Expanding citrus market access using a systems approach to control black spot

Andrew Miles
Department of Employment, Economic Development & Innovation

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Purpose of Project:
The aim of this project was to develop, implement and evaluate a systems approach for the management of citrus black spot (CBS) (*Guignardia citricarpa*) to facilitate the export of citrus from CBS endemic regions in Australia to markets such as the USA. CBS and fruit fly are both considered quarantine organisms by the USA, and the presence of either in a citrus production area currently limits fresh citrus exports. It is likely that fruit fly quarantine restrictions can be overcome by currently available preharvest and postharvest treatments, but there are currently no equivalent protocols for overcoming the CBS barrier to market access. The systems approach will utilise recognised integrated disease management practices for CBS to facilitate negotiations for market access to countries such as the USA; a desirable market for Australian fruit due to seasonality and exchange rates.

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Media Summary

Citrus fruit grown in certain areas of Australia (e.g. Central Burnett, Qld) can be externally blemished by a fungal disease called 'citrus black spot' (CBS). Whilst the effects of CBS are typically only cosmetic, the occurrence of the fungus in these areas prevents the export of fruit to certain destinations where the disease is not known to occur; such as parts of the United States and New Zealand. Whilst CBS is under successful routine management in the Central Burnett, the potential to more intensively and sustainably control the disease to standards acceptable to foreign quarantine agencies was explored by this project to facilitate market access negotiations.

The control of CBS currently relies on the application of fungicides to developing fruit to prevent infection by the fungus. However, past researchers have generated evidence for improved CBS control using cultural practices such as the application of mulch over the leaf litter from which the fungal spores are liberated, and regular tree pruning to promote tree health, improve fungicide effectiveness and reduce the favourability of the canopy microclimate to fungal growth. Based on this evidence a CBS management system was devised and trialled under commercial conditions in the Central Burnett district to demonstrate the low levels of CBS that can be achieved by integrated cultural and improved chemical practices.

The commercial scale trials conducted during this project demonstrated that ≥99.7% of premium graded fruit harvested from blocks managed by a combination of improved fungicide practices, application of mulch over leaf litter, and annual selective hand pruning, were free of infective CBS lesions. In addition to improved CBS control, mulch application and annual pruning are expected to improve control of other citrus diseases and pests, soil health and water use efficiency.

Technical Summary

Citrus produced in areas of Australia where citrus black spot (CBS), caused by *Guignardia citricarpa*, is endemic currently faces market access restrictions to countries for which *G. citricarpa* is exotic. In particular, mandarins produced in the Central Burnett region of Queensland are unable to be exported to potentially lucrative markets such as the USA. As no highly efficacious postharvest disinfestation methods exist for CBS-infected fruit, intensive field control of the pathogen using integrated disease management (IDM) is the most likely means of producing fruit for export that are free of *G. citricarpa* infection.

As the CBS disease cycle provides an opportunity for the implementation of IDM using a combination of cultural and chemical strategies, commercial scale field trials were undertaken in this project on 'Murcott' tangor (*C. reticulata* × *C. sinensis*) in the Central Burnett to demonstrate the efficacy of IDM. The IDM strategy combined a number of treatments previously demonstrated to reduce CBS infection in the field; 1) the suppression of ascospore inoculum by the application of mulch over fallen leaves (Schutte and Kotze 1997); 2) hand pruning (Loest 1968); and 3) fungicide application (Kiely 1967; Miles *et al.* 2004; Schutte *et al.* 1997). Also undertaken was a district-wide survey of the occurrence of *G. citricarpa* infection in 'Murcott' blocks in the Central Burnett to characterise the distribution of the disease in a commercial production district undertaking routine CBS management. In addition, the distribution in the tree canopy of fruit infected by *G. citricarpa* was studied to determine a CBS sampling strategy, along with the effect of harvest time on the postharvest expression of CBS in fruit and whether expression could be enhanced by the treatment of fruit with ethephon.

IDM of CBS was found to produce fruit nearly completely free of CBS; even after CBS expression was maximised by incubation of the fruit in a manner highly unlikely to be experienced in a commercial supply chain. The specified fungicide, pruning and mulching regime produced export specification fruit that ranged from ≥99.69 to ≥99.99% (95% confidence) free of CBS lesions containing reproductive structures (lesions with pycnidia) after incubation, and ≥98.5% (95% confidence) free of any lesions possibly caused by *G. citricarpa* (i.e. lesions with and without pycnidia). We have also shown that even in the absence of IDM, the distribution of CBS in the Central Burnett was greatly skewed towards the majority of blocks in the district having an incidence of 5% or less fruit with CBS; a distribution found to be best represented by an Inverse Gaussian or Beta General model. Also demonstrated was the ability to achieve maximum CBS expression in fruit harvested up to 9 weeks prior to commercial harvest, providing up to 6 weeks notice to growers of the CBS incidence in their export blocks. It was also found that systematic sampling of fruit from all regions of the tree canopy is preferable to sampling canopy regions favouring symptom expression but not necessarily favouring infection.

Further improving CBS control for export markets is likely to be challenging, however, aspects of preharvest control that could be improved in future studies include usage of fungicide chemistries such as strobilurins (Miles *et al.* 2004), and more accurate timing of protectant fungicide (e.g. copper, mancozeb) application based on rates of fruit expansion (Timmer *et al.* 1998); both of which are yet to be adopted in Australia.

Whilst the IDM strategy implemented in this project was investigated for export purposes as part of a systems approach, the strategy represents a best practice protocol for CBS management in general, that is likely to benefit the industry if able to be more widely adopted; particularly if mulching and pruning can lead to a reduced reliance on fungicides for CBS control. Benefits from mulching are also likely to include increased tree vigour and yield (BangChu *et al.* 2007; Huang and Liu 1987; Ingle *et al.* 2001; Mohanty *et al.* 2002; Patil *et al.* 2002; Verdu and Mas 2007). However, it is recognised that implementation of IDM for CBS is linked to the cost and availability of labour and mulch.

Introduction

Since 1992 citrus from certain southern production districts of Australia has been exported to the United States (Biggs 2001), largely made possible because of the absence from these areas of important quarantine threats to the USA such as citrus black spot (CBS) and fruit fly. In Australia CBS only occurs in parts of Queensland, the Northern Territory and coastal New South Wales, where summer rainfall is prevalent. Extensive surveys have demonstrated an absence of CBS from the inland, winter rainfall areas of the Riverland (South Australia), Sunraysia (New South Wales and Victoria border), and Riverina (southern New South Wales) regions, resulting in the internationally recognised area freedom status of these areas (Barkley 1988; Broadbent 1995; Corporate Author 1998; Wall 1989). Internationally, CBS is an economically important disease in some citrus producing regions of South America, Africa and Asia (Calavan 1960; European and Mediterranean Plant Protection Organization 2009; Kiely 1948; Korf *et al.* 2001; Kotze 1981; McOnie 1964; Wager 1952), and has recently been discovered in Florida, USA (Bouffard 2010). The presence of CBS and fruit fly in Queensland, and the Central Burnett citrus district in particular, has prevented market access to the USA and other markets such as New Zealand. Another disease, citrus scab (*Sphaceloma fawcettii*), is also a potential concern to foreign countries due to the existence of a number of different strains of the scab pathogens around the world (Donovan *et al.* 2009; Hyun *et al.* 2009; Tan *et al.* 1996; Timmer *et al.* 1996; Whiteside 1978). However, citrus scab in Australia is primarily of concern only in lemon varieties (Broadbent 1995; Hyun *et al.* 2009). Whilst extensive research has been undertaken for the area wide management of fruit fly (*Bactrocera tryoni*) in the Central Burnett (Lloyd *et al.* 2010), and incorporated with highly efficacious postharvest disinfestation treatments to satisfy market access requirements (De Lima 1993; De Lima *et al.* ; Heather *et al.* 1996; Hill *et al.* 1988), the potential to manage CBS to market access acceptable levels has not been demonstrated. In January 2004 this project was commissioned to investigate the potential of integrated disease management (IDM) strategies to reduce CBS to quarantine acceptable levels. In the project planning phase it was decided that 'Murcott' tangor (*C. reticulata* × *C. sinensis*) would be the most suitable variety for export, based on extensive plantings, suitable shelf life, and existing consumer-base in the USA. Increased market access for citrus from CBS-endemic areas would significantly increase the profitability of citrus production in these areas.

CBS is caused by the fungus *Guignardia citricarpa* (Kiely). The fungus infects fruit in the field during the first 20-24 weeks of fruit development (Baldassari *et al.* 2006; Kotze 1981; Wager 1952), but after infection the fungus remains in a dormant state, generally not producing symptoms until after harvest (McOnie 1967). The latent nature of *G. citricarpa* infection makes field management difficult, as the consumer rather than the grower may be the one to see any symptoms produced. The symptoms produced by *G. citricarpa* are variable, with up to five lesion types having been described (de Goes *et al.* 2000; Kiely 1948). The classic CBS lesion is the 'hard spot' (Fig. 1a) and is typically observed as red to black-rimmed depressed lesions with a light grey or brown centre on maturing citrus fruits (Kiely 1948; McOnie 1964). Hard spot has been considered the least severe form of CBS, and reportedly has its development halted by the host, and the pathogen eventually killed (Kiely 1948). 'Freckle spot' (Fig. 1b) is characterised as slightly depressed, orange to brick red spots, but unlike hard spot the lesion development is not halted by the host, and

further infection can occur. Freckle spot can develop into 'virulent spot' (Fig. 1c,e); the most severe lesion type. These lesions are large, involve the entire thickness of the rind, and cause extensive fruit drop. The other fruit symptoms are 'speckled blotch' (Fig. 1d) and 'cracked spot', the latter of which has not been reported in Australia. Speckled blotch is reported to occur when weather conditions in late summer and early autumn are abnormally hot and dry. CBS can also be observed on leaves, typically appearing as hard spot-like lesions, often accompanied by a chlorotic halo. An important diagnostic feature of CBS lesions is the production of small, black, raised fruiting bodies ('pycnidia') within the lesion (Fig. 1f); particularly hard, freckle and virulent spots. However, accurate diagnosis by eye can be difficult if pycnidia are absent from a lesion, or lesion formation is ambiguous. Therefore methods to increase symptom expression and pycnidia formation would be beneficial in diagnosis. High storage temperature (~27ºC), humidity (80%) and 24 hour fluorescent lighting have been shown to accelerate symptom development (Brodrick and Rabie 1970; Korf 1998; Timossi *et al.* 2003), as well as treatment with ethephon (Baldassari *et al.* 2007). However, the effect of ethephon treatment on symptom production, at different times before and after commercial harvest, has not been evaluated in Australia. Diagnostic accuracy in this project was also improved by the use of a DNA-based detection assay which was developed by a Queensland government funded project (Asian Markets for Horticulture Initiative – "Enhancing citrus black spot management to facilitate market access opportunities for Queensland citrus") undertaken in conjunction with this project.

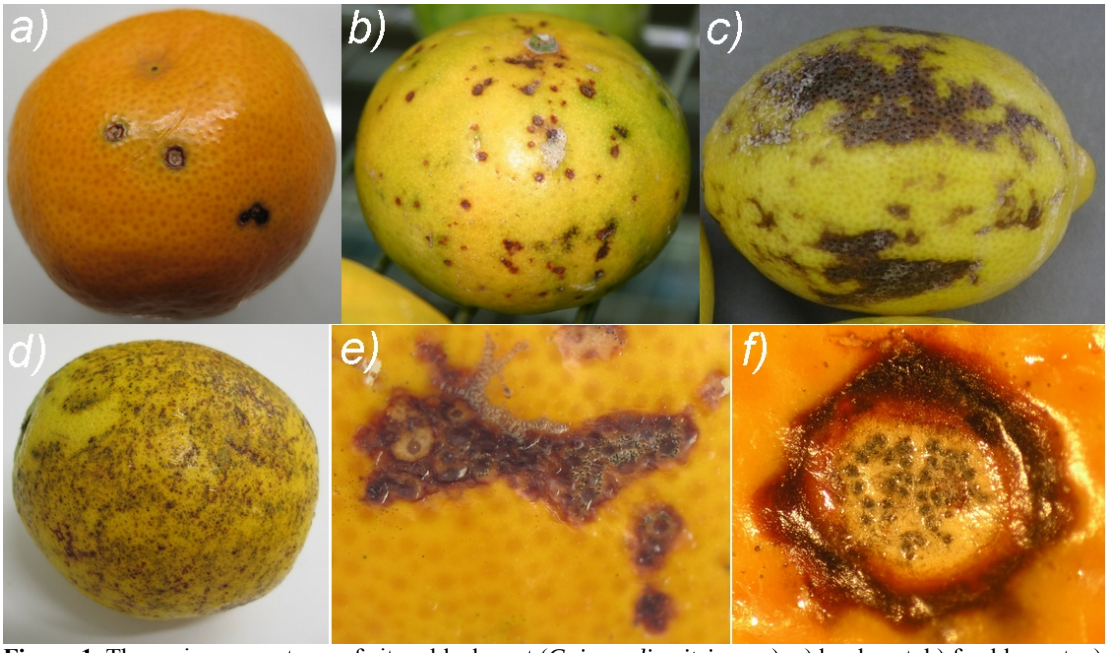

Figure 1. The various symptoms of citrus black spot (*Guignardia citricarpa*): a) hard spot, b) freckle spot, c) virulent spot, d) speckled blotch, e) virulent spot with pycnidia, and f) pycnidia contained within a hard spot lesion.

IDM of CBS is possible based on existing knowledge of the disease cycle (Fig. 2). The primary disease cycle involves windborne 'ascospores' (sexually reproducing stage) of the fungus that are ejected from fruiting bodies ('ascomata'/'perithecia') formed on decaying leaf litter, particularly during conditions of wetting and drying (Kiely 1948). When an ascospore lands on a young fruit or leaf, it geminates, forms

infection structures (appressoria and infection peg), and penetrates the epidermis (Kiely 1948; Kotze 1981). At this point, the fungus will survive asymptomatically as a small knot of mycelium between the cuticle and the epidermis (Brodrick and Rabie 1970; McOnie 1967). The length of the latent period following infection is influenced by fruit maturity, temperature, light intensity, water status and tree vigour (Kiely 1948; Kotze 1981). When latency breaks, the fungus commences actively parasitising the host cells resulting in lesion formation. The secondary disease cycle involves the production of pycnidiospores (asexually reproducing stage) from within the pycnidia described previously as forming within CBS lesions (Kiely 1948; Wager 1952). Pycnidiospores (or conidia) can also be found on dead twigs, leaves, and occasionally on fruit stalks (Kotze 1981). The pycnidiospores are released from the pycnidia after a few minutes of exposure to water (Wager 1952), facilitating water drip/splash dispersal. Pycnidiospores are considered likely to only be important for fruit infection when symptomatic fruit remain on the tree long enough to coincide with the next crop (Kiely 1948; Kotze 1981). The CBS disease cycle is completed when infected leaves abscise from the canopy to the orchard floor and eventually produce more ascospores. An initial wilting of a fallen leaf, followed by cycles of wetting and drying greatly enhances the production of perithecia on leaves (Kiely 1948). Formation of perithecia and ascospores on fruit has never been observed.

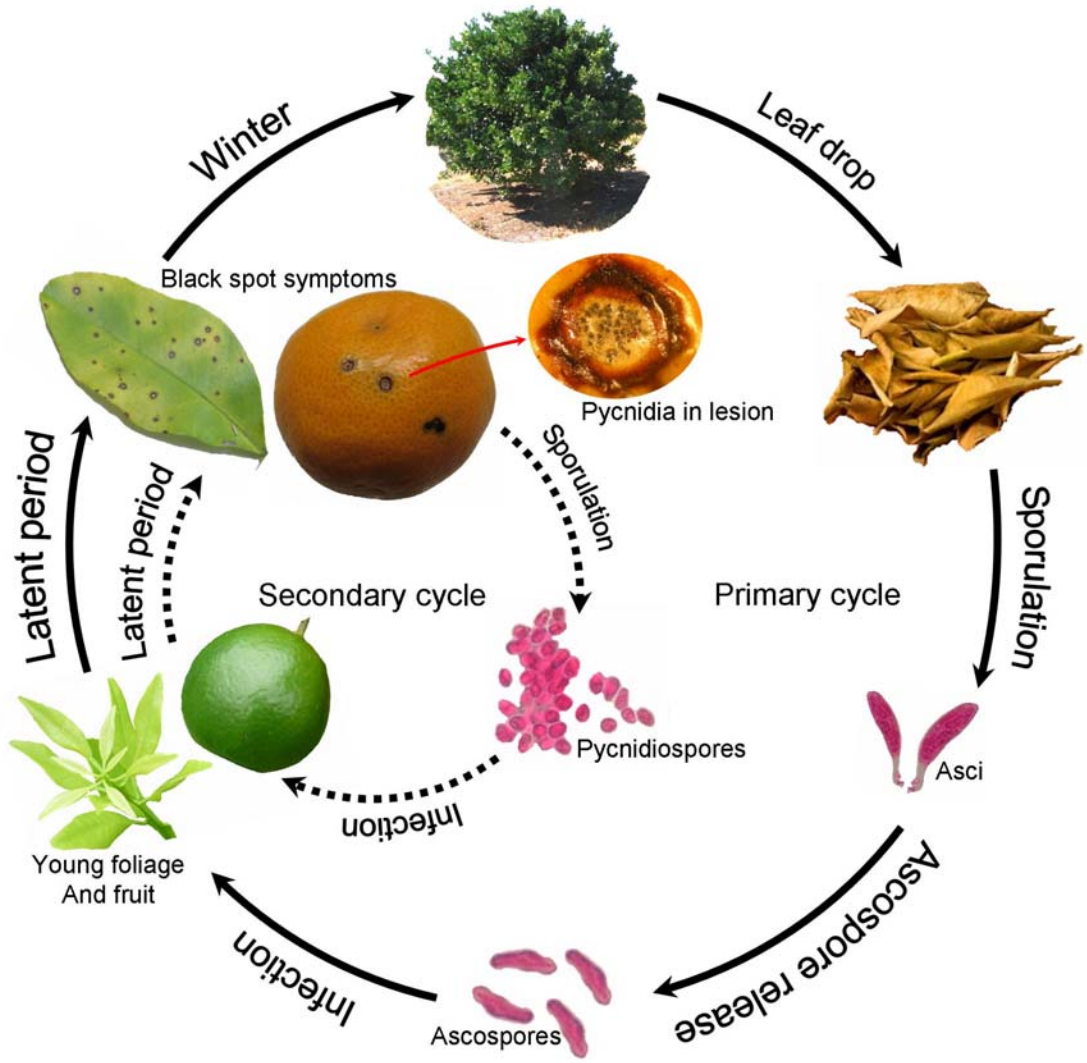

Figure 2. The citrus black spot (*Guignardia citricarpa*) disease cycle.

The application of fungicides to prevent the fungus infecting fruit is the industry standard approach to CBS control, and would form an important part of an IDM system (Fig. 3). The efficacy of protectant (e.g. copper and dithiocarbamate) and systemic (e.g. benomyl) fungicides has been extensively studied for CBS, and found to be effective when applied at the appropriate stage in fruit development (Agostini *et al.* 2006; Beattie *et al.* 1989; Bertus 1981; Kiely 1950; 1967; 1976; Miles *et al.* 2004; Rodriguez and Mazza Gaiad 1996). For 30 years benomyl was a very effective addition to the fungicide options for CBS control, but by the end of 2006 the supply and use of benomyl was prohibited by the Australian Pesticides and Veterinary Medicines Authority. As a result, copper and dithiocarbamate protectant fungicides are the only fungicide options for CBS control in Australia. For these fungicides to be effective it is crucial that fruit are well covered with the fungicide prior to pathogen challenge. Application of these fungicides after infection is of little value. The typical fungicide regime for CBS control consists of two copper applications commencing at petal fall, followed by up to four dithiocarbamate applications; the number and frequency of which varies with disease pressure/history, weather conditions etc.

However, management of CBS to market access levels may require an increased number and frequency of protectant fungicides in order to maintain the coverage required to be effective. This is particularly notable when considering the effect of fruit expansion on coverage, whereby increases in fruit diameter of 40% over 2 weeks, and 50% over one month have resulted in metallic copper residue reductions of ~70% and ~90%, respectively, due to the disproportionate rate at which fruit surface area increases relative to diameter (Timmer *et al.* 1998).

To a far lesser extent than chemical control for CBS, the efficacy of cultural control methods with potential as components of an IDM strategy for CBS control have been studied. These cultural control practices include inoculum reduction and canopy management (Fig. 3). A reduction in the amount of ascospore inoculum through the application of a layer of grass mulch over fallen leaves on the orchard floor has been reported to increase the production of CBS-free fruit by approximately 20% (Schutte and Kotze 1997). The mulch layer is believed to act as a physical barrier to prevent the release of ascospores into the air. This approach has also been effective in reducing leaf litter-borne inoculum in apples (Holb 2006). Further to mulching, there is evidence that canopy management can reduce CBS. Pruned lemon trees were found to have significantly less fruit with CBS (Loest 1968). The exact reason for the reduction in CBS is not explicitly known, but reduced susceptibility of healthy, vigorous hosts has been suggested; as such pruning has been recommended to improve tree vigour (Calavan 1960; Kiely 1950; Kotze 1961; Loest 1968). Pruning may also remove potential sources of pycnidiospore inoculum such as dead twigs, as well as improve fungicide penetration by reducing canopy density, a factor affecting spray application efficiency (Stover *et al.* 2002). As a result of the above findings, these various control options are suggested for CBS control in commercial orchards (Kotze 2000; Mayers and Owen-Turner 1987).

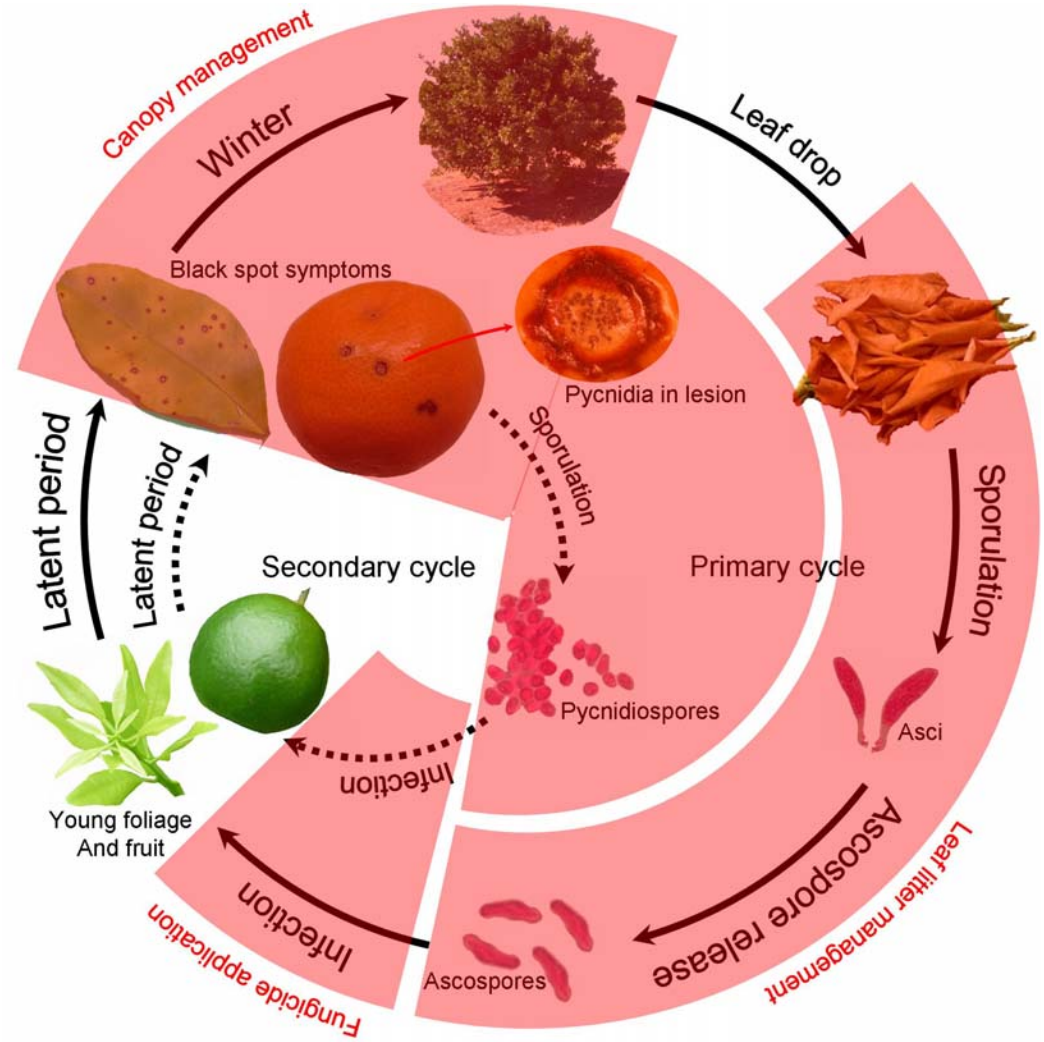

Figure 3. The components of an integrated disease management strategy for citrus black spot (*Guignardia citricarpa*) control (red shading) as related to the disease cycle; fungicide application to prevent infection, leaf litter management to suppress ascospore inoculum, and canopy management to reduce pycnidiospore inoculum and improve tree vigour.

As well as providing control of CBS, the fungicide and pruning components of the proposed IDM strategies for CBS are also likely to be effective against scab; any benefits of mulching for scab control are likely to be an indirect result of improved tree health through mulching. Fruit are most susceptible to infection by *S. fawcettii* for the first 6-8 weeks after petal fall (Timmer 2000; Timmer *et al.* 2003). At this time, the fungicides that are being applied for CBS control would also be protecting against *S. fawcettii* infection if the pathogen is present (Mondal *et al.* 2007). Pruning may also reduce scab through the removal of any existing scab pustules from which spores are produced (Timmer 2000), as well improving fungicide penetration, reducing the favourability of the canopy microclimate and maintaining tree vigour. In addition to the efficacy of fungicides and pruning, the occurrence of scab on 'Murcott' tangor in Australia has never been confirmed, probably due an absence in Australia of *S. fawcettii* pathotypes pathogenic to 'Murcott' (Hyun *et al.* 2009).

In addition to IDM strategies to control CBS, market access requirements for CBS have been met in the past by preharvest fruit sampling and exporting fruit harvested

only from certain portions of the canopy believed to be less likely to harbour CBS-infected fruit. In Australia, market access to Japan incorporated preharvest fruit sampling and incubation as well as a specific harvesting procedure. The preharvest sampling was undertaken to indicate the level of CBS in the candidate export block prior to commercial harvest. It is likely that future market access protocols would also require preharvest sampling and incubation, however it is unclear how soon before commercial harvesting sampling can be undertaken. Growers and exporters need to know as far in advance as possible if a particular block of trees will be eligible for export, however it is unknown if the amount of time before sampling is likely to effect the incidence of CBS in the sample. The specific harvesting procedure used for the Japanese market avoided picking any fruit from the inner and skirt areas of the canopy where fruit were considered to be more likely to be infected (Mayers and Owen-Turner 1989). A more recent study also reports that diseased fruit tend to be more common in the middle and lower canopy positions (Sposito *et al.* 2008). Regulatory bodies have in the past required that fruit be sampled for CBS inspection from the portion of the canopy where infected, symptomatic fruit are most likely to be found; in this case suggesting near the outer, upper part of the canopy on the sides of the tree that receives the most sunlight (Anonymous 2000). However, this is in contradiction to Mayers and Owen-Turner (1989) and Sposito *et al* (2008) reporting infection to be more likely in the inner and skirt canopy positions, as well as Kiely (1948) that reported the distribution of CBS in trees to be considered uniform, with fruit from the north to north-western sector of the tree expressing more severe symptoms earlier due to a more favourable microclimate for symptom development (Kiely 1948). Considering the existing inconsistencies, further work should be undertaken to clarify the distribution of CBS-infected fruit in the tree canopy.

The distribution of the incidence of CBS at the district level (i.e. mow many orchards have 0, 10 20 or 50% etc of fruit infected with *G. citricarpa*) also requires clarification, as the mathematical models having been used to predict the distribution for import risk analysis purposes may overestimate the amount of disease present. This overestimation is likely to inflate the likelihood of introduction of the disease, and negatively impact on the chances of attaining market access. Therefore, the true distribution should be estimated experimentally and compared with different mathematic models to identify which models are most appropriate for import risk analysis.

In order to investigate the potential of IDM to control CBS to acceptable levels for market access we sought to answer the following questions: 1) does canopy position influence CBS infection enough to warrant a targeted sampling/harvesting procedure, 2) does sampling time and ethephon treatment influence CBS expression, 3) what is the distribution of CBS in the Central Burnett production district, 4) what is the incidence of CBS and scab under a specified fungicide and pruning regime, 5) what is the incidence of CBS and scab under a preliminary fungicide, pruning and mulching regime, and 6) what is the incidence of CBS and scab under a specified fungicide, pruning and mulching regime? Answering these questions will provide the Australian citrus industry and Biosecurity Australia much of the necessary information required for the negotiation of market access for citrus from an area such as the Central Burnett.

Materials & Methods

Influence of canopy position on disease incidence

To determine if citrus black spot (CBS) (*Guignardia citricarpa*) and scab (*Elsinoe* sp.) incidence in the field are influenced by the position of fruit in the canopy, with possible implications for a survey sampling strategy, disease was assessed in fruit sampled from various positions within the canopy. In a single production year a field experiment was conducted at three commercial orchards near Gayndah and Mundubbera. At each of the three orchards four replicate trees were selected for the experiment within a plot of approximately 50, 16 to 20-year-old 'Murcott' tangor (*C. reticulata* × *C. sinensis*) trees having not received any fungicides for the first 20 weeks of fruit development; allowing natural infection by *G. citricarpa* and *Elsinoe fawcettii* while fruit are susceptible (Baldassari et al. 2006; Kotze 1981; Timmer et al. 2003; Timmer et al. 2000; Wager 1952). Ten fruit were sampled from each of 16 canopy positions based on north, south, east and west compass points × inner, outer, upper and lower portions, from each replicate tree. Sampled fruit were returned to the laboratory and incubated at ~27ºC, 80% relative humidity and 24 hour fluorescent lighting for 3 weeks to break latency of any *G. citricarpa* infections (Fig. 4) before disease assessments were made.

Figure 4. Fruit holding in a controlled environment room with constant lighting.

Influence of sampling time and ethephon treatment on citrus black spot expression

To determine the influence of fruit sampling time and ethephon treatment on the expression of CBS symptoms, fruit were sampled at different times before commercial maturity and treated with and without ethephon prior to incubation to break latency. Pairs of fruit were sampled from each of the 16 previously described canopy positions of 5 trees within a plot of approximately 50, 16 to 20-year-old 'Murcott' tangor trees having not received any fungicides for the first 20 weeks of fruit development; allowing natural infection by *G. citricarpa* while fruit are susceptible (Baldassari et al. 2006; Kotze 1981; Timmer et al. 2003; Wager 1952). Replicate fruit samples were collected from three commercial orchards near Gayndah and Mundubbera at 9, 5, 3 and 1 week prior to, and 1 and 3 weeks after, commercial maturity in each production year for a total of 2880 fruit for the experiment. At each

sampling time half the fruit were dipped for 5 minutes in 750ppm ethephon (Ethrel®, Bayer CropScience Pty Ltd, Australia), and the other half remained untreated. Dipped fruit were then air dried and incubated along with the untreated fruit to break latency of any *G. citricarpa* infections before disease assessments were made. The experiment was repeated over two consecutive production years.

Distribution of citrus black spot and scab in the Central Burnett citrus production district

To determine the distribution of CBS and scab in the Gayndah/Mundubbera citrus production district, a survey was undertaken of fruit sampled from blocks representative of the district. In total 22 blocks (Fig. 5.) of 'Murcott' tangor trees under routine commercial fungicide treatments (Table 1) were selected for survey over the 2004-05 to 2007-08 production years. Five trees were randomly chosen from across each block for sampling 4-6 weeks prior to commercial harvest in each production year. Two pieces of fruit were randomly selected from each of the 16 previously described canopy positions of each tree, for a total of 160 fruit per block. Sampled fruit were returned to the laboratory and incubated to break latency of any *G. citricarpa* infections before disease assessments were made. In addition to determining the CBS incidence for each block, data were gathered where possible for variables likely to affect CBS incidence at each site; namely 1) age of the trees surveyed, 2) age of the oldest block on the orchard, 3) number of fungicides applied between fruit set and the end of March, 4) distance from the orchard to the next nearest orchard, 5) the township (Gayndah or Mundubbera) the survey orchards were located nearest, 6) high (>6000 l/ha) or low (<6000 l/ha) fungicide application volume, and 7) the CSIRO soil classification of the orchard (De Mooy *et al.* 1977).

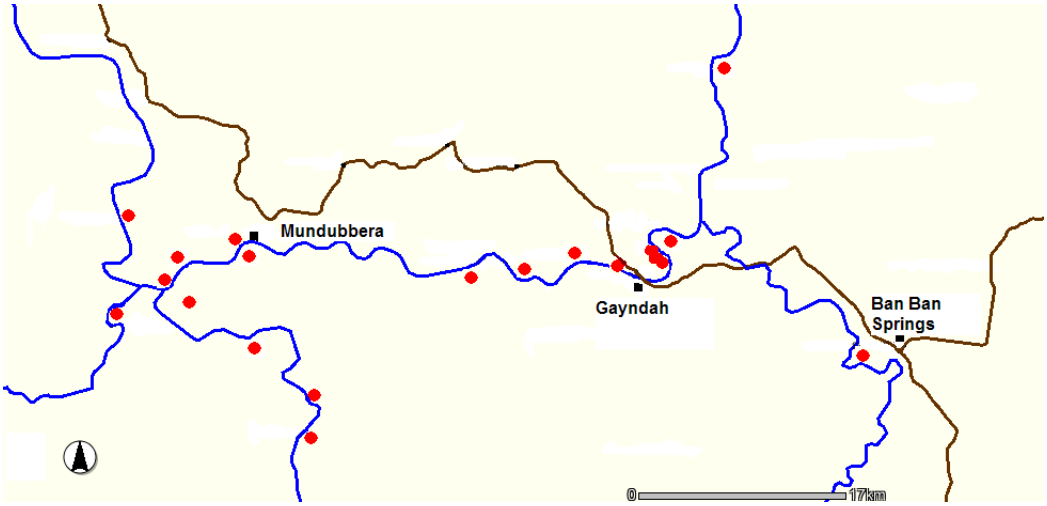

Figure 5. Map of the Central Burnett district showing the locations of the district survey sites (red dots), relative to the townships of Mundubbera and Gayndah. Note that in the case of two locations, two sites were surveyed at each location.

Table 1. The range of routine commercial fungicide treatment regimes typically adopted in the Central Burnett district for control of fungal pathogens during the first 20 weeks of fruit development.

Approximate weeks after petal-fall	Lower spray frequency blocks	Higher spray frequency blocks
0	Copper fungicide	Copper fungicide
1-2		Copper fungicide
4-6	Dithiocarbamate fungicide	Dithiocarbamate fungicide
8	**Fruit increasing in resistance to *S. fawcettii*** †	
8-10		Dithiocarbamate fungicide
10-12	Dithiocarbamate fungicide	
12-14		Dithiocarbamate fungicide
16-18		Dithiocarbamate fungicide
>20	**Fruit increasing in resistance to *G. citricarpa*** Ω	

†(Timmer 2000; Timmer *et al.* 2003)
Ω(Baldassari *et al.* 2006; Kotze 1981; Wager 1952)

Incidence of citrus black spot and scab under a specified fungicide and pruning regime

To determine the incidence of CBS and scab in commercial orchards in the Central Burnett production region under a specific fungicide application and pruning program for potential export markets, large scale disease assessments were made of fruit sampled from treated commercial-scale plots. The representative plots of 'Murcott' tangor were located near the townships of Gayndah (a and b) and Mundubbera (Table 2), and assessed in the 2004-05 and 2005-06 production years.

Table 2. Details of the case study blocks located in the Central Burnett production region of Queensland, Australia.

Location (latitude, longitude)	Planting year	Rootstock	Plot design (rows × trees)	Spray volume (L/tree)	Copper & dithiocarbamate fungicides
Gayndah (a) (-25.615515, 151.631756)	1984	Troyer (*C. sinensis* × *Poncirus. trifoliata*)	14 × 23	24	cupric hydroxide (37.5 g/100 L) mancozeb (160 g/100 L)
Gayndah (b) (-25.612013, 151.588830)	1985	Troyer and Swingle citrumelo (*C. paradisi* × *P. trifoliata*)	13 × 29	23	cuprous oxide (37.5 g/100 L) mancozeb (160 g/100 L)
Mundubbera (-25.718493, 151.343343)	1988	Troyer	14 × 25	36	cupric hydroxide (35 g/100 L) mancozeb (160 g/100 L)

The trees at each site were all selectively hand pruned prior to flowering in each production year. The fungicide program consisted of a copper fungicide application at 0-10% and 75-100% petal fall, followed by four dithiocarbarmate fungicide applications every 4 weeks thereafter to protect fruit during the period of susceptibility. At each site in the 2004-05 production year an untreated control plot of

12-16 trees (3-4 trees long by 4 rows wide), surrounded by a buffer zone of two additional trees deep, was located adjacent to the treated trees and left unsprayed with fungicides during the period of susceptibility. In the 2005-06 production year the untreated plots were moved to another block on the orchard to avoid a build-up of inoculum directly adjacent to the fungicide treated trees. Fruit were harvested from the treated and untreated trees 8 weeks prior to commercial harvest in 2004-05, and 1-3 weeks prior to commercial harvest in 2005-06, from every second tree where possible in the plots to improve sampling accuracy under conditions of low disease incidence and non-uniform disease distribution, as has been reported for CBS (Sposito *et al.* 2007). In 2004-05, a total of 100 trees were sampled from each treated block, with 15 fruit sampled from two of the previously described 16 canopy positions of each tree, for a total of 3000 fruit per plot. A total of eight trees were sampled from the untreated plot, with 25 fruit sampled from four of the 16 canopy positions of each tree for a total of 800 fruit per plot in 2004-05. In 2005-06, a total of 44 trees were sampled from each treated plot, with two fruit from each of the 16 canopy positions, for a total of 1408 fruit per plot. In the untreated plots, five fruit were sampled from each of the 16 canopy positions of 8 trees for total of 640 fruit. Sampled fruit were returned to the laboratory and incubated to break latency of any *G. citricarpa* infections before disease assessments were made.

Evaluation of a preliminary fungicide, pruning and mulching regime for the control of citrus black spot and scab

In order to develop an effective regime of fungicide applications, pruning and mulching for the control of citrus black spot and scab to levels acceptable for export from the Central Burnett production region, best practice methods from existing literature (see introduction) were implemented in commercial scale trial plots, and refined over two production years. The representative blocks of 'Murcott' tangor were as specified in Table 2, but adjacent to the plots used in the previous experiment. Untreated control data from the previous experiment were used in this experiment also. The trees at each site were all selectively hand pruned prior to flowering in each production year. The plots were treated and assessed in the 2004-05 and 2005-06 production years, and treated with the same fungicide program as the previous experiment. Prior to flowering in the 2004-05 production year, hay mulch was applied under the trees along the length of each row of the treated plots, to the width of the drip line of the canopy, at a depth of mulch sufficient to cover any leaf litter (Fig. 6). A second application of mulch was required at two of the trial sites in 2004-05 due to excessive drop of senescing leaves remaining from winter, as a result of the high pressure spray application of the first fungicide applied for the production year. In 2005-06 the hay mulch was applied after the first fungicide application, relieving the need for a second mulch application. Fruit were harvested at the same time with the same sampling strategy as the previous experiment, except in 2005-06 where two fruit were sampled from each of the 16 canopy positions of 94 trees for a total of 3008 fruit per plot. At commercial harvest in the 2005-06 production year, the remaining fruit in the treated plots were commercially harvested, sorted and packed. To determine the incidence of CBS affected fruit after commercial sorting and packing, a sample of approximately 3000 export specification fruit from each of the treated plots at each trial site was collected at random intervals throughout the packing run. In the packing shed fruit received treatment with sodium orthophenylphenate (SOPP), followed by

de-scaling, treatment with carbendazim[1] and dimethoate[2], then imazalil impregnated wax. The sampled fruit were returned to the laboratory and incubated to break latency of any *G. citricarpa* infections before disease assessments were made.

Figure 6. Hay mulch applied under trees to cover any leaf litter and suppress the release of ascospores.

Incidence of citrus black spot and scab under a specified fungicide, pruning and mulching regime

To determine the incidence of CBS and scab in commercial orchards in the Central Burnett production region under a specified fungicide, pruning and mulching regime, large scale disease assessments were made of fruit sampled from treated commercial-scale plots. The representative blocks of 'Murcott' tangor were the same used in the previous experiment. The trees at each site were all selectively hand pruned prior to flowering in each production year. The plots were treated and assessed in the 2006-07 and 2007-08 production years. The fungicide regime consisted of a copper fungicide application at 75% petal fall, followed by five dithiocarbamate fungicide applications every 3 weeks. Hay mulch was applied between the application of the copper fungicide and the first dithiocarbamate fungicide application. The mulch was applied under the trees along the length of each row of the plot, to the width of the drip line of the canopy, at a depth of mulch sufficient to cover any leaf litter. Untreated control plots of 50 trees (two rows of 25), which did not receive any CBS management treatments were established adjacent, but not immediately next to each of the treated plots. Fruit were harvested from the treated and untreated trees 3-4 weeks prior to commercial harvest using the same sampling strategy as the previous experiment (treated: 94 trees × 2 fruit × 16 positions = 3008 fruit; untreated: 8 trees × 5 fruit × 16 positions = 640 fruit). At commercial harvest in each production year the remaining fruit in the treated plots were commercially harvested, sorted and packed. To determine the incidence of CBS affected fruit after commercial sorting, a sample of approximately 3000 export specification fruit from each of the treated plots at each trial site was collected at random intervals throughout the packing run. Sampled fruit were returned to the laboratory and incubated to break latency of any *G. citricarpa* infections before disease assessments were made.

[1] As of January 2010 the use of carbendazim in citrus production was prohibited by the APVMA

[2] Chemical usage currently under review by the APVMA

Disease assessment

To determine the incidence of CBS and scab in the sampled fruit, fruit were individually assessed by eye and light microscope, and the presence or absence of symptoms recorded. Disease incidence was determined as the proportion of fruit with one or more CBS or scab lesions. In all the experiments lesions were diagnosed as CBS if pycnidia of *G. citricarpa* were present on the lesion surface after incubation (European and Mediterranean Plant Protection Organization 2003). In experiments conducted in the 2004-05 and 2005-06 production years symptoms were diagnosed as CBS based on the visual comparison to the described CBS lesion types, including those symptoms without pycnidia (de Goes *et al.* 2000; European and Mediterranean Plant Protection Organization 2003; Kiely 1948; 1960; Kotze 1981; 2000). In the 2006-07 and 2007-08 production years, diagnosis of lesions without pycnidia relied on a realtime PCR assay for *G. citricarpa* based on an existing protocol (van Gent-Pelzer *et al.* 2007) modified to include an internal control (Li *et al.* 2006) and locally validated (van Brunschot *et al*. *unpublished*). A subsample of lesions without pycnidia from all the CBS field experiments in each production year was taken for testing; 39 lesions in 2006-07, and 115 lesions in 2007-08. In each production year, the proportion of tested lesions without pycnidia that were positive for CBS using the realtime PCR assay was used to extrapolate the final number of lesions without pycnidia likely to have been caused by *G. citricarpa* in the total sample. *E. fawcettii* infection in all production years was diagnosed based on the visual comparison to described scab lesions (Fawcett 1936; Timmer 2000).

Weather data

To account for any effects of weather on the incidence of CBS over the four experimental production years, daily rainfall and temperature data (Gayndah only) were obtained from the Bureau of Meteorology from October 2004 until October 2008 for both townships. The number of raindays (days for which rainfall was recorded) were determined as ascospore release occurs during rain spells, with the amount of rain having little effect on the number of spores released (Kotze 1981). To evaluate the effect of temperature on disease development each year, daily temperature data (Gayndah) were used to determine the number of heat units suitable for disease (CBS) development using the formula [(maximum daily temperature + minimum daily temperature) ÷ 2] – cold threshold temperature (Lovell *et al.* 2004). The cold threshold temperature used was 11ºC, and the maximum temperature threshold used was 40ºC as determined based on weather parameters found to fit the global distribution of CBS (Paul *et al.* 2005).

Data analysis

Disease incidence data from the canopy position experiment were analysed using GenStat 8.1 (GenStat 2005) as a split plot design with upper/lower and inner/outer positions nested within compass position. Means were compared using Fisher's protected Least Significant Difference test (LSD).

Disease incidence data for the sample time and ethephon treatment experiment for fruit with pycnidia (and/or speckled blotch) after 20 days was analysed as a split plot design (timing split for ethephon treatment); an arcsin transformation was applied to the data to stabilize the variance.

District survey data were summarised as the number of orchard blocks falling into various disease incidence classes (e.g. 0%, 0.01 to 5.00% etc) for each production year, and disease incidence for each survey block in each year presented in a histogram. To identify a mathematical distribution that bests fits the survey data, the data from all seasons were applied to a range of distributions (Inverse Gaussian, Beta General, Beta Subjective, Person5, Pert, Exponential, Extreme Value, Logistic, Normal, Triangular and Uniform) using @Risk 5.5 (Palisade Asia-Pacific Pty Ltd, Australia) and the goodness of fit tested by chi-square analysis. For the data gathered for variables possibly explaining the amount of CBS, quantitative data were summarised using scatterplots and explored by correlation analysis, multiple linear regression and principal component analysis. Qualitative variables were summarised by boxplots.

Data for the field control regime trials were summarised for each site and tabulated as the total number of fruit assessed (after losses to mould or breakdown disorders other than CBS), total number of fruit with CBS, and percent of fruit with CBS for each site and production year. An upper confidence limit for the true unknown disease proportion (p_u) based on the number of diseased fruit in each of the samples of fruit was determined based on the following formula for determining p_u (Cannon and Roe 1982; Couey and Chew 1986): $\sum_{x=0}^{x=s} \frac{n!}{x!(n-x)!} p_u^x (1-p_u)^{(n-x)} = 1-C$ where n is the number of fruit sampled, s is the number of diseased fruit found, C is the confidence level used (95%). This equation is mathematically difficult to handle except in the simple case of no diseased fruit (s=0). To simplify the calculations for the case where there are diseased fruit this is approximated by the following equation and solved for p_u: $\sum_{x=0}^{x=s} \frac{e^{-np_u}(np_u)^x}{x!} = 1-C$ The approximation is preferable for large n and p close to zero: applicable to sample data from the plots in these trials where CBS management procedures are in place.

Any relationships between the number of raindays and number of CBS heat units, and the district survey data were explored by generating scatter plots of the seasonal (spring, summer, autumn, winter) weather data against the % CBS for each survey block. Promising relationships were further explored by correlation analysis.

Results

Influence of canopy position on disease incidence

During the disease assessment only symptoms of CBS were observed in the sampled fruit; no scab symptoms were observed. Analysis of the CBS incidence data from the canopy position experiment indicated that the three way interaction between compass position, inner/outer and upper/lower was significant (P = 0.031). The incidence of CBS was highest (30.7%) in the North/Inner/Lower position, but there was no significant difference between this position and the East/Inner/Lower and South/Outer/Lower positions (Table 3). There was no strong indication that sampling should be done preferentially from a particular canopy position rather than a systematic sampling strategy of the entire canopy.

Table 3. Mean percent of citrus black spot infected fruit at various positions within the tree canopy†

Compass	Inner		Outer	
Position	Lower	Upper	Lower	Upper
East	21.2	11.6	8.3	17.5
North	30.7	16.7	14.4	12.6
South	15.8	11.7	19.2	6.7
West	11.3	15.5	16.0	16.1

† LSD (95%) = 12.8 except when comparing means with the same compass × inner/outer position (= 10.3) or means with the same compass × upper/lower position (= 12.3)

Influence of sampling time and ethephon treatment on citrus black spot expression

The incidence of CBS was higher in the fruit after 3 weeks of incubation under permanent light, than it was at the time of sampling (Fig. 7); demonstrating that latent infections were expressed by the incubation method. Sampling time before or after commercial harvest had no significant effect on disease expression. Ethephon treatment had no significant effect on disease expression; the results showed no significant interaction with sampling time (P=0.580) and no significant difference in CBS incidence between ethephon-treated and un-treated fruit (P=0.07). Also there were no significant differences between the incidence of CBS at the various sampling times (P=0.57). The experiment was repeated in the following production year, but the low levels of disease incidence in the untreated control prevented any useful data collection.

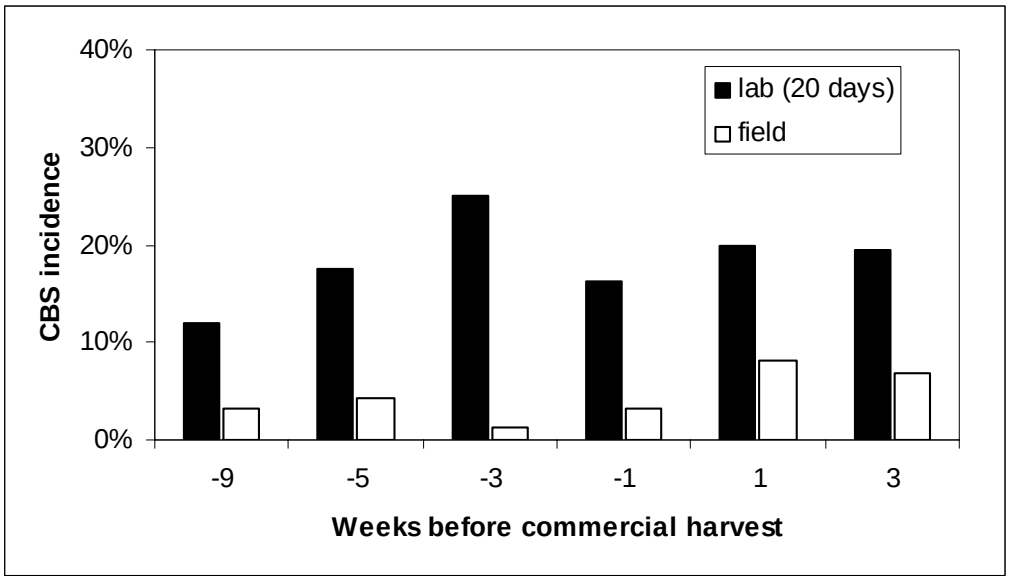

Figure 7. Mean citrus black spot (CBS) incidence in fruit (displaying pycnidia and/or speckled blotch) at the time of sampling (field), and after holding under constant lighting for 20 days at 27ºC and 80% RH (lab), with fruit picked between late May and late August.

Distribution of citrus black spot and scab in the Central Burnett citrus production district

Fruit sampled from the district survey were found to develop CBS, but no scab lesions were observed in any of the fruit. In the 2006-07 production year no lesions without pycnidia (0 of 39 tested) were realtime PCR-positive for *G. citricarpa* DNA, whilst in the 2007-08 production year 9.57% (11 of 115 tested) were positive. The district survey showed the distribution of CBS in the Central Burnett to be skewed towards the majority of blocks (15-20 of 22 surveyed) having a CBS incidence of less than 5% following the incubation of fruit to break the latency of *G. citricarpa* (Fig. 8). The number of blocks with a CBS incidence greater than 5% in each production year was very low (2-7 of 22 surveyed). In each production year a single, different, block was found with >15% CBS incidence (Fig. 8 & 9). CBS was consistently undetectable in the sampled fruit of one of the 22 survey blocks (Fig. 9).

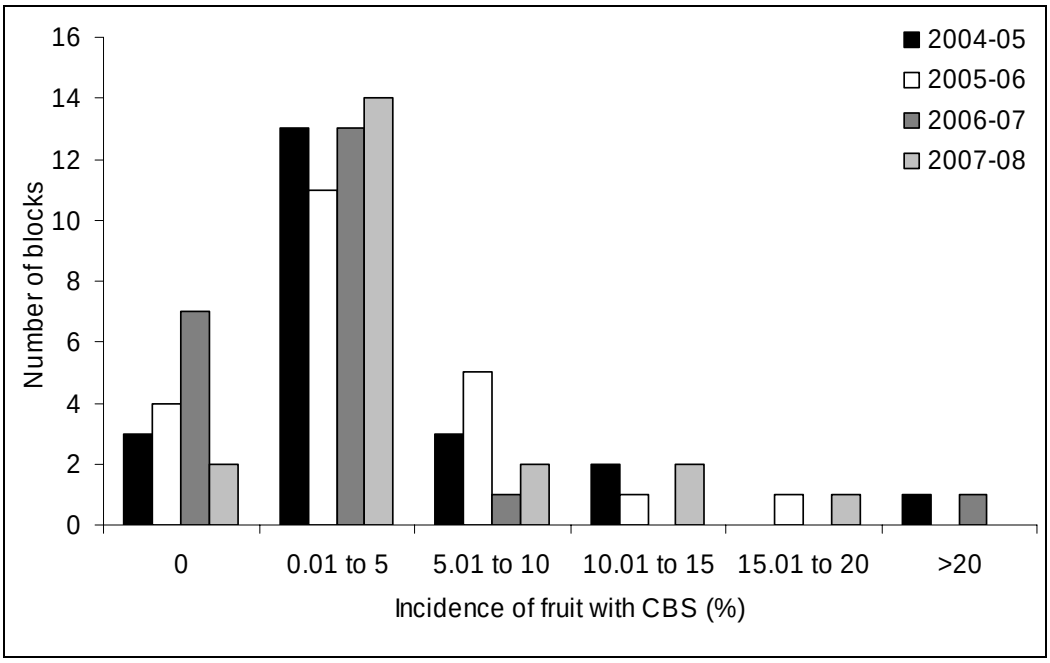

Figure 8. Distribution of citrus black spot in the Gayndah/Mundubbera citrus production region in each production year.

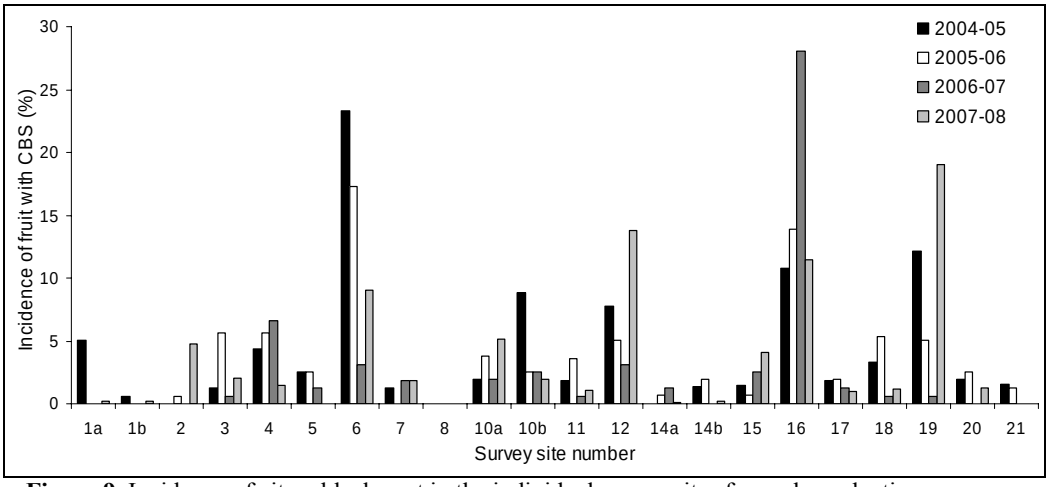

Figure 9. Incidence of citrus black spot in the individual survey sites for each production year.

Several of the distributions fit to the survey data (Fig. 10), however only the Inverse Gaussian and the Beta General were found to have a non-significant chi-square goodness of fit statistic. The remaining models are listed in descending goodness of fit: Person5, Exponential, Extreme Value, Logistic, Normal, Triangular and Uniform.

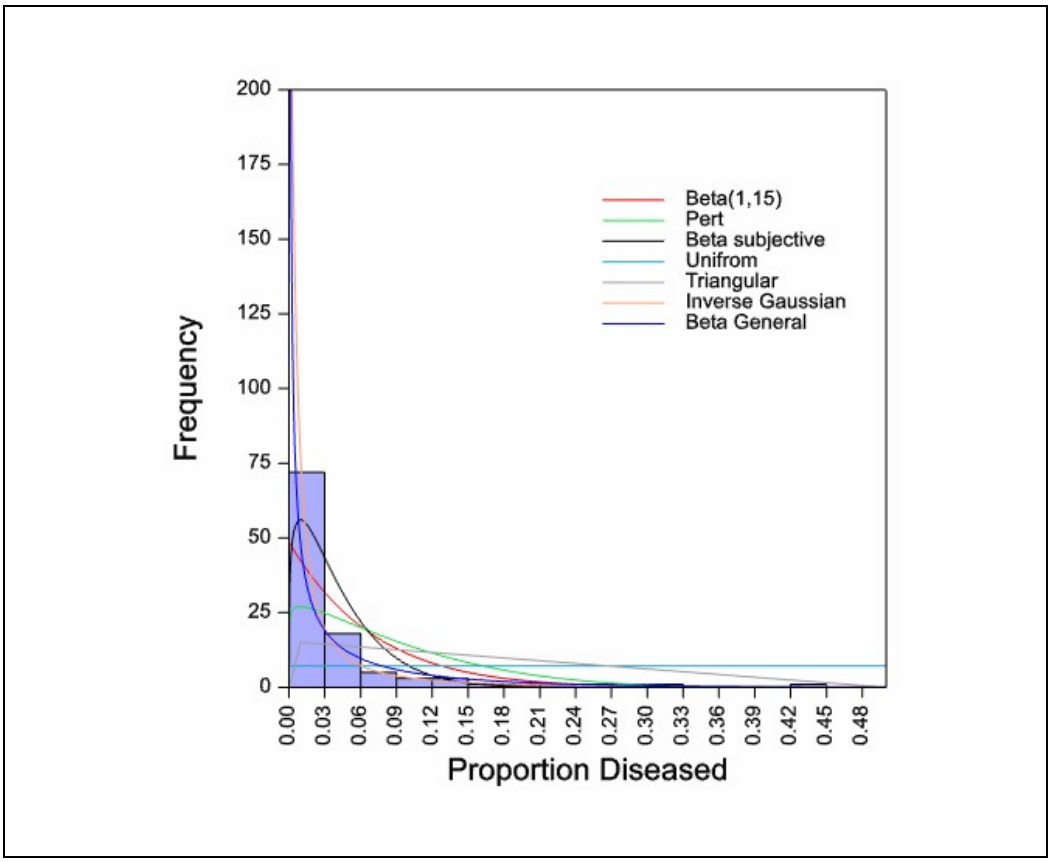

Figure 10. Percentage of fruit with citrus black spot aggregated across all survey years compared to various distributions potentially used in pest risk analysis.

Of the variables possibly explaining the amount of CBS in the different survey blocks, the strongest correlation with the incidence of CBS was the number of fungicides applied to the survey block (Fig. 11), however the correlation was not significant ($r = -0.2025$, $p = 0.0952$). Multiple linear regressions and principal component analysis also failed to identify robust relationships between the different variables and the incidence of CBS, with the highest amount of variance accounted for being only 60.7% using a multiple linear regression model with the following terms:
"Distance from nearest orchard + Age of trees surveyed + Number of fungicides applied + Season + CSIRO soil classification + Fungicide application volume + Fungicide application volume.CSIRO soil classification + Distance form nearest orchard.Fungicide application volume + Season.Fungicide application volume + Number of fungicides applied.CSIRO soil classification".

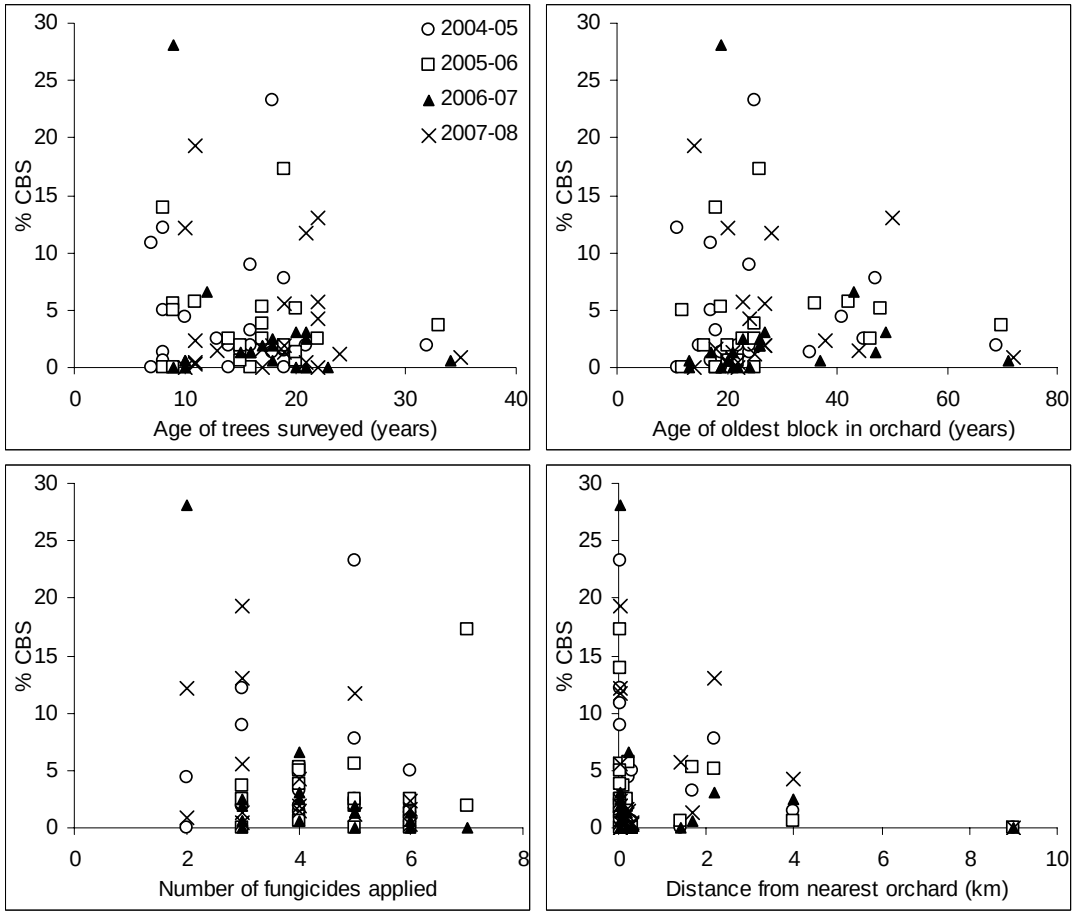

Figure 11. Scatter plots of the age of the trees surveyed (top left), age of the oldest block in the orchard (top right), the number of fungicides applied (bottom left), and the distance of the surveyed orchard from the nearest orchard (bottom right), each plotted against the incidence of citrus black spot (CBS) in fruit harvested over four consecutive production years.

For the qualitative variables, the amount of CBS in blocks located near Gayndah was marginally less than blocks in Mundubbera (Fig. 12); however the blocks in Mundubbera generally received fewer fungicide applications than Gayndah. Blocks sprayed with high fungicide volumes showed slightly lower amounts of CBS than those sprayed with low volumes, however the latter were under represented in the survey with only 5 of the 22 blocks receiving low volumes. The amount of CBS was also variable across the different soil classifications, but the different soil classifications were not equally represented across the survey.

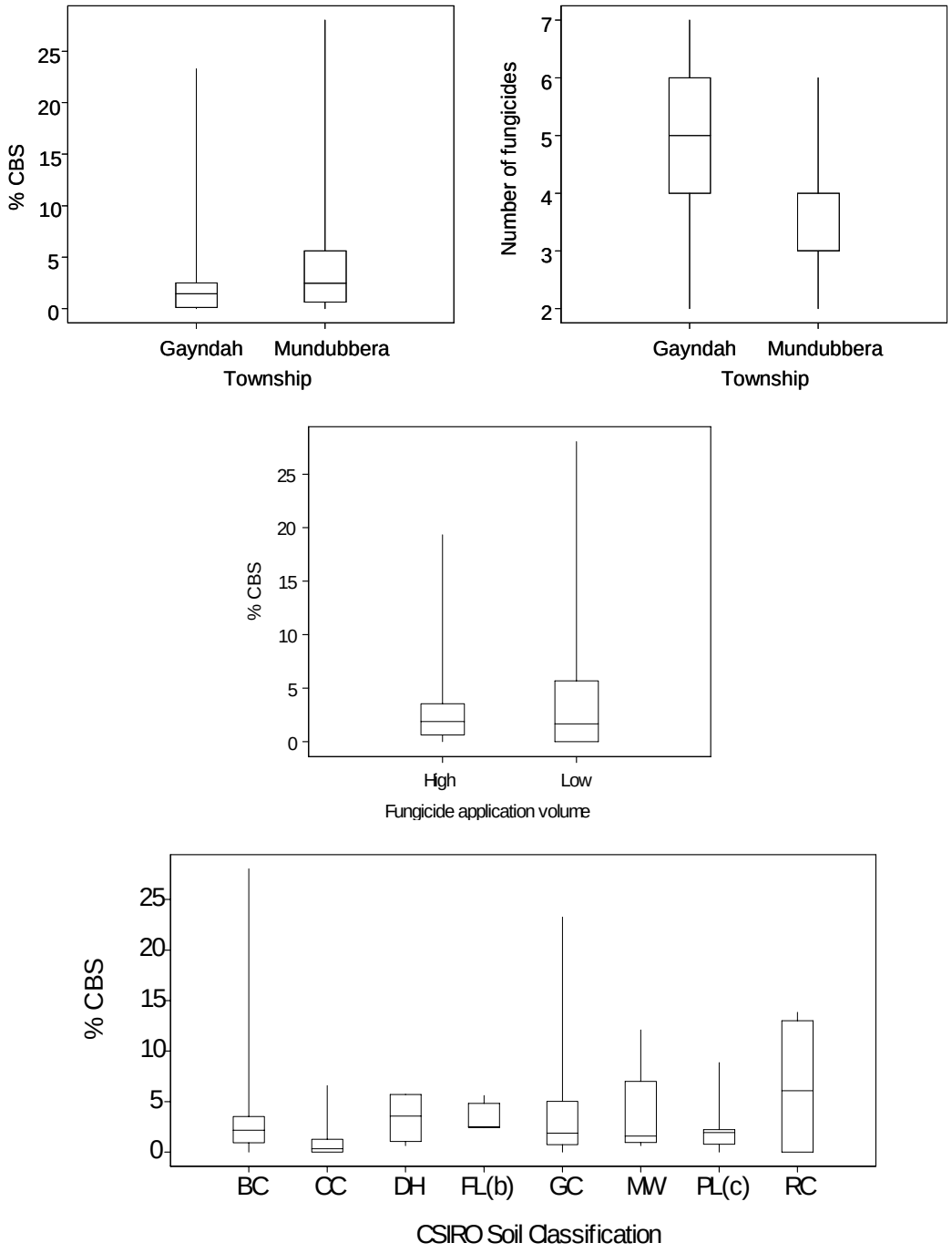

Figure 12. Boxplots of the incidence of citrus black spot (CBS) in fruit harvested over four consecutive production years as related to the qualitative factors of; the township the surveyed orchards were located nearest (top left) and number of fungicides applied in the surveyed blocks in each township (top right), the fungicide application volume (high = >6000 l/ha, low = <6000 l/ha)(centre), and the soil classification of the surveyed orchard (De Mooy *et al.* 1977) (bottom).

Incidence of citrus black spot and scab under a specified fungicide and pruning regime

Assessment of the sampled fruit found CBS symptoms developed in both production years, however no scab symptoms were ever observed. The incidence of CBS in the treated and untreated plots was consistently higher in the 2004-05 production year, and consistently higher in the untreated plots (Table 4). The upper confidence limit for the CBS incidence in fruit of the treated plots in 2004-05 ranged from ≤1.78% to levels exceeding the computational restrictions of the upper limit formula; the highest observed incidence was 5.02%. However, the upper confidence limit was consistently lower in the treated plots in 2005-06 than 2004-05, ranging from ≤0.42 to ≤1.07%.

Evaluation of a preliminary fungicide, pruning and mulching regime for the control of citrus black spot and scab

Assessment of the sampled fruit found CBS symptoms to develop in both production years, however no scab symptoms were ever observed. The incidence of CBS in the treated and untreated plots was consistently higher in the 2004-05 production year, and consistently higher in the untreated plots (Table 5). The upper confidence limit for the CBS incidence in fruit of the treated plots in 2004-05 ranged from ≤1.26% to levels exceeding the computational restrictions of the upper limit formula; the highest observed incidence was 7.76%. However, the upper confidence limit was consistently lower in the treated plots in 2005-06 than 2004-05, ranging from ≤0.26 to ≤0.82%. In the export specification fruit the upper confidence limit for CBS incidence was consistently lower than the field sampled fruit, ranging from ≤0.10 to ≤0.19%; CBS was only detected in 3 out of the 9394 export specification fruit that were harvested.

Incidence of citrus black spot and scab under a specified fungicide, pruning and mulching regime

Assessment of the sampled fruit found CBS symptoms developed in both production years, however no scab symptoms were ever observed. In the 2006-07 production year no lesions without pycnidia (0 of 39 tested) were realtime PCR-positive for *G. citricarpa* DNA, whilst in the 2007-08 production year 9.57% (11 of 115 tested) were positive. The incidence of CBS in the treated and untreated plots was generally higher in the 2007-08 production year (Table 6). The upper confidence limit for the CBS incidence in fruit of the treated plots prior to postharvest handling ranged from ≤0.03 to ≤0.10% across the three sites in 2006-07, but in the 2007-08 production year ranged from ≤0.10 to ≤1.02% for lesions with pycnidia only, and ≤0.21 to ≤1.13% after the inclusion of lesions without pycnidia likely to be positive for *G. citricarpa* DNA. In the export specification fruit the upper confidence limit for CBS incidence across all sites ranged from ≤0.01 to ≤0.16% fruit with CBS lesions in 2006-07, and ≤0.21 to ≤0.31% for lesion with pycnidia only, and ≤0.42 to ≤1.47% after the inclusion of lesions without pycnidia in 2007-08. The incidence of CBS in the field sampled fruit was typically higher than the export specification fruit, however the lower numbers of diseased fruit in the export specification samples tended to result in high upper confidence CBS incidence.

Table 4. The number of fruit assessed and levels of citrus black spot (CBS) in fruit harvested from plots treated with a specified fungicide and pruning regime for CBS control, compared with fruit harvested from untreated control plots.

	No. fruit		No. with CBS		% CBS		95% confidence % CBS ≤[A]	
Site and treatment	2004-05	2005-06	2004-05	2005-06	2004-05	2005-06	2004-05	2005-06
Gayndah (a)								
Fungicide & pruning	2988	1402	41	4	1.37	0.29	1.78	0.65
Untreated	798	634	317	125	39.72	19.72	*	22.87
Gayndah (b)								
Fungicide & pruning	3018	1473	47	9	1.56	0.61	1.99	1.07
Untreated	800	633	112	36	14.00	5.69	16.38	7.51
Mundubbera								
Fungicide & pruning	2926	1484	147	2	5.02	0.13	*	0.42
Untreated	784	554	98	4	12.50	0.72	14.79	1.65

[A]Upper confidence limit for the true unknown disease proportion based on the number of diseased fruit in each of the samples of fruit (Cannon and Roe 1982; Couey and Chew 1986).

*Indicates values for which the number of diseased fruit exceeded the number for which an upper confidence limit could be determined.

Table 5. The number of fruit assessed and levels of citrus black spot (CBS) in fruit harvested from plots treated with a preliminary fungicide, pruning and mulching regime for CBS control, compared with fruit harvest from untreated control plots and export specification fruit from the packhouse.

	No. fruit		No. with CBS		% CBS		95% confidence % CBS ≤[A]	
Site and treatment	2004-05	2005-06	2004-05	2005-06	2004-05	2005-06	2004-05	2005-06
Gayndah (a)								
Fungicide, pruning & mulch	2967	2942	27	3	0.91	0.10	1.26	0.26
Untreated	798	634	317	125	39.72	19.72	*	22.87
Export specification		3158		1		0.03		0.15
Gayndah (b)								
Fungicide, pruning & mulch	2934	2977	50	16	1.70	0.54	2.16	0.82
Untreated	800	633	112	36	14.00	5.69	16.38	7.15
Export specification		3260		2		0.06		0.19
Mundubbera								
Fungicide, pruning & mulch	2925	3030	227	3	7.76	0.10	*	0.26
Untreated	784	554	98	4	12.50	0.72	14.79	1.65
Export specification		2976		0		0.00		0.10

[A]Upper confidence limit for the true unknown disease proportion based on the number of diseased fruit in each of the samples of fruit (Cannon and Roe 1982; Couey and Chew 1986)

*Indicates values for which the number of diseased fruit exceeded the number for which an upper confidence limit could be determined.

Table 6. The number of fruit assessed and levels of citrus black spot (CBS) in fruit harvested from plots treated with a specified fungicide, pruning and mulching regime for CBS control, compared with fruit harvest from untreated control plots[A].

	No. fruit		No. with CBS		% CBS		95% confidence % CBS ≤[B]	
Site and treatment	2006-07	2007-08	2006-07	2007-08	2006-07	2007-08	2006-07	2007-08
Gayndah (a)								
Fungicide, pruning & mulch	3026	3046	0	11 (17.03)	0.00	0.36 (0.56)	0.10	0.60 (0.84)
Untreated	649	601	6	19 (22.06)	0.92	3.16 (3.67)	1.82	4.64 (5.23)
Export specification	3368	3004	0	2 (33.39)	0.00	0.07 (1.11)	0.01	0.21 (1.47)
Gayndah (b)								
Fungicide, pruning & mulch	3026	3080	4	22 (25.06)	0.13	0.71 (0.81)	0.03	1.02 (1.13)
Untreated	648	611	50	28 (28.00)	7.72	4.58 (4.58)	9.77	6.28 (6.28)
Export specification	3160	3434	0	5 (8.35)	0.00	0.15 (0.24)	0.09	0.31 (0.42)
Mundubbera								
Fungicide, pruning & mulch	2951	2982	0	0 (2.58)	0.00	0.00 (0.09)	0.10	0.10 (0.21)
Untreated	635	624	4	23 (31.13)	0.63	3.69 (4.99)	1.44	5.22 (6.70)
Export specification	3000	NS[A]	1	NS	0.03	NS	0.16	NS

[A]Values in parenthesis include lesions that failed to develop pycnidia after incubation, but are likely to be positive for *G. citricarpa* DNA by realtime PCR based on extrapolation (i.e. 9.57% of all lesions without pycnidia were likely to be PCR positive). NS = no sample taken.

[B]Upper confidence limit for the true unknown disease proportion based on the number of diseased fruit in each of the samples of fruit (Cannon and Roe 1982; Couey and Chew 1986)

Weather data

Rainfall occurred in each season and tended to be most frequent during spring and summer (Fig. 13). Similarly, the number of CBS heat units peaked in summer, and troughed in winter (Fig. 14). No significant relationships were observed between the weather variables and the amount of CBS. However, the strongest trend was for the generally low number of spring raindays in 2006-07 to correspond to generally low levels of CBS.

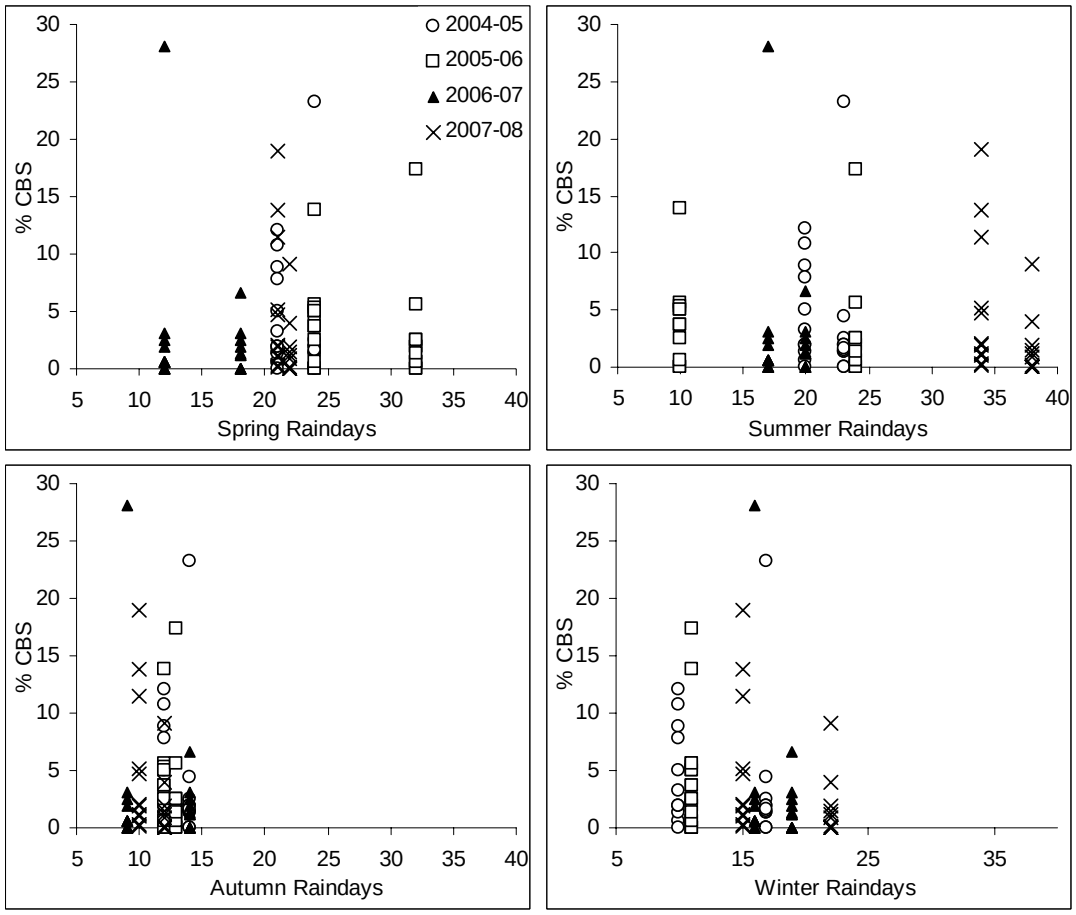

Figure 13. Number of raindays in each season (days with recordable rainfall) plotted against the incidence of citrus black spot (CBS) over four consecutive citrus production years.

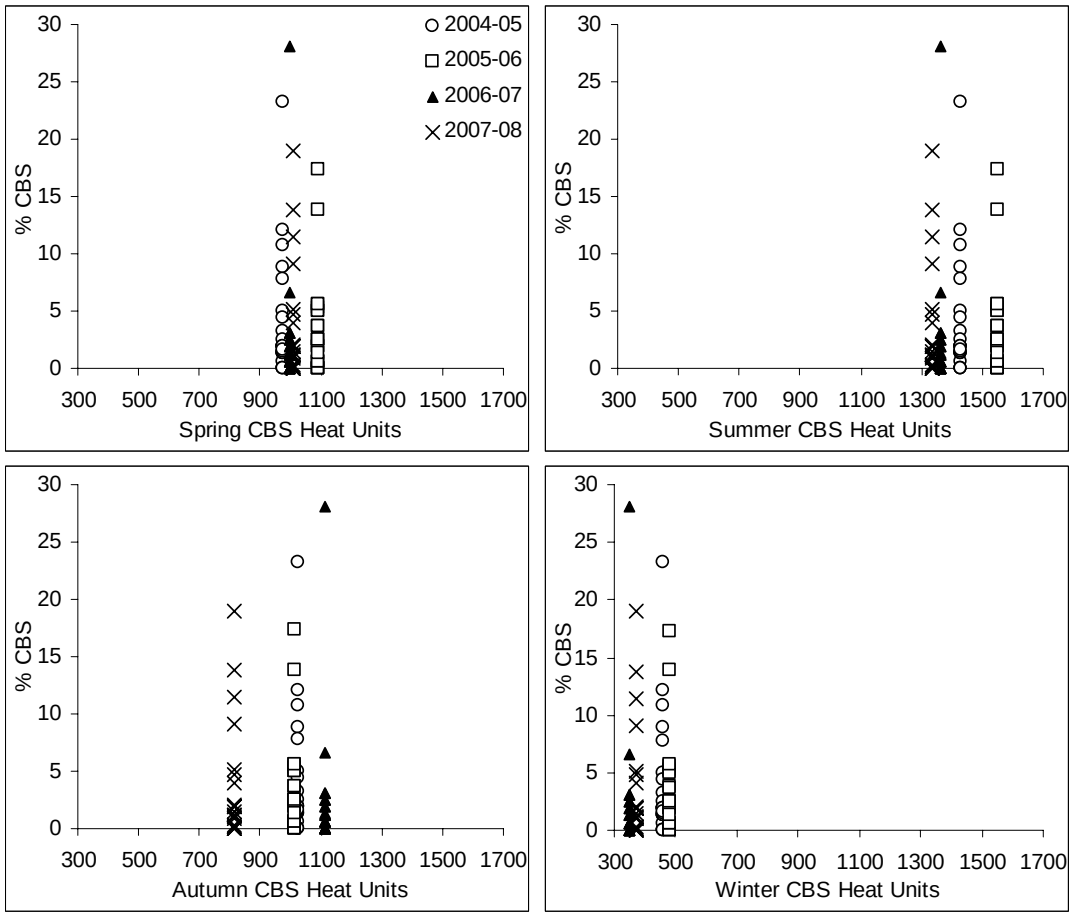

Figure 14. Number of CBS heat units ([(maximum temperature + minimum temperature) ÷ 2] – 11) in each season plotted against the incidence of citrus black spot (CBS) over four consecutive citrus production years.

Discussion

In this project we have demonstrated the potential for integrated disease management (IDM) in the Central Burnett region to result in the production of 'Murcott' tangor nearly completely free of CBS; even after CBS expression was maximised by incubation of the fruit in a manner highly unlikely to be experienced in a commercial supply chain. The specified fungicide, pruning and mulching regime produced export specification fruit that ranged from ≥99.69 to ≥99.99% (95% confidence) free of CBS lesions containing reproductive structures (lesions with pycnidia) after incubation, and ≥98.5% (95% confidence) free of any lesions possibly caused by *G. citricarpa* (i.e. lesions with and without pycnidia). Fruit sampled under this same management regime, but sampled before commercial sorting and packing, were generally observed to have lower numbers of CBS-free fruit, suggesting commercial sorting and packing has a positive effect on the final number of fruit free of CBS. However, the upper confidence levels ranged from ≥98.98 to ≥99.97% of fruit free of CBS lesions with pycnidia, and ≥98.87 to ≥99.79% of fruit free of lesions with and without pycnidia. The regime of pruning and fungicides, without leaf litter management, resulted in the production of generally fewer CBS-free fruit, ranging from an observed occurrence of 94.98% CBS-free fruit in the sampled fruit, up to ≥99.58% (95% confidence) of fruit free of CBS lesions. We have also shown the distribution of CBS in the Central Burnett to be greatly skewed towards the majority of blocks in the district having an incidence of 5% or less fruit with CBS; a distribution found to be best represented by an Inverse Gaussian or Beta General model. Attempts to determine which variables significantly affected the incidence of CBS at the different survey sites were largely unsuccessful, suggesting that the level of survey intensity and data capture were not sufficient in our study to identify the variables of significance, or that unidentified variables more strongly influence CBS at the local level. The ability to achieve maximum CBS expression in fruit harvested up to 9 weeks prior to commercial harvest has been demonstrated, providing up to 6 weeks notice to growers of the CBS incidence in their export blocks. Finally, we have devised and validated a systematic sampling protocol for sampling of fruit from the tree canopy, and postharvest incubation conditions, to maximise CBS detection and expression for CBS surveys.

A high level of disease control was expected to be achieved in the trials undertaken in this study, as each component of the implemented IDM regimes had been previously reported as significantly reducing CBS in the field (Beattie *et al.* 1989; Bertus 1981; Kiely 1950; 1967; 1976; Loest 1968; Miles *et al.* 2004; Rodriguez and Mazza Gaiad 1996; Schutte and Kotze 1997). However, consistent production of 100% CBS-free fruit was not achieved. This could be due to a number of factors, and possibly their interactions, including incomplete suppression of inoculum sources (ascospores and pycnidiospores), contamination from external inoculum sources, incomplete fungicide coverage, and infection occurring outside the commonly accepted fruit susceptibility period. The known *G. citricarpa* inoculum sources are ascospores produced on leaf litter, and pycnidiospores produced on senescing leaves, twigs and fruit lesions (Kiely 1948; Kotze 1981; Timmer 1999). Whilst all of the known leaf litter under the trees was covered by the mulch applications (Fig. 6), leaf escapes cannot be discounted and may have become sources of ascospores. Similarly, removal of all possible sources of pycnidia from the canopy (particularly twigs) cannot be assured. Regardless of this, the trend of mulch application to result in higher numbers and more consistent production of CBS-free fruit suggests a significant reduction in inoculum was

achieved. Therefore it is reasonable to assume that inoculum was greatly reduced in the trial blocks, but not necessarily eradicated. In addition, leaf litter management was not undertaken in all blocks of trees surrounding the trial blocks and movement of airborne ascospores from the surrounding blocks is possible. However it has been hypothesised that *G. citricarpa* ascospores would be dispersed within a similar radius as for *Venturia inaequalis* of only 6 m (Sposito *et al.* 2007), and each of our trial blocks was surrounded by a buffer zone of treated trees measuring at least 14 m to best minimise external inoculum. Whilst protectant fungicides were applied to protect fruit from any inoculum that did reach susceptible fruit it is unlikely that all fruit were entirely covered with sufficient fungicide to confer 100% efficacy, as reports indicate 90-95% coverage is typical for commercial citrus spray equipment in Australia (Chapman *et al.* 1981). It also remains possible that 'Murcott' fruit are susceptible to infection for longer than the commonly accepted 20-24 weeks following fruit set (Baldassari *et al.* 2006; Kotze 1981; Wager 1952), as we are unaware of any experimental work confirming the susceptible period for 'Murcott' specifically. Regardless of these potential opportunities arising for fruit infection, CBS control by the specified fungicide, pruning and mulching regime remained highly effective; even after fruit were incubated in a manner highly unlikely to be experienced in a commercial supply chain.

The trend of lower numbers of fruit with CBS after postharvest packing and sorting may be the result of the postharvest practices undertaken in the packing shed. Whilst postharvest treatments of SOPP, carbendazim (or benzimidazole fungicdies in general), or imazalil have not shown significant efficacy against CBS (Agostini *et al.* 2006; Agostini *et al.* 2004; Seberry *et al.* 1967), the application of wax to fruit has been shown to reduce postharvest development of CBS, possibly as a result of reduced fruit respiration (Seberry *et al.* 1967; Wild 1981). No data could be found regarding the effect of dimethoate on postharvest CBS development. Another aspect of the typical commercial supply chain that may provide further reductions/delays in CBS expression is the recommended storage of mandarin fruit at 5°C (Tugwell 1999). Low temperature storage is considered the most effective means of reducing postharvest CBS expression (Agostini *et al.* 2006; Agostini *et al.* 2004; Calavan 1960; Kotze 2000), and is widely adopted in the commercial supply chain in Australia. Specifically for Australian citrus entering the USA, there are requirements for fruit fly disinfestation by cold treatment of fruit at 1°C for 16 days (Heather *et al.* 1996; Hill *et al.* 1988). This cold treatment was found to reduce CBS expression by 25.4% compared to fruit stored at room temperature for the same amount of time; disease assessments were made after the cold treated and room temperature control fruit were incubated at ~27ºC, 80% relative humidity and 24 hour fluorescent lighting for 3 weeks to break latency of any *G. citricarpa* infections (Wyatt *et al.* 2008). It is therefore reasonable to conclude that commercially adopted postharvest handling procedures will provide additional reductions in CBS expression, in addition to those achieved by rigorous field control.

The distribution of CBS in the Central Burnett was found to be strongly skewed towards the majority of surveyed blocks having less than 5% CBS-infected fruit. The observed distribution of the disease best fit an Inverse Gaussian or Beta General model. However, all the models are conservative and tend to underestimate the number of samples which recorded zero incidence of disease and give a higher probability of disease incidence than what was found. It should also be noted that the

Inverse Gaussian and Beta General models produced very high frequencies as the proportion of diseased fruit decreased to zero. In terms of pest risk analyses using any of these models, it is likely that the probability of entry of the pathogen via fruit from the Central Burnett would be overestimated; the use of observed data will improve the accuracy. The generally low observed disease incidence (mostly <5%) in the area is most likely due to CBS having been routinely managed in the district for at least 40 years. The occurrence of CBS in blocks at incidences over 5% may be the result of several variables, such as fungicide strategy, age of the block, proximity to external inoculum sources (e.g. older blocks of more susceptible varieties), soil type, localised weather events and several other possibilities; probably including their interactions. However, attempts to statistically demonstrate the impacts of such variables were not successful. At best, the number of fungicides applied tended to correlate with CBS incidence, which is unsurprising considering the known efficacy of fungicides against CBS. The survey data also show that the nature of the CBS distribution in the district was generally consistent between production years, and that CBS disease pressure was present in all production years. The degree to which the distribution of blocks is skewed to the left could not be related confidently to the rainfall and temperature factors, however, the distribution was most skewed towards the majority of blocks having less than 5% CBS incidence in 2006-07; the production year where both the number of raindays in Spring and incidence of CBS were lowest. Increased rainfall in spring is likely to favour infection of young fruit, and stimulate the production of perithecia in the leaf litter.

Sampling of fruit to determine the distribution of CBS in the Central Burnett district was undertaken by systematic sampling of fruit from all positions within the tree canopy based on the findings of field experiments investigating the distribution of infected fruit in the canopy. Despite a trend towards increased infection of fruit in the inner and lower canopy positions, which supports previous studies and harvesting strategies (Mayers and Owen-Turner 1989; Sposito *et al.* 2008), there was no strong indication that sampling fruit only from these canopy positions would improve the probability of detection of CBS over the systematic sampling of the entire canopy. The trend towards increased incidence of CBS in the south, lower, inner portion of the tree canopy is most likely due to i) the lower portion of the canopy being physically closer to the primary inoculum source (Kiely 1948), ii) the inside of the tree canopy providinge a microclimate more suitable to microbial growth (Stirling *et al.* 1999), and iii) the lower intensity of sunlight on the southern side of the tree possibly delaying the evaporation of dew/rain from the fruit surface and favouring microbial growth. However, the degree to which these factors can affect the distribution of CBS in the canopy is likely to change depending on variables such as tree age/size, canopy management, climate, irrigation type and fungicide application. Therefore sampling of all canopy positions is likely to reduce the variation imposed by these variables on the distribution of CBS. The results also indicate that the canopy position recommended for survey in the past (north × outer × upper) (Anonymous 2000) was not anymore likely a position for fruit infection than the other positions, and supports the explanation that infected fruit in this position are simply more likely to break latency before fruit in other positions (Kiely 1948).

Incubation of fruit at ~27ºC, 80% relative humidity and 24 hour fluorescent lighting for 3 weeks significantly increased the expression of CBS in fruit as expected (Brodrick and Rabie 1970; Korf 1998; Timossi *et al.* 2003), however treatment with ethephon was of no

apparent value. There is no clear explanation for the lack of response to ethephon as observed by others (Baldassari *et al.* 2007), but does perhaps suggest that maximum disease expression is being obtained by the incubation conditions alone. Furthermore, our results suggest that maximum disease expression occurs in fruit sampled between 9 weeks prior to, and 3 weeks after, commercial harvest. It is particularly useful to know that fruit can be sampled 9 weeks prior to commercial harvest, incubated for 3 weeks, then assessed, giving growers up to 6 weeks notice as to whether or not export is likely to be permitted from any particular block.

A challenging aspect of the assessment of CBS incidence was the occurrence of CBS-like lesions that lacked pycnidia after incubation of fruit under ideal conditions. The occurrence of lesions without pycnidia and the difficulty it presents for diagnosis has been widely reported (European and Mediterranean Plant Protection Organization 2009; Peres *et al.* 2007; van Gent-Pelzer *et al.* 2007), however no studies of the reasons for pycnidia failing to be produced could be found. It is likely that lesions that still lack pycnidia after incubation, and are found by realtime PCR to contain DNA of *G. citricarpa*, whilst probably caused by *G. citricarpa* are likely to be of lesser phytosanitary risk than lesions containing pycnidia. In the absence of pycnidia, mycelium of the pathogen is the only likely infective material of *G. citricarpa* within these lesions (Bonants *et al.* 2003). However, the ability for mycelia to successfully infect citrus tissue and lead to symptom production is not known. Mycelial invasion of citrus fruit from mycelium previously established within pedicel tissues was hypothesised (Schuepp 1961; Sueda 1941), but was later dismissed after field experiments demonstrated that covering fruit with paper bags prevented infection, suggesting an external inoculum source such as pycnidia and ascospores (McOnie 1964). In the absence of a consensus of the epidemiological role of lesions lacking pycnidia, we have separated results into lesions with pycnidia, and any lesions possibly caused by *G. citricarpa* (i.e. lesions with and without pycnidia) where appropriate.

Symptoms of scab were never observed on any fruit during this project. In Australia, the recognised pathotypes of *E. fawcettii* are primarily pathogens of lemon varieties, with some instances of effecting mandarin varieties (Donovan *et al.* 2009; Timmer *et al.* 1996). Whilst biotypes pathogenic and highly problematic to 'Murcott' production are known to exist in Florida (Broadbent 1995; Whiteside 1978), we are not aware of any confirmed reports of scab occurring on 'Murcott' in Australia.

From these studies it can be concluded that IDM for CBS can give rise to very low levels of CBS infection in the field. Rigorous field control, in conjunction with the further reductions in CBS expected by following recommended postharvest handling procedures and cold treatment for fruit fly, greatly reduces the phytosanitary risk to countries importing mandarin fruit from the Central Burnett. The field control data generated during this project can be used for both a market access systems approach and demonstrating the level of CBS control achievable by implementing IDM for CBS.

Technology Transfer

Reports, papers and presentations
September 2004, paper, "Expanding citrus market access using a systems approach to control black spot", Citrus Insight.

October 2004, oral presentation, "Expanding market access using a systems approach to control black spot", Queensland Citrus Growers research forum.

February 2005, poster presentation, "Characterising the incidence of citrus black spot in Central Queensland orchards", Thredbo, NSW, Thredbo Statistical Meeting.

March 2005, oral presentation, "Expanding market access using a systems approach to control black spot", Australian Citrus Growers National Conference, Bundaberg.

May 2006, oral presentation, "Expanding market access using a systems approach to control Citrus Black Spot", Pakistan citrus industry delegation meeting.

October 2006, paper, "Controlling CBS to expand access to the US market", Citrus Insight.

October 2006, oral presentation, "Expanding market access using a systems approach to control Citrus Black Spot", Queensland Citrus Growers research forum.

November 2006, oral presentation, "Citrus Black Spot Research", 'Asian Markets for Horticulture Initiative' Brisbane Markets Exporters Forum.

December 2006, oral presentation, "Citrus to the USA & NZ", Horticulture Australia Working Group on Market Access Research and Development.

August 2007, oral presentation, "Citrus Black Spot Research", 'Asian Markets for Horticulture Initiative' Brisbane Markets Exporters Forum.

October 2008, paper and oral presentation "Integrated Disease Management of Citrus Black Spot (*Guignardia citricarpa* Kiely) in Queensland, Australia", Wuhan, China, 11th International Citrus Congress.

March 2009, oral presentation, "Integrated Disease Management of Citrus Black Spot (*Guignardia citricarpa* Kiely) in Queensland, Australia" (prepared for the International Citrus Congress in China, 2008), Gayndah DPI&F citrus grower R&D meeting.

September 2009, oral presentation, "Citrus black spot", Mareeba Cittgroup

HAL milestone reports were completed throughout the life of the project

Industry Reference Committee
Throughout the life of the project an Industry Reference Committee (IRC) was consulted regarding practical aspects of the project. The IRC members were: Rod Baker (Cooranga Citrus), Craig Meyer (Ventnor Grove), Troy Emmerton (Quebec),

Bevan Young (Ban Ban Orchards), Ian Shepherd (Shepherd Citrus), Tim Ulcoq (Ulcoq Citrus Ent.), Steve Jameson (Tombrie), John Owen Turner (JT P/L), Neville Harris (Grower/GayPak), Greg Zahl (Zahl's), Dan Papacek (Bugs for Bugs), Malcolm Wallis (Citri Care) and Brian Gallagher (Citrus Monitoring Services).

IRC meetings were held on the following dates:
8th October 2003
6th April 2004
27th July 2004
5th April 2005
8th September 2005
11th April 2006
19th September 2006
9th October 2007

National Advisory Group
Throughout the life of the project a National Advisory Group was consulted regarding the market access submission aspects of the project. The NAG members have included David Letham, Emmanuel Mireku, Darryl Barbour, Liam Whyte, Mahmood Nasir, Robert Duthie, and David Heinrich from Biosecuity Australia, Chris Simpson from Queensland Citrus Growers, and Pat Barkley from Citrus Australia Limited.

NAG meetings were held on the following dates:
2nd December 2004
29th June 2006
7th November 2007
11th August 2009

Recommendations

Based on the findings of the project it is recommended that:

- Where possible, IDM is adopted for the control of CBS in endemic areas.
- Future research of the preharvest control of CBS focus on:
    - identifying peak infection periods in the field,
    - confirming the susceptibility period for target export varieties (e.g. 'Murcott'),
    - optimisation of protectant fungicide use based on fruit expansion – though we attempted to account for the reduction in fungicide coverage occurring with fruit expansion in this work, it may be necessary to determine spray timing based on measurements of fruit size,
    - new chemistry.
- Future research of the postharvest control of CBS focus on:
    - understanding the mechanisms controlling symptom expression, and investigate options for their manipulation to prevent lesion formation,
    - novel chemistry such as plant defence promoters (e.g. chitosan),
    - non-chemical control options (e.g. microwave technology) that may have efficacy whilst avoiding chemical residue issues.

Note: postharvest CBS research is often hindered by a lack of sufficiently high quantities of CBS-infected fruit (particularly 'Murcott') due to the routine control

of CBS in commercial orchards – trial fruit needs to accessed from a site where CBS levels are promoted, whilst other diseases such as brown spot (*Alternaria alternata*) are managed.

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- The members of the National Advisory Group
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Appendices

Appendix 1. Circulated summary of final National Advisory Group meeting – 11th August 2009

HAL CT03005: Expanding market access using a systems approach to control Citrus Black Spot

National Advisory Group meeting
11th August 2009

Conference Room, Entomology Building, Indooroopilly Research Centre
80 Meiers Rd, Indooroopilly, Brisbane

In attendance: Andrew Miles (QPIF), Darryl Barbour (BA), Rosemary Kopitke (QPIF), Pauline Wyatt (former QPIF), Rick Ada (QPIF), Pat Barkley (Citrus Australia), Kevin Parr (grower/citrus Australia, Chris Simpson (Citrus Australia), Andre Drenth (Tree Pathology Centre), Irene Kernot (QPIF)

Meeting outcomes and relevant decisions:

- Citrus from outside the fruit fly exclusion zone is currently 5th on the HMAC priority list (3 of the 4 commodities ranked higher are currently in the APHIS 'rule making' stage).
- A preliminary submission for citrus from black spot areas will be developed by BA (with input from Citrus Australia and QPIF) requesting market access based on 'current practice' levels of black spot.
    - Market access will be requested for all varieties to all regions of the USA.
- The trialled 'management system' data will be required only on request from APHIS for a specific 'phytosanitary measure' for black spot.
- Publication of a journal article on the 'management system' will not be pursued at this stage.
- Further postharvest research will not be pursued at this stage.
- The following table outlines a target timeline of activities to progress the HAL project and market access negotiations:

Approximate Date	Action	Lead agency	Notes
August 2009	NAG meeting held	QPIF – HAL project	
By December 2009	Write up 'district survey' for inclusion in a preliminary export submission	QPIF – HAL project	Also write survey data up as a short communication in Australasian Plant Pathology
			Spray records and orchard details (i.e. surrounding blocks etc) would need to

			be collected for the 22 survey blocks from 2004 onwards. This information was not required at the time for the original purpose of the district survey.
	Write up industry background information for preliminary export submission	Citrus Australia	
	Write up seasons 1&2 of 'current practice' field trial data for inclusion in industry background section of preliminary export submission	QPIF – HAL project	
	Compile preliminary export submission	BA	
By end 2009	Submit preliminary export submission to APHIS	BA	
Mid 2010	Prepare 'management system' data as specific phytosanitary measure in case such measures suggested in APHIS feedback	QPIF – HAL project	
Mid-end 2010	Receive feedback from APHIS	APHIS	
+12 months	Compile supplementary submission	BA	

Technical outcomes and relevant decisions:

- District survey data will be presented with relevant background information to make every effort to identify reasons for variation in black spot levels between survey sites. Details of what constitutes 'current practice' on all survey and trial blocks will be provided.
- It will be argued in the submission that fruit lesions that are positive for *G. citricarpa* DNA by real time PCR, but fail to develop any reproductive structures (i.e. pycnidia) are of a lesser phytosanitary risk than lesions with reproductive structures.
- Weather data should be compiled to determine if the experimental seasons were 'typical' of the district.

Appendix 2. District survey submission provided to Biosecurity Australia

Attention: Biosecurity Australia, Department of Agriculture, Fisheries and Forestry

December 2009

Survey methods and results to determine the distribution of citrus black spot and citrus scab in 'Murcott' tangor grown in the Gayndah/Mundubbera citrus production district in Qld, Australia.

Horticulture Australia Limited

CT03005 Expanding Market Access Using a Systems Approach to Control Citrus Black Spot

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Background

In January 2004 Horticulture Australia Limited (HAL) project CT03005 "Expanding Market Access Using a Systems Approach to Control Citrus Black Spot" commenced under the leadership of Primary Industries and Fisheries Queensland. The principal goal of the project was to investigate the potential for a systems approach to control citrus black spot (CBS) (*Guignardia citricarpa*) to levels acceptable to export markets for which CBS is a quarantine concern. Two specific aims of the project were to (i) validate the fruit sampling methods required by the USDA for fruit from other CBS-endemic regions (Anonymous 2000), and (ii) characterise the distribution of CBS, and citrus scab (*Sphaceloma fawcettii*), in 'Murcott' tangor grown under existing disease control strategies implemented in the Central Burnett (townships of Gayndah and Mundubbera) citrus production region of Queensland.

The USDA sampling strategy needed validation as it was not conclusively supported in the existing literature. The USDA strategy required that fruit be sampled from the portion of the canopy where infected, symptomatic fruit are most likely to be found (i.e. near the outer, upper part of the canopy on the sides of the tree that receives the most sunlight). However, the existing literature reported that the distribution of CBS in trees was considered uniform, with fruit from the north to north-western sector of the tree expressing more severe symptoms earlier due to a more favourable microclimate for symptom development (Kiely 1948). Mayers and Owen-Turner (1989) report that CBS is more prevalent in the inner lower portion of the canopy. In a more recent study diseased fruit tended to be more common in the middle and lower canopy positions (Sposito *et al.* 2008). Therefore it was necessary to undertake field experiments to clarify the best fruit sampling strategy. It was also necessary to quantify the existing disease pressure in the Central Burnett district,

record any changes in disease pressure between seasons, and characterise the distribution of the disease for the pest risk analysis. Therefore an annual district survey was undertaken over the 2004-05 to 2007-08 production seasons using the sampling methods determined from the above field experiments.

In August 2009 a meeting of the project's National Advisory Group (project team, industry representatives and Biosecurity Australia) was held. A key decision resulting from this meeting was to present Biosecurity Australia with the methods and results of the sampling strategy validation and district survey components of the project for use in the development of a preliminary export submission to the USA.

Methods

Influence of canopy position on disease incidence

To determine if citrus black spot (CBS) (*Guignardia citricarpa*) and scab (*S. fawcettii*) incidence in the field are influenced by the position of fruit in the canopy, with possible implications for a survey sampling strategy, disease was assessed in fruit sampled from various positions with in the canopy. In the 2004-05 production season a field experiment was conducted at three different commercial orchards within the Gayndah/Mundubbera citrus production district. At each of the three orchards four replicate trees were selected for the experiment within a plot of approximately 50, 16 to 20-year-old 'Murcott' tangor (*C. reticulata* × *C. sinensis*) trees having not received any fungicides for the first 20 weeks of fruit development; allowing natural infection by *G. citricarpa* and *S. fawcettii* when fruit are susceptible (Baldassari *et al.* 2006; Kotze 1981; Timmer *et al.* 2003; Timmer *et al.* 2000; Wager 1952). Ten fruit were sampled from each of 16 canopy positions based on north, south, east and west compass points × inner, outer, upper and lower portions, from each replicate tree. Sampled fruit were returned to the laboratory for assessment.

District survey

To determine the distribution of CBS and scab in the Gayndah/Mundubbera citrus production district, a survey was undertaken of fruit sampled from blocks representative of the district. In total 22 blocks (Fig. 1.) of 'Murcott' tangor trees under routine commercial fungicide treatment (Table 1) were selected for survey over the 2004-05 to 2007-08 production seasons. Five trees were randomly chosen from across each block for sampling 4-6 weeks prior to commercial harvest in each season. Based on the results of the above canopy position experiment, two pieces of fruit were

randomly selected from each of the 16 previously described canopy positions of each tree, for a total of 160 fruit per block. Sampled fruit were returned to the laboratory for assessment.

Disease assessment

To determine the incidence of CBS and scab in the sampled fruit, fruit were individually assessed by eye and light microscope, and the presence or absence of symptoms recorded. Prior to assessment, the latency of *G. citricarpa* infections was broken by incubation of fruit under ideal conditions for CBS lesion development of 27°C, 80% relative humidity and permanent light (Fig. 2.) for 3 weeks (Brodrick and Rabie 1970). Fruit sampled from the 2004-05 season were incubated in darkness, but all other conditions of incubation were consistent between seasons. After fruit were incubated, disease incidence was determined as the proportion of fruit with one or more CBS or scab lesions. In all the seasons lesions were diagnosed as CBS if pycnidia of *G. citricarpa* were present on the lesion surface after incubation (Anonymous 2003). In the 2004-05 and 2005-06 seasons symptoms were diagnosed as CBS based on the visual comparison to the described CBS lesion types, including those symptoms without pycnidia (Anonymous 2003; de Goes *et al.* 2000; Kiely 1948; 1960; Kotze 1981; 2000). In the 2006-07 and 2007-08 seasons, diagnosis of lesions without pycnidia relied on a realtime PCR assay for *G. citricarpa* based on an existing protocol (van Gent-Pelzer *et al.* 2007) modified to include an internal control (Li *et al.* 2006) and locally validated (van Brunschot *et al*. *unpublished*). A subsample of lesions without pycnidia from all the CBS field experiments in each season was taken for testing; 39 lesions in 2006-07, and 115 lesions in 2007-08. In each season, the proportion of tested lesions without pycnidia that were positive for CBS using the

realtime PCR assay was used to extrapolate the final number of lesions without pycnidia likely to have been caused by *G. citricarpa* in the total sample. *E. fawcettii* infection in all seasons was diagnosed based on the visual comparison to described scab lesions (Fawcett 1936; Timmer 2000).

Data analysis

Disease incidence data from the canopy position experiment were analysed using GenStat 8.1 (GenStat 2005) as a split plot design with upper/lower and inner/outer positions nested within compass position. Means were compared using Fisher's protected Least Significant Difference test (LSD). District survey data were summarised as the number of orchard blocks falling into various disease incidence classes (e.g. 0%, 0.01 to 5.00% etc) for each season, and disease incidence for each survey block in each year presented in a histogram.

Results

Influence of canopy position on disease incidence

During the disease assessment only symptoms of CBS were observed in the sampled fruit; no scab symptoms were observed. Analysis of the CBS incidence data from the canopy position experiment indicated that the three way interaction between compass position, inner/outer and upper/lower was significant ($P = 0.031$). The incidence of CBS was highest (30.7%) in the North/Inner/Lower position, but there was no significant difference between this position and the East/Inner/Lower and South/Outer/Lower positions (Table 2). There was no strong indication that sampling should be done preferentially from a particular canopy position rather than a systematic sampling strategy of the entire canopy.

District survey

Fruit sampled from the district survey were found to develop CBS, but no scab lesions were observed in any of the fruit. In the 2006-07 season no lesions without pycnidia (0 of 39 tested) were realtime PCR-positive for *G. citricarpa*, whilst in the 2007-08 season 9.57% (11 of 115 tested) were positive. The district survey showed the distribution of CBS in the Central Burnett to be skewed towards the majority of blocks (15-20 of 22 surveyed) having a CBS incidence of less than 5% following the incubation of fruit to break the latency of *G. citricarpa* (Fig. 3). The number of blocks with a CBS incidence greater than 5% in each season was very low (2-7 of 22 surveyed). In each season a single, different, block was found with >15% CBS incidence (Fig. 3 & 4). CBS was consistently undetectable in the sampled fruit of one of the 22 survey blocks (Fig. 4).

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Figures and Tables

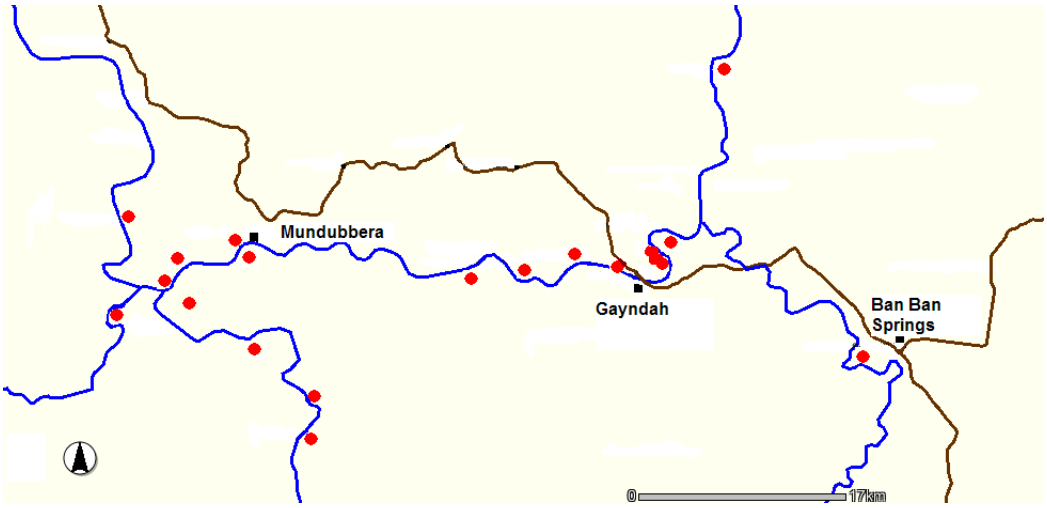

Figure 1. Map of the Central Burnett district showing the locations of the district survey sites (red dots), relative to the townships of Mundubbera and Gayndah.

Figure 2. Incubation of citrus fruit at 27°C, 80% relative humidity and permanent light to break latency of *Guignardia citricarpa* and express citrus black spot symptoms.

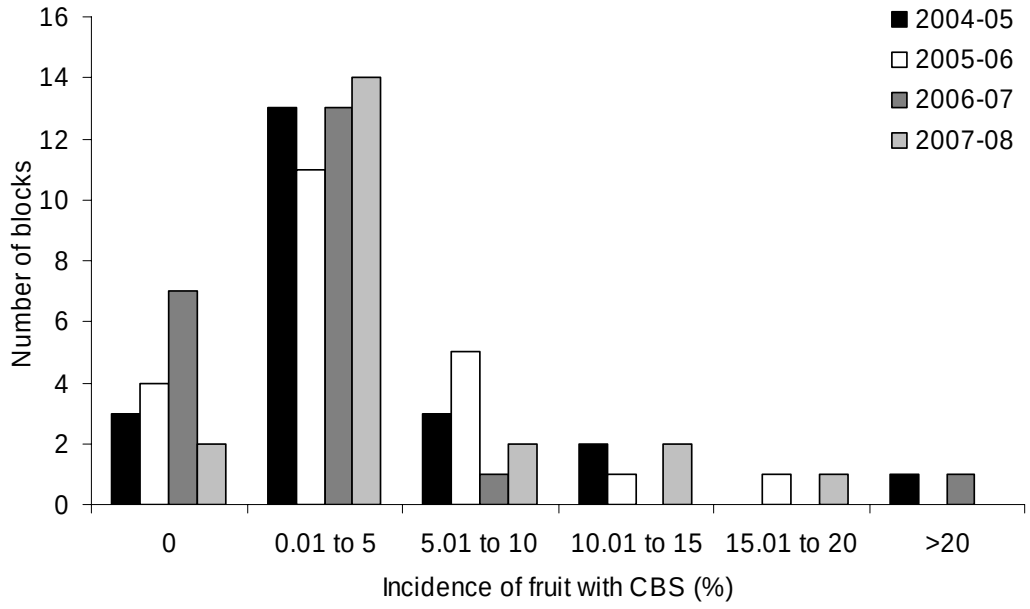

Figure 3. Distribution of citrus black spot in the Gayndah/Mundubbera citrus production region in each season.

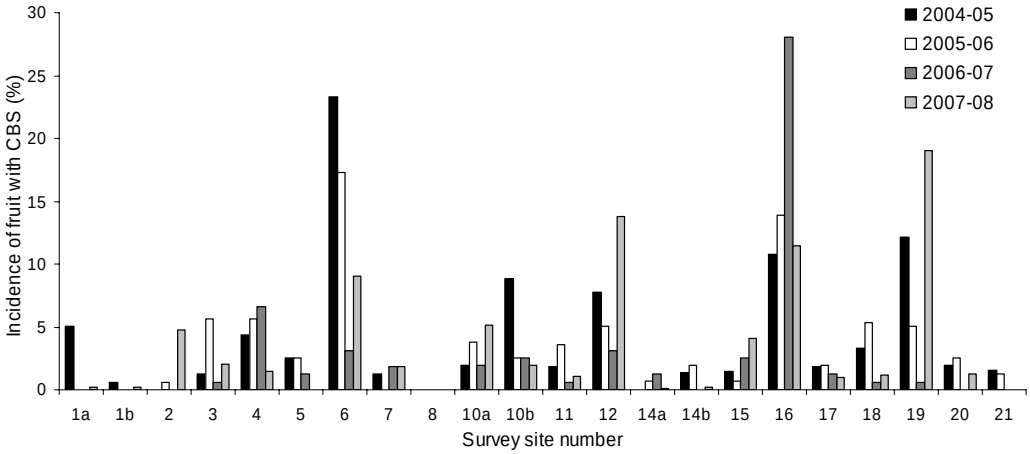

Figure 4. Incidence of citrus black spot in the individual survey sites for each season.

Table 1. The range of routine commercial fungicide treatment regimes typically adopted in the Central Burnett district for control of fungal pathogens during the first 20 weeks of fruit development.

Approximate weeks after petal-fall	Lower spray frequency blocks	Higher spray frequency blocks
0	Copper fungicide	Copper fungicide
1-2		Copper fungicide
4-6	Dithiocarbamate fungicide	Dithiocarbamate fungicide
8	Fruit increasing in resistance to *S. fawcettii* [†]	
8-10		Dithiocarbamate fungicide
10-12	Dithiocarbamate fungicide	
12-14		Dithiocarbamate fungicide
16-18		Dithiocarbamate fungicide
>20	Fruit increasing in resistance to *G. citricarpa* [Ω]	

[†](Timmer 2000; Timmer *et al.* 2003)
[Ω](Baldassari *et al.* 2006; Kotze 1981; Wager 1952)

Table 2. Mean percent of citrus black spot infected fruit at various positions within the tree canopy†

Compass	Inner		Outer	
Position	Lower	Upper	Lower	Upper
East	21.2	11.6	8.3	17.5
North	30.7	16.7	14.4	12.6
South	15.8	11.7	19.2	6.7
West	11.3	15.5	16.0	16.1

† LSD (95%) = 12.8 except when comparing means with the same compass × inner/outer position (= 10.3) or means with the same compass × upper/lower position (= 12.3)

Appendix table 1. 2004-05 season survey results.

	2004-05								
			No. fruit with CBS-like lesions[†]			% fruit with CBS-like lesions			
Survey site no.	No. fruit assessed	No. fruit with citrus scab	Pycnidia present	Pycnidia absent	**Total no. fruit with CBS**	Pycnidia present	Pycnidia absent	**Total % fruit with CBS**
1a	2926	0	-	-	**147**	-	-	**5.02**
1b	160	0	-	-	**1**	-	-	**0.63**
2	159	0	-	-	**0**	-	-	**0.00**
3	157	0	-	-	**2**	-	-	**1.27**
4	159	0	-	-	**7**	-	-	**4.40**
5	158	0	-	-	**4**	-	-	**2.53**
6	159	0	-	-	**37**	-	-	**23.27**
7	159	0	-	-	**2**	-	-	**1.26**
8	153	0	-	-	**0**	-	-	**0.00**
10a	158	0	-	-	**3**	-	-	**1.90**
10b	158	0	-	-	**14**	-	-	**8.86**
11	160	0	-	-	**3**	-	-	**1.88**
12	154	0	-	-	**12**	-	-	**7.79**
14a	159	0	-	-	**0**	-	-	**0.00**
14b	2988	0	-	-	**41**	-	-	**1.37**
15	136	0	-	-	**2**	-	-	**1.47**
16	158	0	-	-	**17**	-	-	**10.76**
17	160	0	-	-	**3**	-	-	**1.88**
18	153	0	-	-	**5**	-	-	**3.27**
19	157	0	-	-	**19**	-	-	**12.10**
20	153	0	-	-	**3**	-	-	**1.96**
21	3018	0	-	-	**47**	-	-	**1.56**

[†]Lesions were not separated according to the presence or absence of pycnidia after incubation of fruit.
CBS = citrus black spot.

Appendix table 2. 2005-06 season survey results.

	2005-06							
			No. fruit with CBS-like lesions[†]			% fruit with CBS-like lesions		
Survey site no.	No. fruit assessed	No. fruit with citrus scab	Pycnidia present	Pycnidia absent	**Total no. fruit with CBS**	Pycnidia present	Pycnidia absent	**Total % fruit with CBS**
1a	159	0	0	0	**0**	0.00	0.00	**0.00**
1b	159	0	0	0	**0**	0.00	0.00	**0.00**
2	160	0	1	0	**1**	0.63	0.00	**0.63**
3	161	0	2	7	**9**	1.24	4.35	**5.59**
4	159	0	8	1	**9**	5.03	0.63	**5.66**
5	160	0	3	1	**4**	1.88	0.63	**2.50**
6	156	0	22	5	**27**	14.10	3.21	**17.31**
7	160	0	0	0	**0**	0.00	0.00	**0.00**
8	158	0	0	0	**0**	0.00	0.00	**0.00**
10a	160	0	6	0	**6**	3.75	0.00	**3.75**
10b	159	0	3	1	**4**	1.89	0.63	**2.52**
11	165	0	0	6	**6**	0.00	3.64	**3.64**
12	158	0	7	1	**8**	4.43	0.63	**5.06**
14a	157	0	1	0	**1**	0.64	0.00	**0.64**
14b	158	0	3	0	**3**	1.90	0.00	**1.90**
15	157	0	1	0	**1**	0.64	0.00	**0.64**
16	159	0	20	2	**22**	12.58	1.26	**13.84**
17	158	0	3	0	**3**	1.90	0.00	**1.90**
18	150	0	4	4	**8**	2.67	2.67	**5.33**
19	159	0	1	7	**8**	0.63	4.40	**5.03**
20	159	0	4	0	**4**	2.52	0.00	**2.52**
21	**158**	**0**	**2**	**0**	**2**	**1.27**	**0.00**	**1.27**

[†]Lesions were separated according to the presence or absence of pycnidia after incubation of fruit, however lesions without pycnidia were assumed to be caused by *G. citricarpa* and are included in the totals.
CBS = citrus black spot.

Appendix table 3. 2006-07 season survey results.

	2006-07							
			No. fruit with CBS-like lesions			% fruit with CBS-like lesions		
Survey site no.	No. fruit assessed	No. fruit with citrus scab	Pycnidia present	Pycnidia absent	**Total no. fruit with CBS**[†]	Pycnidia present	Pycnidia absent	**Total % fruit with CBS**[†]
1a	161	0	0	16	0	0.00	9.94	0.00
1b	160	0	0	0	0	0.00	0.00	0.00
2	157	0	0	3	0	0.00	1.91	0.00
3	159	0	1	26	1	0.63	16.35	0.63
4	167	0	11	0	11	6.59	0.00	6.59
5	160	0	2	1	2	1.25	0.63	1.25
6	159	0	5	7	5	3.14	4.40	3.14
7	160	0	3	16	3	1.88	10.00	1.88
8	158	0	0	9	0	0.00	5.70	0.00
10a	154	0	3	5	3	1.95	3.25	1.95
10b	160	0	4	2	4	2.50	1.25	2.50
11	159	0	1	2	1	0.63	1.26	0.63
12	159	0	5	1	5	3.14	0.63	3.14
14a	160	0	2	1	2	1.25	0.63	1.25
14b	158	0	0	14	0	0.00	8.86	0.00
15	160	0	4	2	4	2.50	1.25	2.50
16	157	0	44	22	44	28.03	14.01	28.03
17	158	0	2	1	2	1.27	0.63	1.27
18	159	0	1	60	1	0.63	37.74	0.63
19	160	0	1	10	1	0.63	6.25	0.63
20	160	0	0	3	0	0.00	1.88	0.00
21	162	0	0	4	0	0.00	2.47	0.00

[†]Only lesions with pycnidia were found to be PCR-positive for *G. citricarpa*, therefore only fruit with lesions with pycnidia are included in totals.
CBS = citrus black spot.

Appendix table 4. 2007-08 season survey results.

2007-08

			No. fruit with CBS-like lesions				% fruit with CBS-like lesions			
Survey site no.	No. fruit assessed	No. fruit with citrus scab	Pycnidia present	Pycnidia absent	Pycnidia absent and +ve PCR[†]	**Total no. fruit with CBS[Φ]**	Pycnidia present	Pycnidia absent	Pycnidia absent and +ve PCR	**Total % fruit with CBS[Φ]**
1a	159	0	0	4	0.38	0.38	0.00	2.52	0.24	0.24
1b	156	0	0	3	0.29	0.29	0.00	1.92	0.18	0.18
2	159	0	7	6	0.57	7.57	4.40	3.77	0.36	4.76
3	170	0	2	16	1.53	3.53	1.18	9.41	0.90	2.08
4	162	0	2	3	0.29	2.29	1.23	1.85	0.18	1.41
5					no data[Ω]					
6	160	0	10	47	4.50	14.50	6.25	29.38	2.81	9.06
7	160	0	3	0	0.00	3.00	1.88	0.00	0.00	1.88
8	159	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00
10a	161	0	8	3	0.29	8.29	4.97	1.86	0.18	5.15
10b	163	0	3	2	0.19	3.19	1.84	1.23	0.12	1.96
11	160	0	1	7	0.67	1.67	0.63	4.38	0.42	1.04
12	162	0	22	3	0.29	22.29	13.58	1.85	0.18	13.76
14a	147	0	0	1	0.10	0.10	0.00	0.68	0.07	0.07
14b	160	0	0	3	0.29	0.29	0.00	1.88	0.18	0.18
15	156	0	6	3	0.29	6.29	3.85	1.92	0.18	4.03
16	166	0	18	10	0.96	18.96	10.84	6.02	0.58	11.42
17	155	0	1	5	0.48	1.48	0.65	3.23	0.31	0.95
18	153	0	0	18	1.72	1.72	0.00	11.76	1.13	1.13
19	164	0	31	2	0.19	31.19	18.90	1.22	0.12	19.02
20	162	0	2	0	0.00	2.00	1.23	0.00	0.00	1.23
21	158	0	0	0	0.00	0.00	0.00	0.00	0.00	0.00

†9.57% of lesions without pycnidia were found to be PCR positive for *G. citricarpa* in the 2007-08 season, therefore the number of fruit with lesions without pycnidia that are likely to have been caused by *G. citricarpa* (i.e. 'Pycnidia absent and +ve PCR') = 'Pycnidia absent' × 0.0957.

[Φ]Totals = 'Pycnidia present' + 'Pycnidia absent and +ve PCR'

[Ω]Permission to survey site number 5 in the 2007-08 season could not be obtained.
CBS = citrus black spot, +ve = positive.
z

Appendix 3. October 2008, paper and oral presentation "Integrated Disease Management of Citrus Black Spot (*Guignardia citricarpa* Kiely) in Queensland, Australia", Wuhan, China, 11th International Citrus Congress.

Integrated Disease Management of Citrus Black Spot (*Guignardia citricarpa* Kiely) in Queensland, Australia

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Additional index words: Murcott, integrated disease management, Phyllosticta

Abstract. **Citrus black spot (CBS) (*Guignardia citricarpa*) is a significant disease of citrus grown in the Central Burnett district of Queensland, Australia. Disease control currently relies primarily on the application of protectant fungicides (e.g. copper or dithiocarbamate) to reduce infection, and appropriately timed picking and cooling of fruit to avoid expression of symptoms postharvest. However, evidence in the literature suggests cultural practices such as pruning and mulching can help reduce CBS. Despite this it is uncommon in the Central Burnett that these cultural practices are integrated with fungicide applications to control CBS. The desire to produce CBS-free fruit, as well as the reduction in fungicide options with the recent loss of the systemic fungicide benomyl, has generated an incentive to demonstrate if integrating chemical and cultural strategies can significantly reduce CBS incidence relative to the district-wide incidence. A district survey was undertaken to measure the level of CBS found in blocks treated with the "current management program" typically adopted throughout the district, and found the majority of blocks (91%) to have less than 5% of fruit affected by CBS. In the same season as the survey, a "best practice**

program" for CBS control was devised based on strategies reported in the literature, and applied to three commercial-scale trial plots in the district. The best practice program integrates: i) altered fungicide application timing to account for losses of fungicide coverage as fruit rapidly expand, ii) pruning to reduce pycnidiospore inoculum, promote tree vigour and spray penetration, and iii) application of hay mulch over the leaf litter to suppress liberation of ascospores from fallen leaves. The highest proportion of CBS-affected fruit were harvested from untreated control trees (3.17%), followed by the current management program trees (2.54%), and the least CBS affected fruit were harvested from the best practice program trees (0.05%). Our research shows that significant improvements in CBS management are possible by integrating protectant fungicide application with pruning and mulching.

Citrus black spot (CBS) is primarily a cosmetic disease of citrus fruit caused by the fungus *Guignardia citricarpa* (Kiely). The reduced marketability of fruit affected by CBS has led to the disease being of economic significance in many citrus producing countries of the world, including certain regions of Australia (Calavan, 1960; Kiely, 1948b; Korf et al., 2001; McOnie 1964; Wager 1952). In Australia CBS only occurs in parts of Queensland, the Northern Territory and coastal New South Wales, where summer rainfall is prevalent. Extensive surveys have demonstrated an absence of CBS from the inland, winter rainfall areas of the Riverland (South Australia), Sunraysia (New South Wales and Victoria border), and Riverina (southern New South Wales) regions, resulting in the internationally recognised area freedom status of these areas (Barkley, 1988; Broadbent, 1995; Corporate, 1998; Wall, 1989). This area freedom status has allowed the successful export of citrus to countries for which CBS at present is a quarantine concern, such as the United States of America (Biggs, 2001).

Research on the pathogen biology, disease cycle, epidemiology and chemical control conducted in Australia initiated effective CBS management in the CBS-endemic regions of the country (Kiely, 1948a; Kiely, 1948b; Kiely, 1950). Further improvements in CBS control in Australia followed the demonstration under local conditions of the high efficacy of the systemic fungicide benomyl for CBS control (Kiely, 1976). For 30 years benomyl was a very effective addition to the fungicide options for CBS control, but by the end of 2006 the supply and use of benomyl was

prohibited by the Australian Pesticides and Veterinary Medicines Authority. The loss of benomyl has reduced the diversity of CBS management options in Australia.

Symptoms caused by *G. citricarpa* on mature citrus fruit are characterised by red to black-rimmed depressed lesions with a light grey or brown centre filled with black pycnidia (Fig. 1.). Other lesion types such as hard spot, freckle spot, spreading or virulent spot and speckled blotch have also been described as occurring on fruit (Kiely, 1948b; Kiely, 1960). Under certain circumstances fruit abscission can occur in addition to the cosmetic affects of CBS on fruit (Benson, 1895; Kiely, 1948a). Several published descriptions of the CBS disease cycle exist (Kiely, 1948b; Kiely, 1950; Kotze, 1981; Kotze, 1996). The disease cycle consists of a primary cycle involving typically-airborne ascospores, and a secondary cycle involving typically-water dispersed pycnidiospores. Ascospores are forcefully ejected from perithecia that develop on infected citrus leaves after they fall to the ground and are subjected to cycles of wetting and drying. Pycnidiospores form within the small black pycnidia that are commonly found in the centre of lesions on mature fruit, and sometimes within lesions on leaves, or on the surface of dead twigs, leaves, and occasionally on fruit stalks. Airborne ascospores arising from the leaf litter are reported to play a relatively more important, but not exclusive, role in CBS epidemics than pycnidiospores (Kiely, 1948b; Kotze, 1981). Citrus fruit are reported to be most susceptible to infection for approximately the first 20-24 weeks of development (Baldassari et al., 2006; Kotze, 1981; Wager, 1952).

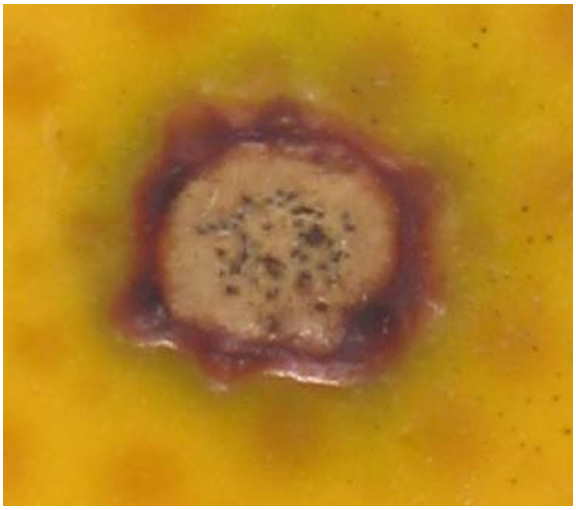

Fig.1 A typical lesion of citrus black spot (*Guignardia citricarpa*), on the surface of a mature Murcott mandarin fruit, appearing as a red to black-rimmed depressed lesion, with a light grey or brown centre containing black pycnidia.

Several different methods for reducing CBS, including both chemical and cultural means, have arisen from gaining an understanding of the CBS disease cycle. The efficacy of protectant (e.g. copper and dithiocarbamate) and systemic (e.g. benomyl) fungicides has been extensively studied, and found to be effective when applied at the appropriate stage in fruit development (Beattie et al., 1989; Bertus, 1981; Kiely, 1950; Kiely, 1967; Kiely, 1976; Miles, et al., 2004; Rodriguez and Mazza, 1996). The effect of fruit expansion on residual fungicide levels has also been studied. It has been observed that with fruit diameter increases of 40% over 2 weeks, and 50% over one month, the metallic copper residue on fruit reduces by ~70% and ~90%, respectively, due to the disproportionate rate at which fruit surface area increases relative to diameter (Timmer et al., 1998). This suggests a need for the revision of timing of fungicide applications.

To a far lesser extent than chemical control, the efficacy of cultural control methods for CBS such as inoculum reduction and canopy management has also been studied. A reduction in the amount of ascospore inoculum through the application of a layer of grass mulch over fallen leaves on the orchard floor has been reported to increase the production of CBS-free fruit by approximately 20% (Schutte and Kotze, 1997). The mulch layer is believed to act as a physical barrier to prevent the release of ascospores into the air. This approach has also been effective in reducing leaf litter-borne inoculum in apples (Holb, 2006). Further to mulching, there is evidence that canopy management can reduce CBS. Pruned lemon trees were found to have significantly less fruit with CBS (Loest, 1968). The exact reason for the reduction in CBS is not explicitly known, but reduced susceptibility of healthy, vigorous hosts has been suggested; as such pruning has been recommended to improve tree vigour (Calavan, 1960; Kiely, 1950; Kotze, 1961; Loest, 1968). Pruning may also remove potential sources of pycnidiospore inoculum such as dead twigs, as well as improve fungicide penetration by reducing canopy density, a factor affecting spray application efficiency (Stover et al., 2002). As a result of the above findings, these various control options are suggested for CBS control in commercial orchards (Mayers and Owen-Turner, 1987; Timmer et al., 2000).

Despite both chemical and cultural practices being developed for CBS control, in the CBS-endemic regions of Australia the control of CBS presently relies almost entirely on fungicide applications. In the absence of the systemic fungicide benomyl, a fungicide application program was developed consisting of 5-weekly application of

protectant fungicides with either copper or dithiocarbamate active ingredients when fruit are susceptible to *G. citricarpa*. Timely harvesting and appropriate storage conditions are also undertaken to reduce CBS symptom expression. Since it would be very rare to find a commercial citrus orchard that routinely integrates cultural and chemical practices for CBS, and in the absence of any studies conducted in such orchards, the potential improvements in CBS control under an integrated "best practice program" are unknown.

The Central Burnett district in the state of QLD is the largest citrus production area in Australia affected by CBS; Murcott mandarins being the most common variety. CBS control in Murcott mandarins for fresh markets is therefore a priority in the Central Burnett. In order to demonstrate the CBS control that is possible using a best practice program, we sought to address the following questions: (i) what is the present level of CBS in the Central Burnett district, and (ii) can CBS be significantly reduced using a best practice program incorporating canopy management, inoculum reduction measures and revised fungicide applications? Increasing the amount and reliability of supply of CBS-free citrus fruit would be of benefit to citrus producers in the CBS-endemic regions of Australia.

Materials and Methods

District survey.

In order to determine the levels of CBS throughout the Central Burnett citrus production region, 22 representative blocks (Fig. 2.) of Murcott mandarin trees under typical CBS management were selected at the end of the 2006-07 season. The "current management program" typically applied for CBS control in the district is detailed in Table 1. To determine the levels of CBS in each block, 4 to 6 weeks prior to commercial harvest five trees were randomly chosen from across each block and two pieces of fruit randomly selected from each of 16 canopy positions (north, south, east and west, by upper and lower canopy, and by inner and outer canopy) of each tree, giving a total of 160 fruit per block. Sampled fruit were then incubated at 27 °C, 80% relative humidity and permanent light for 3 weeks to promote disease development (Brodrick and Rabie 1970). Visual confirmation of symptoms of CBS was considered sufficient if lesions were typical red to black-rimmed depressed spots with a light grey or brown centre containing pycnidia (Fig. 1.). All other suspect

lesions were diagnosed by incubation of affected tissue on potato dextrose agar or by a species-specific real time PCR assay to demonstrate the presence/absence of *G. citricarpa*. In total approximately 3500 fruit were assessed for symptoms of CBS.

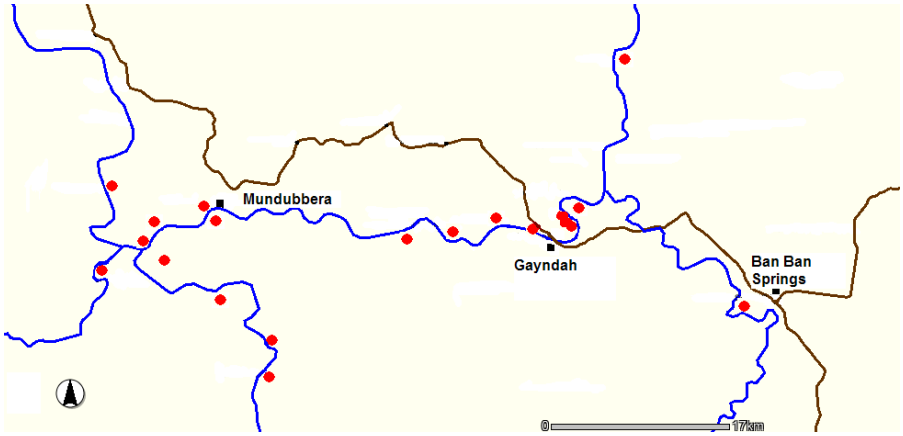

Fig.2 Map of the Central Burnett district showing the locations of the district survey sites (dots), relative to the townships of Mundubbera and Gayndah.

Field management trials.

To determine if CBS levels could be reduced relative to disease incidence levels obtained under the current management program, as determined in the district-wide CBS survey above, a best practice program was devised based on integrating a number of different strategies previously reported in the literature. The best practice program involved applying these published strategies including pruning, mulching and revised fungicide applications, in a single "best practice program" treatment under large-scale field conditions. In the 2006-07 season the best practice program detailed in Table 1 was applied to at least 300 Murcott mandarin trees arranged in a plot of approximately 12 rows of approximately 25 trees. Single, large plots replicated at three different sites in the district were adopted instead of multiple, smaller plots replicated at the same site, to reach a compromise between the need to: i) suppress airborne ascospores potentially able to spread between rows or small experimental plots in the same orchard, and ii) sample sufficiently high numbers of trees and fruit to detect treatment effects under the relatively low disease incidence found under intensive disease management conditions. Two replicate plots of this design were established near the township of Gayndah, and a third replicate plot established near the township of Mundubbera (Fig. 2.). The trees had been planted in 1984, 1985 and 1988 for the two Gayndah sites and single Mundubbera site, respectively. Control

plots, 50 trees (two rows of 25), which did not receive any CBS management treatments were established adjacent, but not immediately next to each of the three best practice program plots. This selection of a single plot containing fewer trees than the best practice program plot was adopted instead of a balanced design to reach a compromise between the need to: i) avoid the contamination of the best practice program plot with airborne ascospores from the control plot, and ii) reduce the amount of contamination of the commercially valuable trees in the vicinity of the untreated trees.

Table 1 The current management program typically adopted in the Central Burnett district for the control of citrus black spot (*Guignardia citricarpa*), compared with an experimental best practice program.

Weeks post anthesis	Current management program	Best practice program
<0	Pruning: optional, typically mechanical if undertaken, no requirement to remove prunings	Pruning: all trees by hand and remove prunings from the canopy and orchard floor
2	Fungicide: copper	Fungicide: copper
3		Mulching: hay mulch mechanically spread[Z] over leaf litter to ~20cm depth and finished by hand if required
5		Fungicide: dithiocarbamate
7	Fungicide: copper	
8		Fungicide: dithiocarbamate
11		Fungicide: dithiocarbamate
12	Fungicide: dithiocarbamate	
14		Fungicide: dithiocarbamate
17	Fungicide: dithiocarbamate	Fungicide: dithiocarbamate
≥20	Fruit increasing in resistance	

[Z]Tomahawk 6060 (Teagle Machinery Ltd., Cornwall, UK) round bale shredder

Data collection in the best practice program blocks commenced 3-4 weeks prior to commercial harvest. Fruit were sampled in a systematic pattern (generally every second tree) to improve sampling accuracy under conditions of low disease incidence and non-uniform disease distribution, as has been reported for CBS (Sposito et al., 2007). A total of 94 trees were sampled from each best practice program plot, within an area of the treated plot surrounded on all sides by a buffer of two treated trees. The sample from each tree consisted of two fruit from all of 16 canopy positions as described previously for the district survey, for a total of 3008 fruit per plot. In the untreated control plot, a total of 640 fruit per plot were sampled by taking five fruit from each of the 16 canopy positions described above, from only the inter-row-facing halves of the canopies of 16 trees. In total just under 11000 fruit were incubated and assessed for symptoms of CBS.

Statistical analysis.

Previous pilot experiments (unpublished) showed no significant difference in CBS between the three trial sites. Assuming no site differences, CBS levels in the three best practice program trial plots were compared with that found in the control plots and under the current management program. The data was analysed in Genstat (GenStat, 2008) using a Generalised Linear Model (GLM) with binomial distribution and logit link.

Results

District survey.

Results of the Central Burnett district surveys conducted in the 2006-07 season are shown in Fig. 3. The majority of blocks (20 out of 22) were found to have less than 5% of fruit affected by CBS. One block was found to have 28% of sampled fruit affected by CBS.

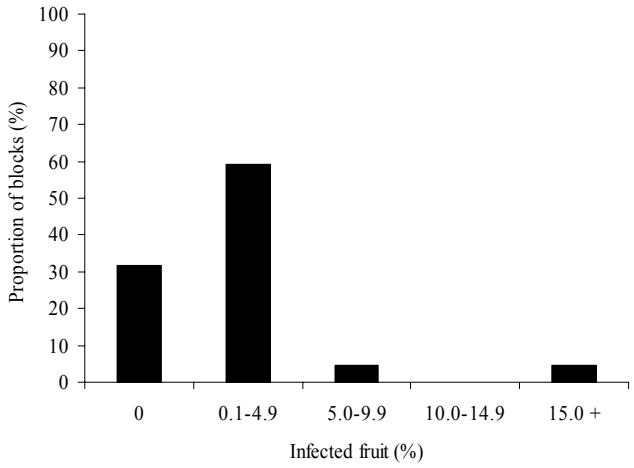

Fig.3 The proportion of blocks in the Central Burnett district with different proportions of fruit with symptoms of citrus black spot, determined in the 2006-07 season by a survey of 22 Murcott mandarin blocks representative of the district.

Field management trials.

In the 2006-07 season, the level of CBS in the best practice program trial plots was found to be significantly ($P<0.001$) lower than the CBS levels measured under the current management program (Table 2). The highest levels of CBS were found in the untreated control plots located at the three best practice program trial sites.

Table 2 The proportion of citrus black spot (CBS) affected Murcott mandarin fruit in commercial blocks treated with the current management program typically adopted in the Central Burnett district, compared to that of samples from trial plots treated with a best practice program integrating canopy management, mulching over leaf litter and fungicide applications, or trial plots with no CBS management (untreated control).

Treatment	Proportion with CBS[Z] ± SE
Current management program	0.0254 ± 0.0092 a
Best practice program	0.0005 ± 0.0008 b
Untreated control	0.0317 ± 0.0140 a

[Z]Means followed by the same letter are not significantly different at the P=0.05 level

Discussion

Our results demonstrate that the incidence of CBS can be significantly reduced in the Central Burnett district using a best practice program integrating the cultural

practices of pruning and mulching in conjunction with a revised protectant fungicide application regime. Furthermore, the results of the district survey indicate that the overall level of CBS present in the district is low, with the majority of blocks surveyed having less than 5% of sampled fruit affected by CBS. The level of CBS was also low in the untreated control plots, most likely due to a history of commercial CBS management resulting in low levels of carry-over inoculum from previous years. Further research is being undertaken to provide additional data on the effectiveness of the proposed best practice program.

The relative contributions of the individual components of the best practice program to the reduction in CBS relative to the current practice program were not measured in this experiment. Whilst determining this is highly desirable, preference was given to determining a benchmark level of CBS control possible using methods that are practically available to producers in a district where CBS occurs at generally low, but consistent levels. Our evidence suggests that this benchmark level for the Central Burnett is ≥99.89% CBS-free fruit (95% confidence) at harvest.

Several important considerations were identified during the formulation of the best practice program. These included the timing of the pruning, mulching and fungicide applications. Pruning was undertaken prior to flowering to ensure the removal of possible pycnidiospore inoculum sources prior to susceptible fruit being present, but also to allow this time intensive task to be undertaken in the break between seasons. Mulch was applied after the first fungicide application, due to the nature of the high-pressure/velocity sprayers used in citrus production inducing significant fall of declining leaves after winter. Additionally, to avoid the production of ascospores from exposed leaves the mulch application preceded the onset of summer rain, which was found in previous studies (unpublished) to coincide with the development of ascocarps in the leaf litter a few weeks later. The importance of rainfall to ascospore inoculum has also been reported from South Africa (Kotze, 1981). Fungicide timing was adjusted to occur more frequently during the susceptible period to reduce the losses in coverage that occur as fruit expand, and to avoid the use of copper-based products in the warmer parts of summer when rind stippling may occur (Schutte et al., 1997). It is acknowledged that the best practice program significantly increases the use of fungicide, however, it is likely that the total quantity of fungicide could be reduced by the adoption of lower volume spray application

systems. It is also unknown if fungicide dosage for CBS control can be reduced when the frequency of application is increased.

Apart from any contribution to CBS control, casual observation suggested mulching had improved irrigation efficiency, reduced weed occurrence under the trees, and preliminary soil studies have shown consistently fewer plant parasitic nematodes in soils under the mulch. Similar observations have been reported in other trials, including additional benefits such as increased tree vigour and yield (BangChu, et al., 2007; Huang and Liu, 1987; Ingle, et al., 2001; Mohanty, et al., 2002; Patil, et al., 2002; Verdu and Mas, 2007). However, increased organic matter in soil can affect the availability of plant nutrients (Stirling and Eden, 2008). The general effects of mulching in agriculture have been reviewed by Jacks et al. (1955).

A cost-benefit analysis of the best practice program is yet to be undertaken, but would be of significant use to citrus growers affected by CBS in Australia. However, hay bail and water costs can be significant variables in a drought-prone country such as Australia. The ability to mechanically apply the mulch increases the practicality and affordability of this management practice under Australian conditions.

Acknowledgments

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Appendix 4. February 2005, poster presentation, "Characterising the incidence of citrus black spot in Central Queensland orchards", Thredbo, NSW, Thredbo Statistical Meeting.

CHARACTERISING CITRUS BLACK SPOT INCIDENCE IN CENTRAL QUEENSLAND

Rosemary Kopittke and Pauline Wyatt
Department of Primary Industries & Fisheries, Queensland

Introduction

- Aimed to assess the distribution of citrus black spot in the Central Burnett as part of a project which is developing and evaluating pre and postharvest disease management practices that will meet phytosanitary requirements allowing the export of Queensland citrus to the USA.
- In general, determination of the probable distribution of disease in pest risk assessments is done on limited data, perhaps just a few values from large samples taken over a few years.
- Surveyed disease incidence under the current management system – 160 fruit from each of 21 blocks on 14 orchards in the Central Burnett area.
- Compared observed data with the distributions typically used by the regulatory arm of the United States Department of Agriculture (USDA) in their pest risk assessments and investigated the impact these choices would have on the chance of success of the application for market access.

Location of properties sampled for citrus black spot in Central Burnett area

Fitting distributions to observed data

- Attempts to fit the data to a range of distributions in GenStat failed to produce models of good fit – almost 40 percent of the samples returned no positive identification of Citrus Black Spot.
- Using Palisade @RISK (the software used by USDA in the Pest Risk Assessments), seven models were successfully fitted: Beta general, Extreme value, exponential, logistic, normal, triangular, uniform (in order of goodness of fit)
- Only two of these had non-significant chi-square goodness of fit statistics – the Beta General and Extreme value models.

Sampling Murcott mandarins

Distributions used in recent Pest Risk Assessments (PRA)

Based on expert opinion – most are essentially reliant only on the minimum, most likely and maximum values

- Uniform (avocados from Mexico, 1995)
- Triangular (clementines from Spain, 2001)
- Beta (citrus from Argentina, 1997)
- Pert (clementines from Spain, 2001 and 2003) defined as

$$f(x) = \frac{(x-\min)^{\alpha_1-1}}{B(\alpha_1,\alpha_2)(\max-\min)^{\alpha_1+\alpha_2-1}} \quad \mu \equiv \frac{\min+4*\text{mostlikely}+\max}{6} \quad \alpha_1 \equiv 6\frac{\mu-\min}{\max-\min} \quad \alpha_2 \equiv 6\frac{\max-\mu}{\max-\min}$$

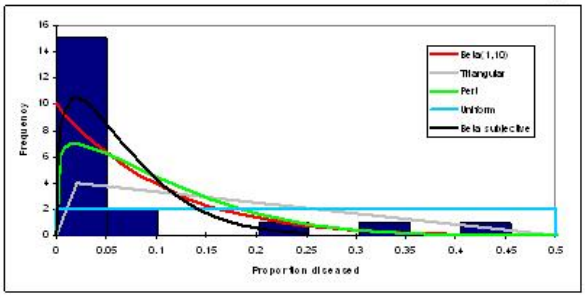
where B is the Beta distribution; min=minimum, mostlikely=mode and max=maximum values

The Pert distribution is less sensitive to the extremes of the data and more sensitive to the most likely value. In a modified version of the distribution increasing the "4" in the formula for the mean produces a progressively more peaked distribution.

Comparison of PRA models with observed data

(a Beta Subjective model which includes the mean as a fourth parameter was also graphed)

- In general, the PRA models are conservative. They give higher disease incidence probabilities than recorded and fail to reflect the large number of samples which recorded zero positives.



Percentage of Murcotts infected by the pathogen citrus black spot compared to the distributions likely to be chosen to characterise the disease distribution for Pest Risk Analysis

- The Beta distribution chosen by manually selecting the parameters is better at reflecting the peak than the uniform and triangular distributions.
- The Pert distribution, which has been used in the most recent PRA examined, also models the peak better; using a modified Pert distribution may improve the match.

Conclusions

When USDA's current distribution options are used

- they overestimate the likelihood of pest establishment in the importing country and hence
- lessens the possibility of Central Burnett orchardists accessing the export market

Recommendation

Collect survey data from additional years and orchards to develop a model which more accurately describes the incidence of citrus black spot disease.

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