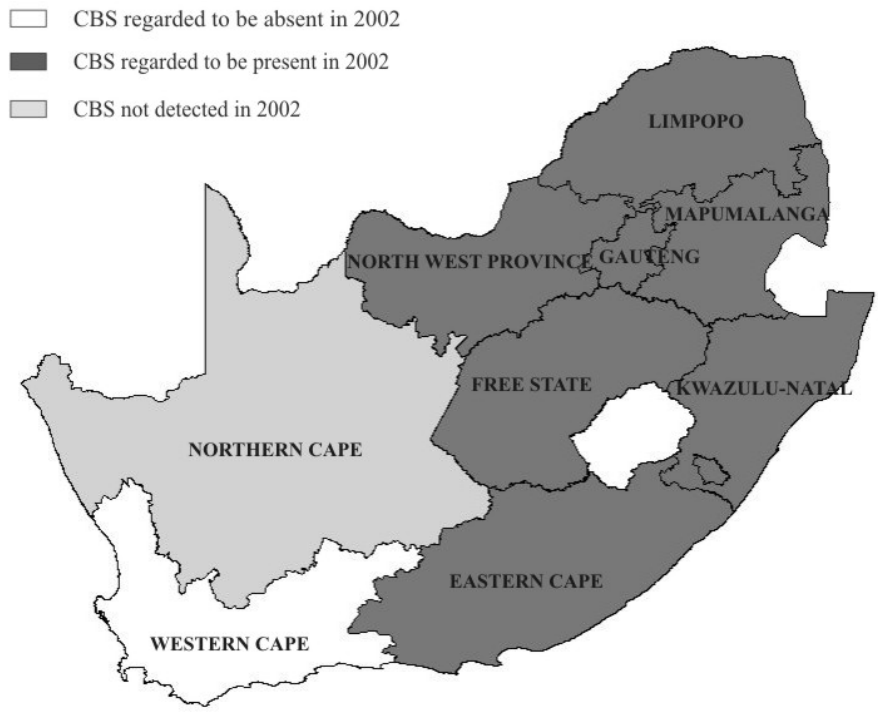

Figure 4.4 — The potential Citrus Black Spot status of provinces in South Africa indicating the Western Cape as a province where the disease was officially considered to be absent in 2002 (European Union, 1998) and the Northern Cape where the disease was not detected in 2002.

4.3.4 Application of the disease similarity index to Australia and Europe

4.3.4.1 Climatic matches with Australia

To confirm that the chosen maximum similarity index is sufficient to describe the potential for establishment of CBS, the 16 South African CBS locations were individually compared to each of 676 locations in Australia (Figure 4.5). According to an official document of the European Community, three states in Australia, namely South Australia, Western Australia and the Northern Territory, have been declared CBS free (European Union, 1998). A matrix, similar to that for South Africa, was drawn up for Australia to indicate the areas where CBS is considered to be present and those areas where CBS is considered to be absent (Figure 4.6). The maximum climatic similarities deemed to be sufficient for the establishment of CBS, as determined in 4.3.3, were mapped onto this matrix to test the validity of the model.

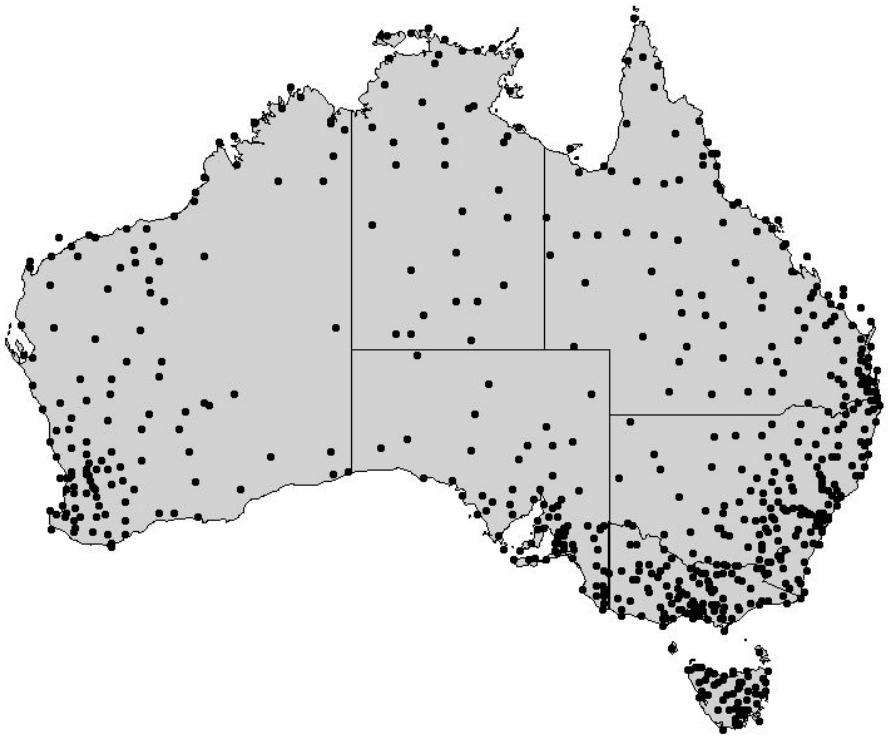

Figure 4.5 — The location (•) of 676 climate stations in Australia.

Figure 4.6 — The potential Citrus Black Spot status of states in Australia.

4.3.4.2 Climatic matches with Europe

The climates of 16 CBS locations in South Africa (Figure 4.3) were compared to 285 locations in Europe (Figure 4.7). Maximum climatic similarity was separately determined for each of the 285 locations.

4.3.5 Mapping the disease similarity index

Results from the Match Climates function are returned as a map, graph or table. All results were exported from CLIMEX to ArcView GIS 3.3 (Environmental Systems Research Institute) following instructions in http://www.ento.csiro.au/climex/climex.htm and are presented as maps. In ArcView, locations were separated into those that had maximum similarity indices ≥60% (which included all localities where CBS was found) and those that had maximum similarity indices <60%.

4.4 Results

4.4.1 Determination of an index of similarity sufficient for disease establishment

All areas where CBS is found in South Africa had maximum similarity indices ≥60% (Figure 4.8). Five localities that fall within provinces with CBS absence, namely Boegoebergdam (62.7%), Kimberley (62.9%), Kuruman (62.3%) (Northern Cape), Riversdale (61.4%) and George (60.3%) (Western Cape) had maximum similarities in climate of ≥60%. This suggests that these localities may be climatically suitable for the potential establishment of CBS. The majority of localities with maximum similarity indices below 60% are from CBS free provinces (Figure 4.9). However, eleven localities with indices below 60% fall within provinces with an official CBS presence, but these include sites where no citrus is produced (southern coastal areas, and areas at very high elevations, e.g. Belfast, Mapumalanga, the highest point in South Africa). Therefore a maximum similarity index of greater than 60% can be considered sufficient for disease establishment and a maximum similarity index of 60% or less can be considered as a climate probably unsuitable for CBS.

4.4.2 Validating the climate similarity index using Australian locations

At maximum similarity indices above 60%, all of the locations from Australia that were considered to be CBS positive at the time of assessment were successfully mapped (Figure 4.10), and no locations were mapped in areas considered CBS free.

4.4.3 Application of the disease similarity index to determine the likelihood of establishment of the disease in European countries

As maximum climatic similarities ≥60% were found to represent the level of similarity that would be suitable for CBS establishment, this similarity level is used to test the potential establishment of CBS in Europe. At this level, none of the 285 locations analysed from Europe had a climate similar to the 16 South African locations (Figure 4.11).

Figure 4.7 — The location (•) of 285 climate stations in Europe.

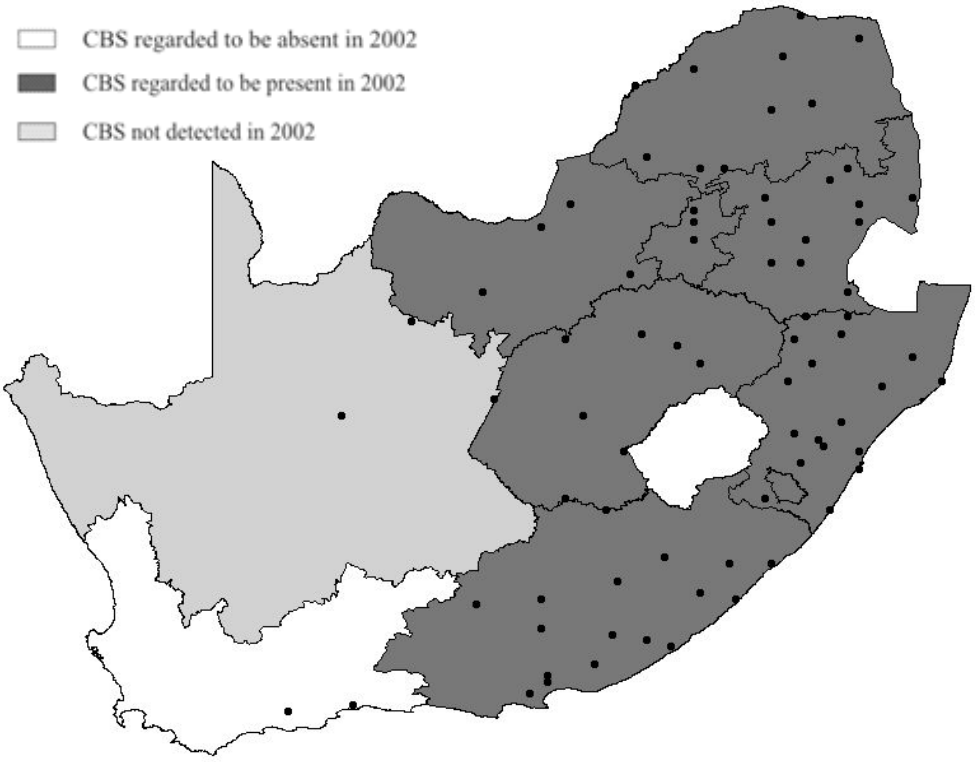

Figure 4.8 — Locations (•) in South Africa that have a maximum climatic similarity of ≥60% to the 16 South African locations which fall in the proximity of areas where CBS is found (see section 4.3.3). Provinces are colour coded to indicate the observed occurrence of CBS at a provincial level.

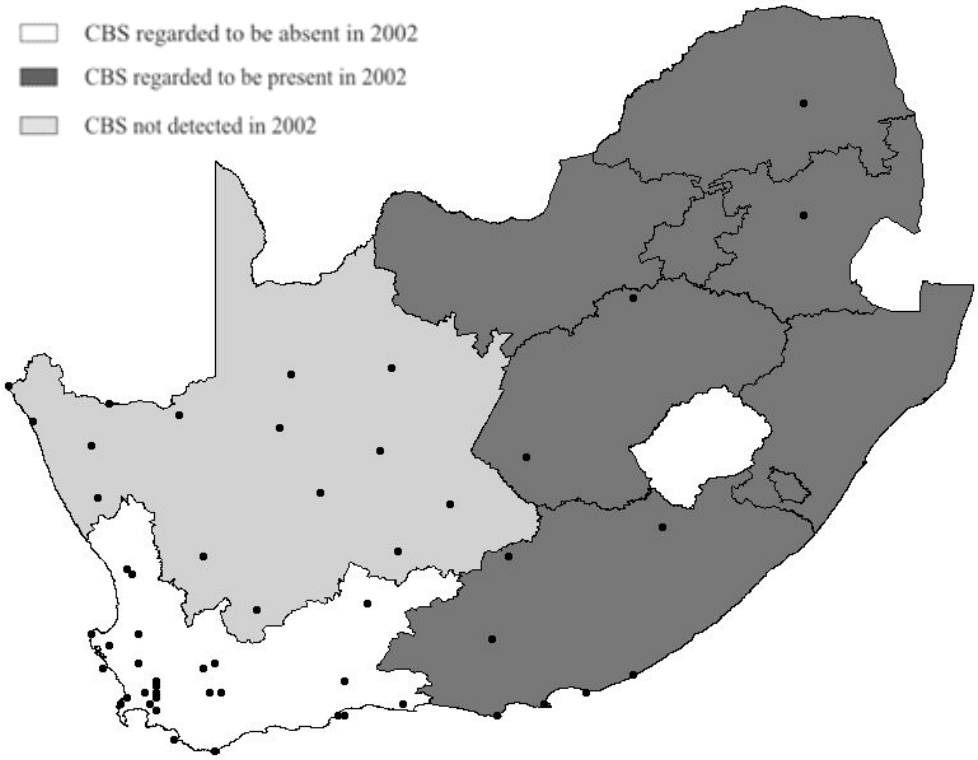

Figure 4.9 — Locations (•) in South Africa that have a maximum climatic similarity of <60% to the 16 South African locations which fall in the proximity of areas where CBS is found (see section 4.3.3). Provinces are colour coded to indicate the observed occurrence of CBS at a provincial level.

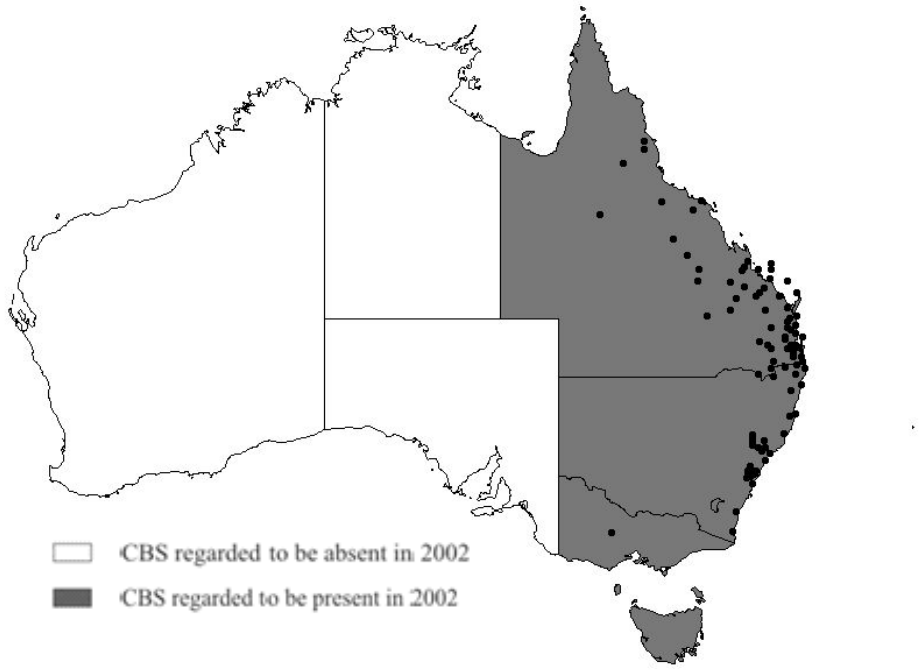

Figure 4.10 — Australian locations (•) with a maximum similarity index ≥60% to 16 South African locations which fall in the proximity of areas where CBS is found.

Figure 4.11 — None of the 285 locations in Europe had a maximum climatic similarity ≥60% to the 16 South African locations which fall in the proximity of areas where CBS is found.

4.5 Discussion

In this study it was found that a similarity index of ≥60% is sufficient to circumscribe a high potential for the establishment of CBS. These results are comparable to those found by Bennett et al. (1998), who used the Match Climates function to determine where certain plant species useful as genetic resources for plant breeding may occur. These authors selected a similarity index of 65% as the minimum level of similarity required for the successful establishment of plant species.

Results obtained in the present study support the absence of CBS in the Western Cape and Northern Cape Provinces, with the majority of weather data localities having a maximum similarity in climate of <60% to the chosen localities in South Africa.

Locations within the two Australian states with known CBS presence, New South Wales (Cobb, 1897; Kiely, 1960; Bertus A.L., 1980) and Queensland (Wager, 1945), are consistently included in the potential distribution of the disease when using a similarity level ≥60% to model the potential for establishment of CBS. Regions of Australia known to be free of CBS were not modelled as areas of potential occurrence at this similarity level. It is interesting to note that the inland localities of NSW (Bourke and Narromine), where the pathogen has been introduced and where it has not been able to establish, are not mapped as localities where the disease might occur. This verifies the hypothesis that a similarity index of ≥60% discriminates between

localities where the climate is conducive for the establishment of the disease and those localities where the climate is not suitable for CBS establishment.

None of the 285 locations in Europe had a similarity of ≥60% when compared with any of the 16 chosen locations circumscribing positive CBS occurrence in South Africa. Consequently, it appears that the disease will not be able to establish itself in Europe. The knowledge that the disease does not currently occur in Europe supports these findings (European Union, 1998). Moreover, South Africa has been exporting citrus to Europe since 1906 (Oberholzer, 1969) and CBS has been present in South Africa from 1929 (Doidge, 1929). However, restrictions on the import of citrus from South Africa to the EU were first enforced in 1977 (European Union, 1977) and specific restrictions on imports from CBS infected areas were only introduced in the 1990s (European Union, 1992). Presumably CBS infected fruit entered the European Union over the 48-year period of free trade in citrus from CBS infected areas. During this time CBS did not establish in Europe. In practice, this situation is similar to that experienced in South Africa in terms of free-trade of citrus fruit between the rest of the country and the Western Cape region, where the disease has similarly not established to date.

Finally, it should be taken into consideration that the Match Climates Function in CLIMEX only considers the similarity in the meteorological data. The species requirements and interactions are not taken into consideration. Therefore outputs from this model should be interpreted with caution, and only serve as a rough first estimation for the potential establishment of a species.

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