Citrus Pathology Resource Scientist

Dr Andre Drenth
The University of Queensland

Project Number: CT07012

CT07012

This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the citrus industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of:
Syngenta Australia Pty Ltd
the citrus industry

ISBN 0 7341 3273 5

Published and distributed by:
Horticulture Australia Ltd
Level 7
179 Elizabeth Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399

HAL
Horticulture Australia

CT07012 - Citrus Pathology Resource Scientist

Final Report

Dr Andrew Miles
Department of Agriculture, Fisheries and Forestry
&
Assoc. Prof André Drenth
The University of Queensland, Centre for Plant Science

August 2013

HAL Horticulture Australia
Citrus Australia
The University of Queensland
Queensland Government

Project Details

Project Title: Citrus Pathology Resource Scientist

HAL Project number: CT07012

Project Leader: A/Prof André Drenth
The University of Queensland,
Centre for Plant Science, Queensland Alliance for Agriculture and Food Innovation (QAAFI) Brisbane.

Ecosciences Precinct, Level 2C West, 41 Boggo Road, Dutton Park, QLD 4102, GPO Box 267, Brisbane, QLD, 4001.
Ph: +61 7 3255 4391; Fax: +61 7 3846 2387;
Email: **a.drenth@uq.edu.au**

Team Member Dr Andrew K Miles
Horticulture and Forestry Science, Department of Agriculture, Fisheries and Forestry (DAFF), Brisbane.
E-mail: **andrew.miles@daff.qld.gov.au**

Collaborators: Andrew Beattie, Grant Chambers, Richard Davis, Nerida Donovan, Bob Emmett, Deborah Hailstones, Sandra Hardy, Paul Holford, Joanne Luck, Ceri Pearce, Malcolm Smith, Lucy Tran-Nguyen, Matthew Weinert, Pauline Wyatt
Added during project
Nancy Cunningham, Peter Dal Santo, Dale Griffin, Andrew Harty, Lauren O'Connor, Dan Papacek, Roger Shivas, Mui-Keng Tan, Yu Pei Tan, Peter Taverner

Expert: Patricia Barkley

Funding sources: This project has been funded by HAL using the citrus industry levy, voluntary contributions from from Syngenta Australia Pty Ltd and matched funds from the Australian Government. The Department of Agriculture, Fisheries and Forestry Qld also provided funds.

Date of report 12 August 2013

Statement of purpose of the project
The project addressed pathology issues within the four strategic industry investment areas of: i) germplasm; ii) productivity; iii) market access and biosecurity; and iv) resource, technical support, extension and training. The objective was to approach disease issues under these areas in an integrated manner. Several diseases were addressed during the project, as documented in the report. Another important objective was training and collaboration at different levels to build capacity to deal with disease issues in the future.

Project commencement date: 20 September 2007; **Project completion date:** 12 August 2013

Media Summary

Project CT07012 addressed strategically important pathology issues in four key areas: i) germplasm; ii) productivity; iii) market access and biosecurity; and iv) resource, technical support, extension and training.

A screening assay we developed resulted in the generation of ~20,000 hybrid *Citrus* resistant to the 'Emperor' brown spot disease for commercial evaluation. In the long term resistance to this disease will significantly reduce losses and increase orchard profitability.

Orchard productivity due to diseases was minimised through improved access to effective fungicides. The project contributed to access to two useful products, and has identified several more promising fungicides for use in the future. Input was also provided to aid growers in recovering from two major flooding events which took place during the project.

In order to strengthen market access and biosecurity, two pathogens that already occur in Australia were characterised in detail to underpin market access submissions and biosecurity decisions. A cost effective way to improve Biosecurity is to increase surveillance by training industry pest scouts and consultants to identify exotic pests and diseases. We collaborated with Biosecurity Queensland and provided targeted training to 45 pest scouts and surveillance officers.

Four pathology workshops attended by representatives of government, universities, and Citrus Australia Limited were organised as part of the project to discuss in detail pathology resources, technical support, extension and training. The outcomes of research activities discussed at the workshops were then extended to growers through presentations at the Citrus Australia National Conferences, regional forums, and more than 10 industry media articles.

To ensure pathology expertise will continue to be available to the industry a suite of new projects covering various aspect of germplasm; productivity; market access, biosecurity, technical support, extension and training have been developed and submitted to Horticulture Australia Limited for consideration.

Technical Summary

Project CT07012 addressed pathology issues in the four connected areas: i) germplasm; ii) productivity; iii) market access and biosecurity; and iv) resource, technical support, extension and training. These are four strategically important areas for the Australian citrus industry, which all have the potential to be negatively impacted on by plant pathogens.

The development of a method for screening hybrid mandarin varieties for susceptibility to the 'Emperor' brown spot disease, caused by the fungus *Alternaria alternata*, resulted in 20,000 resistant hybrids for commercial evaluation. The availability of these resistant hybrids has the potential to significantly reduce the impact of this disease and was possible due to the effective collaboration between CT07012 and the National Scion Breeding Program. We also identified the possibility of breeding for resistance to other diseases such citrus scab, caused by the fungus *Elsinoë fawcettii*.

Productivity in citrus is reduced by diseases, but during this project two major floods took place which had a direct impact on the orchards, as well as indirect impacts by increasing disease pressure. The main diseases were 'Emperor' brown spot and citrus black spot. We estimated that these two diseases cost the industry $19M per season in fruit losses and control costs. Managing disease following the two floods was achieved through Emergency Use Permits, and the evaluation of new fungicides. Candidate fungicides were scrutinised for efficacy, resistance management, and compatibility with export market maximum residue limits (MRLs). At the completion of CT07012, efficacy data had been generated for six novel fungicides, including at least one promising multi-site activity fungicide and three promising succinate-dehydrogenase inhibitor (SDHI) fungicides.

Market access and biosecurity is a priority issue for the citrus industry. Major activities in this area were reviews of surveillance and import risk analyses, biosecurity awareness training, and research to underpin market access. A cost effective way to improve Biosecurity is to increase surveillance by training industry pest scouts and consultants to identify exotic pests and diseases. We collaborated with Biosecurity Queensland and provided targeted training to 45 pest scouts and surveillance officers. Detailed scientific input was provided into import risk analysis to ensure that the analysis was based on sound science. Research in market access involved the use of DNA sequencing to show that the same fungus, *Phyllosticta citricarpa*, causes black spot in Australia and other countries.

Resource, technical support, extension and training were provided through four citrus pathology workshops attended by representatives from government, universities, and Citrus Australia Limited. Research outcomes were extended to growers through presentations at industry conferences, regional meetings, and technical field visits to individual orchards. The project has published three peer-reviewed journal articles, two additional articles in preparation, one book chapter, and more than ten grower-focussed articles.

Project CT07012 has delivered significant outcomes in the four different strategic areas, and to deliver further outcomes for the citrus industry new proposals were developed at the last citrus pathology workshop and submitted to HAL for consideration in November 2012. These projects include CT13020 *Increasing market access, profitability and sustainability through integrated approaches to fungal disease control*, focussed on continued breeding for resistance to diseases and evaluation of fungicides. A specific CBS project CT13021 *Joint Florida and*

Australia citrus black spot research initiative will focus on identifying sources of resistance to black spot, as well gaining a better understanding of the leaf litter cycle. Collaborator project submissions include CT13009 *Protecting Australian citrus germplasm through improved diagnostic tools* to ensure on-going access for growers to high health status planting material. A specific biosecurity project has also been submitted to HAL. This suite of project proposals offers the industry on-going expertise in the areas of germplasm, productivity, market access, biosecurity, resource, technical support, extension and training.

Table of contents

Chapter 1

Germplasm

1.1 Introduction

The objectives of the activities outlined in this chapter are aimed to **provide pathology support to all aspects of *Citrus* germplasm manipulation, such as breeding, rootstock and scion evaluation projects, budwood and seed supply, post-entry quarantine (PEQ), and nursery practices and standards**. The Australian citrus industry invests significant sums of money into these aspects of germplasm including; funding of breeding programs (e.g. Horticulture Australia Limited (HAL)-funded National Citrus Scion Breeding Program); purchase of budwood (e.g. Auscitrus), seed (e.g. regional seed schemes), and nursery trees; and import of new germplasm requiring inspection and post-entry quarantine (PEQ). All these forms of investment are/can be negatively affected by plant pathogens and benefit from the input of this project, and the input of the project collaborators through their various agency supported roles. Just a few examples of pathogens which can negatively impact *Citrus* germplasm include pathogens that impede the:

- productivity of trees e.g. orange stem pitting caused by *Citrus tristeza virus*;
- quality of fruit e.g. Emperor brown spot (EBS) caused by *Alternaria alternata*;
- trade of fruit e.g. citrus black spot (CBS) caused by *Phyllosticta citricarpa*;
- efficiency of evaluation of germplasm produced by the breeding program e.g. *A. alternata*, and scab caused by *Elsinoë fawcettii*.

To varying extents this project has contributed to reducing the negative effects of pathogens in each of the above examples. Specifically, the project team and collaborators have:

- worked together to achieve the construction of new insect-proof screenhouse facilities at Bundaberg to prevent the spread of insect-vectored pathogens to valuable germplasm;
- participated in NSW DPI-led inspections for various graft transmissible diseases, such as orange stem pitting (CTV), in the Auscitrus budwood multiplication scheme;
- screening of hybrids from the National Scion Breeding program for resistance to *A. alternata*;
- conducted pilot experiments to identify *P. citricarpa* host status of accession in the Bundaberg germplasm collection; and
- undertaken surveys for citrus scab susceptibility in progeny blocks and identify parent families that more readily produce resistant progeny.

Deployment of resistance and improvement in the provision of clean planting material is aimed at managing citrus diseases before the orchard phase of production, therefore greatly minimising the impact and costs to growers associated with dealing with disease once established in the orchard.

1.2 Construction of an insect-proof screenhouse facility at the Bundaberg Research Station

Background

Citrus can be negatively impacted by a range of insect-vectored pathogens. In Australia, the most serious of these pathogens are certain strains of *Citrus tristeza virus* (CTV), such as the orange stem pitting strains reported from Queensland (Owen-Turner, 1990; Barkley, 1991). CTV is spread primarily by the brown citrus aphid (*Toxoptera citricida*)(Broadbent *et al.*, 1996). Other insect-vectored pathogens occur outside of Australia, and pose a major biosecurity risk to Australian citrus production. The *Candidatus* Liberibacter spp. causing huanglongbing (HLB), or citrus greening disease, are easily the most concerning of these exotic pathogens (Barkley *et al.*, 2010). In the case of HLB the bacteria are spread by sap sucking psyllid insects (Garnier and Bove, 2000). A critical management tool for diseases such as CTV and HLB is the exclusion of insect vectors from primary germplasm sources (Barkley and Forsyth, 1987; Aubert, 1990). In the case of *Citrus* in Australia, the only facilities for this purpose were the insect-proof screenhouses located at the NSW DPI Elizabeth Macarthur Agricultural Institute, Camden NSW, and the Auscitrus propagation facility at Dareton, NSW. However these facilities are dedicated to their primary function of germplasm protection and propagation. Additional facilities in different regions would therefore be highly beneficial for research purposes such as *Citrus* breeding, as well as quarantine purposes in the case of an outbreak of an exotic disease such as HLB. Therefore the CT07012 project team collaborated to prepare an application to Qld DAFF to construct an insect-proof screenhouse at the Bundaberg Research Facility. An application was prepared and a letter of endorsement (below) was provided by André Drenth. The application was successful and the screenhouse completed in March 2009.

Discussion

The screenhouse facility has been a major step forward for the breeding program, enabling research work on a number of issues critical to the citrus industry. Genetic resistance to CTV has been studied in rootstock hybrids bred locally as well as those imported from overseas. Material from this extensive study has now been planted commercially in central Queensland. Genotypes highly sensitive to CTV have been protected in the screenhouse against virus transmission by aphids and used in hybridisation work. Such genotypes would not have survived without this facility and unique traits would have been lost from the breeding program. An experiment comparing virus-free and virus-infected budwood, and interactions with rootstock, was propagated within the facility and has now been planted in the field to examine infections rates and impacts on yield and quality. Recalcitrant pollen producers are being kept in the facility, and can be allowed to reach full anthesis without fear of pollen cross contamination from insects. Seedlings are being germinated and grown within the facility to develop disease-free budwood sources. Plants resulting from shoot-tip-grafting are also being housed to prevent re-infection. Hybrids from the triploid breeding program are kept in the facility to prevent leaf miner damage and encourage maximum vegetative growth prior to field planting. None of these activities would have been possible without this insect proof facility, which was constructed at no cost to the citrus industry.

THE UNIVERSITY OF QUEENSLAND

Tree Pathology Centre

Queensland Government
Department of Primary Industries and Fisheries

April 14, 2008

Dr Malcolm Smith
Citrus Breeder
Bundaberg Research Station

As part of the DPI&F breeding program for citrus based at Bundaberg Research Station it is paramount that germplasm and advanced breeding material is being kept in an insect proof screen house. The reasons for this are:

- There are a number of damaging stem-pitting strains of citrus Tristeza virus in Queensland which are insect transmitted. Without an insect proof facility breeding material may leave Bundaberg Research Station carrying the virus which is unacceptable in the light that it is easily prevented and leaves the organisation open for litigation.

- One of the main diseases which is widespread in Southeast Asia, but absent in Australia is Citrus Greening. This disease is insect transmitted and introduction of this disease has the potential to destroy citrus production in Queensland and all breeding materials generated in the last few decades. Citrus variegated chlorosis is also a potential threat to Australian citrus and insect transmitted.

- Budwood source trees need to be placed within an insect proof enclosure as from these trees budwood is obtained for the production of nursery trees. A source of trees with a high health status is paramount to control the spread of insect transmitted diseases already in Australia as well as exotic diseases on our doorstep in Southeast Asia.

An insect proof screen house is a low tech but highly effective solution for the production of plant material with a high health status. The use of insect proof screen houses is standard practice in most parts of the world. The current practice of sourcing budwood from field grown trees of unknown health status is simply not acceptable in light that a simple and cheap alternative is available which has been shown to be highly effective for many other crops.

Malcolm, if I can be of any further assistance to help you secure these facilities for the citrus breeding program please do not hesitate to contact me.

Sincerely,

Dr. Andre Drenth
Principal Research Fellow
Leader Tree Pathology Centre
The University of Queensland & Department of Primary Industries and Fisheries

DPI&F Plant Pathology Building, 80 Meiers Road, Indooroopilly, QLD 4068

Ph +61 7 3896 9345
fax +61 7 3896 9533
DrenthA@dpi.qld.gov.au

1.3 Auscitrus Budwood Multiplication Scheme Inspection

Background

Australian citrus growers and nurserymen rely on the Auscitrus propagation scheme for access to high health status, true to type Citrus budwood and rootstock seed (Barkley and Forsyth, 1987). The routine pathogen indexing and inspection of the source material is critical to preventing the spread of damaging graft transmissible viruses and viroids. Following the 2009 Citrus Australia National Conference, Nerida Donovan (citrus pathologist, NSW DPI) and Grant Chambers (Auscitrus Indexing Officer, NSW DPI) invited Andrew Miles (DAFF, Qld) to participate in inspections of the Auscitrus multiplication blocks. Time was also spent conducting various field visits around the region. The full trip report prepared by Nerida Donovan is below.

Report on Auscitrus inspections and field visits

National Citrus Pathology Program

Sunraysia region – November 2009

Nerida Donovan, NSW DPI

Donovan N (2009) Inspection of Auscitrus Budwood Multiplication Blocks, Dareton Agricultural Research and Advisory Station. 11th November. NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Menangle, New South Wales

Citrus Australia National Conference, Mildura

The annual citrus industry conference was held in Mildura on 9-10/11/09. The conference theme was 'Varieties, Commercialisation, Biosecurity' and was attended by citrus growers, nurserymen and service providers, the majority from Australia and New Zealand. Andrew Miles (Citrus Pathologist, Qld DEEDI) and Nerida Donovan (Plant Pathologist, NSW DII-PIE) were present at both days of the conference and Grant Chambers (Auscitrus indexing officer, NSW DII-PIE) attended the second day. Andrew gave a field presentation on 10/11/09 highlighting the recent pathology work being undertaken by pathologists involved with the National Citrus Pathology Program, including brief updates of other R&D work occurring in Queensland.

Dareton Agricultural Research and Advisory Station (ARAS)

Nerida Donovan and Grant Chambers showed Andrew Miles around the NSW DII-PIE Dareton ARAS. The tour included an inspection of severe field symptoms of exocortis viroid on Washington navel trees that form part of a viroid trial planted in 1989. The trial was established to determine the effect of citrus viroids on tree size, health and fruit production per canopy surface area. Also of interest were the mandarin rootstock trials, with several Chinese rootstocks demonstrating excellent graft compatibility.

Inspection of the Auscitrus budwood multiplication blocks

The Auscitrus budwood multiplication blocks at the Dareton ARAS were inspected on 11/11/09 by Grant Chambers, Nerida Donovan and Andrew Miles.

All trees in the budwood blocks 2, 3D and 4, and rows 1 and 2 of budwood multiplication block 3E were checked for disease symptoms and off-type shoots (approx. 1600 trees).

No symptoms were observed of the following diseases:

- citrus canker (*Xanthomonas citri* subsp. *citri)*

- huanglongbing or citrus greening (*Candidatus Liberibacter* sp.)

- stubborn (*Spiroplasma citri*)

- citrus black spot (*Guignardia citricarpa*)

No symptoms of grapefruit stem pitting (ropy or deep pits on trunks or branches) were observed on the grapefruit trees surveyed. However, we know from prior testing that some grapefruit trees in the budwood multiplication blocks contain strains of CTV other than the mild cross protecting strain of PB61.

Observations and recommendations for specific trees were provided in a report to the Auscitrus manager, Tim Herrmann.

A sample was collected from a grapefruit tree in the citrus field repository which was exhibiting leaf symptoms consistent with Australian Citrus Dieback (ACD). The sample will be tested in the laboratory for phytoplasma – the suspected cause of ACD. A further reason for the interest in the ACD sample is the similarity of the symptoms to those of huanglongbing.

Auscitrus AGM

On 11/11/09, Grant Chambers and Nerida Donovan attended the Auscitrus AGM held at the Auscitrus office in River Road Dareton. At the meeting they spoke about the testing of the Auscitrus budwood and rootstock seed supply trees and the citrus repository program. Andrew Miles attended the meeting as an observer in order to learn more about how the industry organisation functions.

Field visits – Sunraysia

On 12/11/09, Steven Falivene (District Horticulturist, NSW DII-PIE) took plant pathologists Andrew Miles and Nerida Donovan to 3 orchards in the Sunraysia region.

Frank Simoneta – Mourquong

The group visited an orchard where the trees were supposed to all be Eureka lemon on Benton citrange rootstock. A number of trees were showing reduced vigour and windows were cut in the bark at the bud union to reveal a brown stain under the bark. Steven had previously visited the site with Sandra Hardy (Industry Leader – Citrus for NSW DII-PIE) and they had diagnosed incompatibility. Eureka lemon is normally compatible with Benton citrange.

Sam Cross Holdings – Curlwaa

Manager – Justin Kassulke

The group visited an orchard of Lanes Late navel on *Citrus trifoliata* rootstock. The trees were planted on mounds and estimated to be around 10 years old. The block was converted to drip irrigation 3 years ago. The manager said they had been working on optimising the irrigation of the block but there were periods when the soil was waterlogged and then dried. The soil was a clay loam but the topsoil was only shallow therefore most of the soil in the mound was still quite heavy.

Trees on the ends of some rows were either in decline or had recently died, with the fruit still on the tree. Where a tree at the end of the row had died, the next tree along the row was in decline. Justin predicted it took around 12 months for most of the affected trees to die. One of the dying trees was pulled out of the ground using a tractor and chain. As the tree was lifted it broke in half revealing a cross section of the trunk. A large section of the root system was dying and had a paucity of feeder roots. The cross section showed a brown discoloration of some of the major roots and up into the trunk, stopping at the bud union.

The decline and death of the trees appears to be consistent with sudden death disorder. It is possible that the wetting and drying cycles on the clay loam soil led to a deterioration of root health. Opportunistic soil organisms invade the dying root system causing further problems. The trees are able to cope until too much of the root system is infected and the roots are no longer able to support the canopy – leading to tree death.

Seven Fields – Sunwest

Manager – Grant King

The group visited a block of Afourer mandarin trees on Troyer citrange and Cleopatra mandarin rootstocks. The trees had been planted in virgin soil in 2006. On average last season, the trees on Troyer had a greater crop load than those on Cleopatra. Scattered trees on Cleopatra had winter yellows last autumn and were now exhibiting yellowing over the whole canopy. Agriphos had been applied a few months before (early spring). The feeder roots of 3 affected trees were inspected and appeared to be healthy.

The manager and his off-sider (Graham) are planning to tag some of the affected trees and monitor their progress over the next 12 months, including observations of crop load. They are also going to send soil and leaf samples for nutrient analysis and nematode counts. Nerida has offered to test samples for graft transmissible pathogens if the problems persist without an identifiable cause. Samples were not collected on the day due to the extremely high ambient temperature (42°C) which is known to greatly reduce viral titre.

Soil variations across the orchard were noticeable from the air when departing Mildura (also noticeable using Google Earth, Fig. 1.3.1). It would be necessary to plot the locations of affected trees in order to detect any influence these variations may have on the incidence of the disorder.

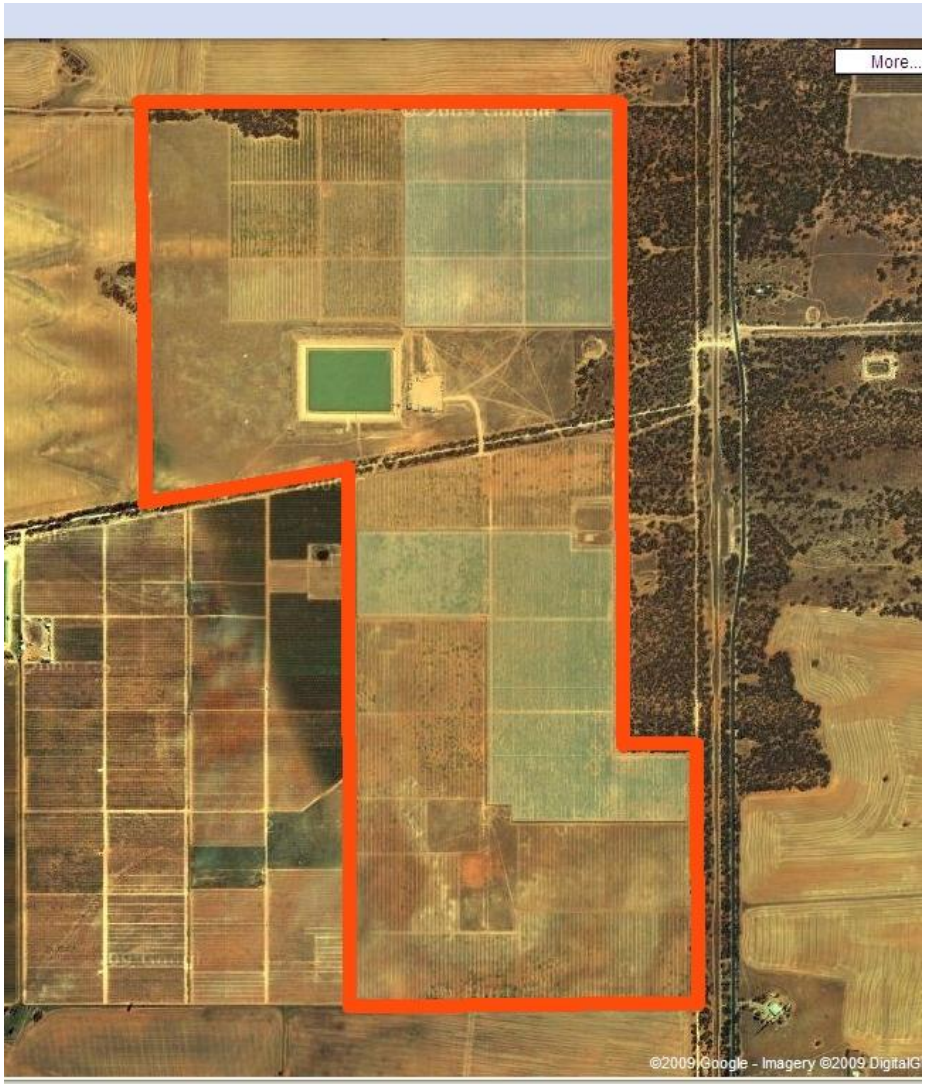


Figure 1.3.1 Google Earth image of Seven Fields orchard. Orange line indicates boundary.

1.4 Characterisation of EBS susceptibility in advanced germplasm

Introduction

The fungus *Alternaria alternata* causes the EBS disease in certain varieties of mandarin (e.g. Emperor), tangelos (e.g. Minneola) and tangors (e.g. Murcott) (Pegg, 1966). The disease can be highly destructive, resulting in at least $5M in crop losses in Queensland annually (Miles *et al.*, 2011). Symptoms of the disease include brown to black spots surrounded by a chlorotic halo on leaves and fruit, defoliation and shoot dieback (Fig. 1.4.1). Some of the disease symptoms result from a host-specific toxin produced by the fungus which kills the host cells before feeding on the dead tissue. Growers currently invest upwards of $1.5M annually in the application of protective fungicides to prevent fungal growth and toxin production. However, relying on fungicides is risky due to the potential for failures caused by poor application timing, coverage, fungicide resistance, or weather patterns that prevent spray application or reduce the efficacy of fungicides. A more reliable solution to EBS is selecting for resistant mandarin varieties.

HAL projects CT09014 'Early season replacement for Imperial mandarin' and CT09023 'Commercial development of subtropical mandarins' aim to develop new mandarin varieties suited to Australian conditions. Under these projects the team located at the Bundaberg Research Station has performed in excess of 5,000 cross pollinations each spring to generate hybrid seedlings with potential to replace existing varieties. Seedlings are then field planted in high density plots (10,000 trees/ha) for horticultural evaluation. A number of advanced selections have been made from this hybridisation program in the past, but the susceptibility to EBS has not been fully characterised for these selections.

Resistance to EBS would significantly reduce the cost of growing varieties such as Murcott in Queensland, improving profit margins for growers and reducing fungicide inputs to the environment and for concerned consumers. The aim of this work was to characterise the susceptibility of advance selections in the Bundaberg-based breeding program. Collaboration between CT07012, CT09014 and CT09023 leading to the release of new varieties resistant to EBS would largely eliminate one of the costliest diseases of *Citrus* in Australia.

Methods

Characterisation of existing selections

To enable screening for resistance to EBS, a detached leaf assay was undertaken to characterise the susceptibility to EBS in advanced scion selections from the breeding program. The leaf assays were performed largely by the same methods as those of Dalkilic *et al.* (2005), however instead of misting the leaves with spore suspension, a single point inoculation was performed. Three replicate susceptible leaves of 30 advanced selections from the scion breeding program were collected from the field, washed and surface sterilized. Also collected were leaves of Daisy and Murcott as known susceptible controls, grapefruit as a tolerant control, and Ellendale as a known resistant control. A fourth leaf of every selection/variety was also collected for use as a negative control. Leaves were placed into petri dishes lined with a filter paper saturated in sterile distilled water. A conidia suspension was prepared from a known

pathogenic isolate of *A. alternata* by flooding a colony on potato dextrose agar (PDA) with sterile distilled water, then lightly scraping the surface of the colony with a sterile spatula to liberate the conidia. The suspension was then adjusted to 8 x 10^5 conidia/mL using a haemocytometer. A single 10µL drop of conidia suspension was then placed on each of the 3 leaves of each selection/variety. A single 10µL drop of sterile distilled water was placed on the fourth leaf of every selection/variety as a negative control. Following inoculation the petri dishes were incubated at room temperature (24±1°C) and high humidity for 4 days. Every 24 hours the average lesion diameter was determined by calculating the mean of the longest and shortest dimensions of the lesion. The area under the disease progress curve (AUDPC) was then determined (Campbell and Madden, 1990) and compared for the different selections/varieties by ANOVA.

Figure 1.4.1 Symptoms of EBS caused by *Alternaria alternata*. Brown spots surrounded by a chlorotic halo on fruit (top left) and a leaf (centre). Necrotic vascular system due to movement of the host specific toxin (bottom left). Blighting of flush tips (right).

Results

Characterisation of existing selections

EBS lesions developed on the susceptible Murcott and Daisy leaves, small lesions developed on the tolerant grapefruit leaves, while no lesions developed on the resistant Ellendale leaves (Table 1.4.1). No lesions developed on leaves treated with water. Based on the results the various selections could be loosely categorized as resistant, tolerant, or susceptible. Resistant selections showed no lesion development (AUDPC = 0), susceptible selections were those with an AUDPC not significantly different from that of Murcott, and tolerant selections were those with an AUDPC >0, but significantly lower than Murcott. Rankings according to AUDPC should be considered as only a guide to the relative susceptibility of selections, as the variables of leaf age and size could not be fully accounted for.

Table 1.4.1 Means of the area under disease progress curve for lesions caused by *Alternaria alternata* on various *Citrus* selections/varieties[A]

Variety/selection	AUDPC	Notes
Ellendale	0.0 a	Resistant control
00C028	0.0 a	
00C032	0.0 a	
02C018	0.0 a	
02C062	0.0 a	
02C065	0.0 a	
07C001	0.0 a	
07C004	0.0 a	
00C013	1.7 ab	
09C013	2.3 abc	
Grapefruit	5.8 abcd	Tolerant control
02C048	7.0 abcde	
08C001	8.0 abcdef	
05C020	10.1 abcdefg	
02C061	12.0 bcdefgh	
09C018	13.0 cdefghi	
02C063	13.5 defghi	
07C005	14.5 defghij	
02C100	15.8 defghijk	
00C018	16.8 defghijk	
01C011	16.8 defghijk	
02C104	17.3 efghijk	
05C007	17.3 efghijk	
02C014	17.4 efghijk	
Daisy	17.8 efghijk	Susceptible control
03C004	19.3 fghijk	
03C043	19.4 fghijk	
02C059	20.1 ghijk	
05C016	20.1 ghijk	
02C001	22.0 hijk	
05C003	23.0 hijk	
00C029	24.3 ijk	
Murcott	25.3 jk	Susceptible control
02C055	26.9 k	
P-value	<0.001	

[A]Mean values within columns followed by the same letter are not significantly different at $P = 0.05$.

Discussion

Detached leaf assays have been used to characterise the susceptibility of advanced mandarin selections from the breeding program. Of the 30 selections tested, 7 were identified as resistant to *A. alternata*, 8 as tolerant, and the remainder susceptible. In this case both tolerant and susceptible selections are sensitive to the toxin produced by the fungus, with slower disease progress in the tolerant selections likely to be due to physiological host factors such as cuticle thickness. Cuticle thickness has been shown to explain the changes in susceptibility of leaves to *A. alternata* (Pegg, 1966).

Characterising the resistance and susceptibility of advanced selections will assist the breeder to select specific parents for use in future hybridizations to maximize the production of resistant progeny. This is particularly important for developing EBS resistant breeding into a commercial-scale practice for the breeding program.

1.5 Commercial-scale EBS resistance screening as the first step in breeding new mandarins for Australia

Andrew K. Miles[1*], Toni K. Newman[2], Debra L. Gultzow[2], S. Carola Parfitt[2], André Drenth[3] and Malcolm W. Smith[2].

[1]The University of Queensland and Queensland Department of Agriculture, Fisheries and Forestry, EcoSciences Precinct, Australia, andrew.miles@daff.qld.gov.au; [2]Queensland Department of Agriculture, Fisheries and Forestry, Bundaberg Research Station, Australia; [3]Centre for Plant Science, The University of Queensland, EcoSciences Precinct, Australia.

Miles AK, Newman TK, Gultzow DL, Parfitt SC, Drenth A, Smith MW (2013) Commercial-scale alternaria brown spot resistance screening as the first step in breeding new mandarins for Australia. *Acta Horticulturae* (**Submitted**).

Abstract

Rapid screening tests and an appreciation of the simple genetic control of EBS susceptibility have existed for many years, and yet the application of this knowledge to commercial-scale breeding programs has been limited. Detached leaf assays were first demonstrated more than 40 years ago and reliable data suggesting a single gene determining susceptibility has been emerging for at least 20 years. However it is only recently that the requirement for genetic resistance in new hybrids has become a priority, following increased disease prevalence in Australian mandarin production areas previously considered too dry for the pathogen. Almost all of the high-fruit-quality parents developed so far by the Queensland-based breeding program are susceptible to EBS necessitating the screening of their progeny to avoid commercialisation of susceptible hybrids. This is done effectively and efficiently by spraying 3-6 month old hybrid seedlings with a spore suspension derived from a toxin-producing field isolate of *Alternaria alternata*, then incubating these seedlings in a cool room at 25°C and high humidity for 5 days. Susceptible seedlings show clear disease symptoms and are discarded. Analysis of observed and expected segregation ratios loosely support the hypothesis for a single dominant gene for susceptibility, but do not rule out the possibility of alternative genetic models. After implementing the routine screening for EBS resistance for three seasons we now have more than 20,000 hybrids growing in field progeny blocks that have been screened for resistance to the EBS disease.

Keywords: alternata, disease, citrus, mandarins, Australia, genetics, susceptibility

Introduction

The EBS disease is caused by the fungus *Alternaria alternata* (Fr.:Fr.) Keissl (Pegg, 1966) with one particular strain affecting certain mandarins (e.g. ‘Emperor’), tangors and tangor hybrids (e.g. ‘Murcott’). Advanced leaf symptoms are typically large necrotic areas, surrounded by a chlorotic halo and often associated with vein darkening, premature senescence and entire shoot death (Pegg, 1966; Swart *et al.*, 1998; Timmer *et al.*, 2000). Symptoms on fruit are expressed as sunken, brown lesions, observed reaching up to 5mm in diameter. A chlorotic halo often surrounds

lesions on green fruit, but becomes indistinguishable on coloured fruit. Worldwide, the disease now causes significant problems in almost all areas where susceptible varieties are grown. In Australia, the economic cost of EBS is estimated to be more than USD $3,000 per hectare in fruit losses and control costs (Miles *et al.*, 2011).

Although the EBS disease cycle is relatively simple, it is very challenging to disrupt through management practices such as the application of fungicides. The ability of the fungus to induce symptoms in the plant tissue and sporulate within a period of only a few days makes the pathogen highly damaging under suitable environmental conditions. Alternaria is a necrotrophic fungus and conidia are produced on dead tissues in the tree canopy and on abscised leaves and twigs on the orchard floor (Timmer *et al.*, 1998a). Production of conidia requires periods of leaf wetness, before they are dislodged and dispersed by wind (Timmer *et al.*, 1998a). When conidia germinate on the surface and infect a susceptible host, cell necrosis occurs within 30 hrs, and before any host penetration occurs (Pegg, 1966). Cell necrosis is related to the production of a host specific toxin (HST) by the fungus (Kohmoto *et al.*, 1991). This necrosis is the result of leakage of electrolytes from host cells after exposure to the HST (Kohmoto *et al.*, 1979). Only young leaves are susceptible to the fungus, becoming resistant once the leaf cuticle is sufficiently developed (Pegg, 1966). In Australia, fruit are susceptible to the disease regardless of age (Miles *et al.*, 2005). Completion of the disease cycle occurs through the production of conidiophores and conidia on infected tissue (Timmer *et al.*, 1998a). Control using protectant fungicides is reliant upon achieving thorough coverage of rapidly expanding leaves and fruit, before pathogen attack; coverage of expanding plant parts is known to be difficult to achieve and maintain (Timmer *et al.*, 1998b). Cultural practices, such as pruning to improve air movement and removal of dead tissues, have proven ineffective under commercial conditions.

A more reliable and long term sustainable approach to controlling EBS would be the development of cultivars that are resistant to the disease. The susceptibility of mandarins and tangors to EBS is determined by the sensitivity of the cultivar to the HST produced by the tangerine pathotype of *A. alternata* (Kohmoto *et al.*, 1991). The inheritance of sensitivity to the HST is hypothesised to be controlled by a single dominant gene (Dalkilic *et al.*, 2005). This simple genetic control creates an opportunity to breed resistant cultivars via conventional hybridisation. Due to the susceptibility of young plants a rapid bioassay of seedlings through direct inoculation may provide excellent results due to the fact that the pathogen: i) grows quickly and readily produces conidia in culture; ii) symptoms are expressed on leaves within very short time periods; and iii) toxin sensitivity under these conditions is an unambiguous trait. Despite these favourable genetic and practical characteristics, breeding for EBS resistance has only recently been considered a priority in Australia. This prioritisation follows a steady increase in EBS disease pressure and fruit losses in production regions traditionally considered too dry for serious EBS epidemics.

Breeding for resistance to EBS is a highly desirable and achievable goal for commercial breeding programs providing screening methods are effective, efficient and low cost. The aim of the research described in this paper is to develop a commercial-scale method for breeding for resistance to EBS in the mandarin breeding program based in Queensland, Australia. The specific aims were to: i) identify and test highly virulent isolates of *A. alternata* for use as an inoculum source; ii) develop a bioassay enabling screening of large numbers of hybrid seedlings; and iii) confirm the genetics of inheritance of resistance to EBS. The methods and findings of this study will assist our *Citrus* breeding program, as well as others, to contribute to the control

of this highly damaging disease. The production, evaluation and commercial release of *Citrus* cultivars resistant to EBS will greatly improve the profitability of citrus production in humid production areas where EBS occurs. Furthermore, resistant cultivars will break the reliance on fungicides for control of EBS.

Materials and Methods

Source of Isolates

In order to identify highly virulent isolates of *A. alternata* for use as an inoculum source in breeding activities, isolates were obtained from fresh EBS leaf specimens. Leaves with typical EBS lesions were collected from trees of 'Daisy' mandarin (*Citrus reticulata* Blanco) and 'Wekiwa' tangelo (a complex hybrid involving *C.* × *paradisi* Macf.) in the Bundaberg region of Queensland. Leaves were briefly surface sterilised by swabbing both sides with 70% ethanol, then allowing the ethanol to evaporate. Small pieces of leaf tissue were excised from the margins of the lesions and plated onto petri dishes containing half strength potato dextrose agar (PDA). The plates were incubated at 25 °C under black light for 3-5 days. Mono-conidial isolates of any *A. alternata* colonies that grew were obtained using standard techniques (Smith, 2002). The mono-conidial isolates were immediately stored at -80 °C as spore suspensions in 15% glycerol.

Confirmation of Pathogenicity

In order to confirm the pathogenicity of the isolates obtained above, detached leaf assays were performed using leaves of 'Murcott' mandarin (*C. reticulata* hybrid) and 'Lockyer' rough lemon (*C.* × *jambhiri* Lush). Leaves were prepared for the detached leaf assay based largely on the methods of Timmer et al. (1996). Cultures of the isolates above were established on PDA from under glycerol storage and incubated at 25 °C under near ultra violet light for 5 days. Conidia were harvested from the colonies by flooding the petri dish with sterile distilled water, and lightly scraping the colony surface with a sterile spatula. The resulting spore suspensions were then each adjusted to 1×10^5 conidia per mL. For each isolate three 20 µL droplets of spore suspension were placed evenly onto the underside of each of three replicate leaves of each *Citrus* cultivar. The detached leaves were then incubated at 25 °C for 5 days to allow lesions to develop. Lesion sizes were measured and compared to evaluate the virulence of isolates.

Large-Scale Bioassay

In order to develop hybrid cultivars resistant to EBS, large numbers of hybrid seedlings need to be screened each year for resistance to EBS using a direct seedling bioassay. A colony of a highly virulent, toxin producing isolate of *A. alternata* was established from under glycerol storage onto PDA. Within 5 days, the colony was subcultured onto 80 PDA plates for large-scale multiplication of conidia. The plates were incubated at 25 °C under black light for 5 days. Spore suspensions were prepared as above, and adjusted to 1×10^5 conidia per mL, resulting in a total of 3-4 L of spore suspension. Each year large populations of hybrid seedlings were produced from the corresponding year of hand pollinations. Seedlings were raised in polystyrene produce boxes (500 × 320 × 280mm) containing potting mix at approximately 60 seedlings per box. Boxes of seedlings were transferred to shelving in a refrigerated cold room programmed to operate at 25 °C. A domestic humidifier (Euky Bear Steam Vaporiser, Extralife, Australia) was added to the cold room to

create saturated air capable of maintaining constant leaf wetness without causing runoff. Seedlings were sprayed with the spore suspension to just before run-off using a hand-operated mister (Fig. 1.5.1). The seedlings were then incubated in the cold room at 25 °C and high humidity for 5 days. Plants remained wet with the spore suspension for the entire 5 days of incubation. After incubation the seedling boxes were returned to a shadehouse and visually inspected for disease symptoms. When clear EBS symptoms were observed on susceptible seedlings, the results were recorded, and the diseased seedlings discarded. Following inspection for EBS, the remaining resistant seedlings were grown for a further 6 months in a shadehouse before field planting at high-density (10,000 trees per ha) for horticultural evaluation.

Figure 1.5.1 Inoculation with *Alternaria alternata* and incubation of hybrid seedlings.

Genetics of Resistance

In order to confirm if the genetics of resistance was following the segregation ratios expected for a single recessive allele for resistance, as observed by Dalkilic et al. (2005), the segregation ratios from the large-scale bioassay were subjected to chi-square analysis.

Results and discussion

Of the 13 mono-conidial isolates retrieved from the lesions on leaves of 'Daisy' and 'Wekiwa', only 5 produced symptoms on 'Murcott' leaves in the detached leaf assay. The remaining 8 isolates failed to produce any symptoms. None of the isolates from 'Daisy' and 'Wekiwa' produced symptoms on the 'Lockyer' leaves, whilst symptoms were produced on these leaves by control isolates cultured from symptomatic rough lemon leaves. Based on these results it was concluded that the 5

isolates from 'Daisy' and 'Wekiwa' were of the tangerine pathotype of *A. alternata*. The relatively low recovery of pathogenic isolates from diseased tissue suggests a high frequency of saprophytic *A. alternata* colonisation of symptomatic tissue. Furthermore, differences in lesion size (data not shown) indicate putative differences in virulence between the 5 pathogenic isolates that produced symptoms. These observations highlight the need for thoroughly characterised isolates to be used in the screening process.

Large-Scale Bioassay

Symptoms of EBS were first observed 24-48 hours after inoculation (Fig. 1.5.2), and continued to develop during incubation. In 2010, 2011 and 2012 totals of 5,843, 7,083 and 17,089 hybrid seedlings were inoculated with *A. alternata* and inspected for EBS symptom development. A combined total of 9,038 out of 30,015 seedlings were culled due to the formation of EBS lesions after inoculation. The effectiveness of inoculation was consistent between years, based on the proportions of susceptible progeny resulting from 24 crosses that were repeated in multiple years. For example, 05C016 × 02C018 resulted in 27% and 33% susceptible progeny in 2011 and 2012, respectively. Cross 05C016 × 02C065 resulted in 39% and 38% susceptible progeny in 2011 and 2010, respectively. Cross DeNules × 09C018 resulted in 0% susceptible progeny in both 2011 and 2010. Some inconsistencies were observed, but only in 7 of the 24 cases where the same cross was made in multiple years.

Figure 1.5.2 EBS symptoms visible within 48 hours of inoculation with *Alternaria alternata* (top left). Inspection for symptoms (top right), discarding of susceptible seedlings (bottom left), and high density field planting of resistant hybrids (bottom right).

Genetics of Resistance

The inheritance of susceptibility to *A. alternata* being hypothesised to be controlled by a single dominant gene (Dalkilic *et al.*, 2005) is only partially supported by our crosses, as shown in Table 1.5.1. The examples in Table 1.5.1 were chosen arbitrarily on the basis of being the crosses with the largest population sizes, and/or include a parent of a well-known cultivar. In most examples there is a trend towards observing fewer susceptible offspring than expected. Crosses between two resistant parents produced almost no susceptible hybrids as expected. However, crosses between a resistant and a susceptible parent generally resulted in only ~30% susceptible hybrids, when the expected value was 50%. Similarly, crosses of two susceptible parents generally resulted in less than 60% susceptible hybrids when 75% was expected. Deviations from expected segregation ratios can occur due to a number of reasons including; i) a tendency for disease escapes during the inoculation procedure; ii) incubation conditions being suboptimal for *A. alternata*; iii) human error in detecting symptomatic plants; iv) genetic control being more complex than a single gene; and v) the influence of cytoplasmic genes. Disease escapes may be the result of incomplete coverage of all plants with spore suspension and/or the absence of young susceptible leaves on particular seedlings at the time of treatment. Incubation conditions being suboptimal for *A. alternata* infection and EBS development is considered unlikely. The ideal conditions for EBS are prolonged periods of leaf wetness at 25 °C (Canihos *et al.*, 1999). The inoculation conditions in the selection procedure mirrored these conditions, with clear EBS symptoms developing on successfully inoculated susceptible seedlings. Human error in detecting symptoms after inoculation cannot be ruled out, even though seedlings were assessed by experienced operators. Indeed, human error might be expected to overestimate disease susceptibility (rather than the underestimate we have observed) considering reports of susceptible reactions on small leaves taken from resistant accessions (Reis *et al.*, 2007). Disease escapes, poor incubation conditions and human error in detection would result in susceptible hybrids inadvertently being field planted. Some of these hybrids could reasonably be expected to later develop disease symptoms in the field; particularly when considering that these field plantings receive no fungicide applications. Of the >20,000 screened hybrids planted in the field, only one plant outside of the positive controls has shown EBS symptoms to date.

While errors in phenotyping cannot be dismissed, the absence of large numbers of diseased hybrids appearing in field plantings, and the consistent 'underestimation' of susceptible progeny across a range of screenings with different parents, in different years, suggests that a single gene model may not always be sufficient to explain segregation when heterozygous parents are used.

Dalkilic et al. (2005) suggest that cytoplasmic genes may explain distorted segregation seen in their reciprocal backcross. To further test this possibility, we identified 5 parental combinations where reciprocal crosses had been made. Using the susceptible parent as the female or male did not consistently increase or decrease the percentage of susceptible hybrids produced (Table 1.5.2). We therefore conclude that cytoplasmic genes do not satisfactorily explain distorted segregation ratios for EBS susceptibility.

Although homozygous susceptible cultivars are known, such as 'Minneola' and 'Orlando' (Dalkilic *et al.*, 2005), none of these have featured heavily in our breeding program because of fruit quality problems, and all were removed from the program before EBS screening commenced. Instead, the crossing and EBS screening have

demonstrated that all susceptible parents in the program are heterozygous and capable of producing disease resistant hybrids. This has important practical implications because all susceptible hybrids can be discarded without concern of loosing desirable traits from EBS-susceptible parents in the breeding program.

Table 1.5.1 Tests for segregation of *Alternaria alternata* resistance in *Citrus* hybrids subject to large scale bio-assay.

				Chi-square		
Crosses	Model	Sus[A]	Res[A]	0:1	1:1	3:1
00C019 × 'Clausellina'	ss × ss	1	327	1.00[B]		
00C019 × 'Miho Wase'	ss × ss	0	179	0.00		
00C019 × 'Okitsu'	ss × ss	1	170	1.00[B]		
00C019 × 11C015	ss × ss	0	512	0.00		
'Daisy' × 09Q035	Ss × ss	32	12	50.28	4.79	13.89
03C024 × 10Q033	Ss × ss	193	330	236.67	18.26	482.07
03C024 × 10Q055	Ss × ss	179	339	216.39	25.31	503.88
03C024 × 11Q034	Ss × ss	139	368	161.08	54.50	229.73
'Fallglo' × 05C016	ss × Ss	51	164	57.86	31.90	113.09
07C004 × 02C122	ss × Ss	144	188	183.88	2.93[2]	68.74
00C019 × 10Q046	ss × Ss	8	252	8.12	142.82	282.57
02C002 × 09Q029	ss × Ss	52	100	62.73	7.77	51.01
'Daisy' × 09Q028	Ss × Ss	7	8		0.03[B]	2.53[B]
03C022 × 11C028	Ss × Ss	315	232		6.33	37.11
05C007 × 09Q032	Ss × Ss	253	187		4.98	30.13
05C003 × 03C066	Ss × Ss	214	153		5.1	23.00

[A]Sus = susceptible to *A. alternata*, Res = resistant to *A. alternata*, [B]$P > 0.05$

00C019: 'Ellendale' × 'Murcott', 11C015: 'Imperial' × 'Nova', 09Q035: 06Q011 × Ellendale, 03C024: 'Fina' × 'Murcott', 10Q033: 'Encore' × 06Q006, 10Q055: 'Ellendale' × 01C007, 11Q034: 'Ellendale' × 06Q010, 05C016: 'Ellendale' × 'Murcott', 07C004: IM111 × 'Fremont', 02C122: 'Ellendale' × 'Murcott', 10Q046: 'Encore' × 06Q010, 02C002: 'Aust Clem' × 'Murcott', 09Q029: 'Ellendale' × 06Q006, 09Q028: 'Ellendale' × 06Q008, 03C022: 'Ellendale' × 'Murcott', 11C028: 'Ellendale' × 01C028, 05C007: 'Aust Clem' × 'Murcott', 09Q032: IM111 × 06Q008, 05C003: 'Imperial' × 'Murcott', 03C066: 'Ellendale' × 'Murcott'.

Table 1.5.2 Tests for segregation of *Alternaria alternata* resistance in reciprocal crosses of *Citrus* hybrids subject to large scale bio-assay.

				Chi-square
Crosses	Model	Sus[A]	Res[A]	1:1
00C019 × 05C016	ss × Ss	16	90	29.41
05C016× 00C019	Ss × ss	34	64	4.70
02C018 × 05C016 2010	ss × Ss	30	69	7.99
05C016 × 02C018 2010	Ss × ss	33	68	6.25
02C018 × 05C016 2011	ss × Ss	12	12	0.00[B]
05C016 × 02C018 2011	Ss × ss	8	22	3.45[B]
02C065 × 05C016	ss × Ss	4	2	0.34[B]
05C016 × 02C065	Ss × ss	27	44	2.06[B]
02C065 × 08C002	ss × Ss	15	55	12.44
08C002 × 02C065	Ss × ss	14	15	0.02[B]
09C018 × 05C016	ss × Ss	4	7	0.42[B]
05C016× 09C018	Ss × ss	16	37	4.33

[A]Sus = susceptible to *A. alternata*, Res = resistant to *A. alternata*, [B]$P > 0.05$

00C019: 'Ellendale' × 'Murcott', 05C016: 'Ellendale' × 'Murcott', 02C018: 'Oroval' × 'Imperial', 02C065: 'Ellendale' × 'Murcott', 08C002: 'Imperial' × 'Nova', 09C018: 'Ellendale' × 01C028,

Conclusions

Breeding mandarins resistant to EBS is an achievable goal that will pay dividends for citrus producers, consumers, and the environment. In the case of EBS, genetic resistance is expected to be highly robust and unlikely to breakdown under field conditions. This is largely due to resistance being the result of an absence in the host of a receptor site for the toxin, as has been demonstrated specifically for toxin sensitivity in rough lemon (Tsuge *et al.*, 2013), rather than the presence of single or multiple resistance genes which need to recognise specific elicitors produced by the pathogen and therefore tend to exert selection pressure upon the pathogen to overcome resistance (Poland *et al.*, 2009). Testament to the robust nature of this form of resistance is the long-standing resistance to EBS of 'Imperial' mandarin (*C. reticulata*) in Queensland despite growing alongside highly diseased varieties such as 'Murcott'.

The distorted segregation ratios, assuming a single gene model, require further investigation to determine whether they are an artefact of the screening methodology, or have a genetic basis. If these consistently distorted ratios are related to methodological problems then very substantial numbers of EBS susceptible hybrids will have been field planted. These populations in the field will be monitored over their life for signs of susceptibility to EBS under field conditions.

Even with the possibility that some susceptible hybrids have escaped the screening process, the methods described herein have removed nearly 10,000 EBS susceptible hybrids at a very early stage in the program. Practicality has come from an inoculation system that uses equipment already used by the program, or that is of very low cost and readily available (e.g. a standard cold room and domestic humidifier vs. a dedicated controlled environment facility). Adopting simple, cost-effective screening methods has been critical to the routine implementation of EBS resistance screening in the breeding program.

Acknowledgements

The authors gratefully acknowledge Citrus Australia Limited, Horticulture Australia Limited, The Queensland Department of Agriculture, Fisheries and Forestry and The University of Queensland. Additional thanks are given to the Gayndah and Mundubbera Fruitgrowers' Associations.

1.6 *P. citricarpa* host status

Introduction

CBS, caused by *P. citricarpa*, is a significant burden to citrus growers in the regions where the disease occurs. Not only can the fungus reduce marketable yield and require costly fungicide applications for control (Miles *et al.*, 2004), the quarantine status of the pathogen in countries such as the USA is preventing access to export revenue worth an estimated $67.5M annually (Harty, 2010). Whilst significant progress has been made on the fungicide and cultural control front to dramatically reduce the incidence of CBS in fruit (Miles *et al.*, 2008; Miles *et al.*, 2004), the development of resistant varieties would be hugely advantageous to growers. However, the key first step in any efforts to develop resistant varieties is to identify genetic sources of resistance in *Citrus*.

Citrus aurantium and its hybrids have been classified as resistant to *P. citricarpa* (Kotze, 1981). The Tahiti lime (*C. latifolia*) has also been classified as resistant in the past, but following further investigation has been reclassified as an 'insensitive' host, i.e. the pathogen can colonise fruit but does not cause CBS symptoms (Baldassari *et al.*, 2008). In contrast, a fruit classified as "resistant" would halt colonisation of plant tissue by the pathogen. It is likely that *C. aurantium* is also insensitive, rather than resistant, as cultures of *P. citricarpa* have been isolated from asymptomatic *C. aurantium* fruit (McOnie, 1964). Reports of *P. citricarpa* herbarium accessions associated with CBS symptoms on fruit of *C. aurantium* further confuse the host status of *C. aurantium* to *P. citricarpa* (Glienke *et al.*, 2011). The key problem this highlights is that identifying resistance is challenging, and would require thorough testing to confirm the resistant status.

Considering confirming resistance is going to be a significant body of research, it may be more efficient to firstly confirm the susceptibility of less well known *Citrus* accessions. Confirming susceptibility to *P. citricarpa* is significantly more straightforward, as an accession can be classified as susceptible simply by confirming the identity of a CBS lesion occurring on fruit. Using this approach it may be possible to quickly identify susceptible accessions amongst a germplasm collection, leaving a smaller, more targeted list of potentially resistant material for more thorough investigation.

The aim of this study was to investigate the feasibility of determining the host status of various *Citrus* accessions by incubating fruit exposed to field infection at the Bundaberg Research Station.

Methods

To investigate the feasibility of determining the host status of various *Citrus* hybrids and rare *Citrus* accessions to *P. citricarpa*, fruit were sampled and incubated to break latency of any field infections of the pathogen. In total 26 accessions from the Bundaberg Research Station *Citrus* arboretum were sampled (Table 1.6.1). Fruit were incubated at 27°C, 80% RH and permanent light for 21 days. Following incubation fruit were inspected by eye and suspect symptoms examined using light microscopy. Lesions were diagnosed as CBS if pycnidia of *P. citricarpa* were present on the lesion surface after incubation as above (Baayen *et al.*, 2003). If pycnidia were not present on the surface of suspect lesions, isolations were performed onto PDA according to Baayen *et al*. (2003). The successful culture of colonies typical of *P. citricarpa* was used to confirm lesions without pycnidia as being CBS, and the accession confirmed

as susceptible to *P. citricarpa*. If colonies of *P. citricarpa* were not obtained, the host status of the accession remained unconfirmed.

Results

Of the 26 accessions sampled, only the Baker's Sweet × *C. inodora* hybrid was confirmed as susceptible to CBS by both symptoms and isolation of *P. citricarpa*. Rough lemon was included as a known susceptible accession, which was confirmed in this experiment. The remaining accession either showed no suspect lesions after incubation, or developed lesions that could not be confirmed by isolations.

Discussion

This experiment aimed to determine if the susceptibility of *Citrus* accessions to *P. citricarpa* could be cheaply and easily confirmed by relying on the natural infection in the Bundaberg Research Station arboretum. The results suggest that infection levels are not sufficient to be useful, even though the pathogen is present, fungicide use is minimal, and the environment conducive. An observation possibly explaining the low disease pressure is the generally low level of leaf litter observed in the arboretum. Leaf litter is the main source of *P. citricarpa* inoculum (ascospores) in orchards (Kiely, 1948). It is hypothesised that the low amount of leaf litter is the result of faster leaf litter decomposition in the soil due to reduced fungicide use; fungicide run-off under commercial conditions is expected to result in reduced fungal saprophyte activity in the leaf litter.

On the basis of the hypothesis above it may be possible to obtain more reliable data concerning the susceptibility of *Citrus* germplasm by introducing a uniform source of inoculum to the arboretum. For example, leaf litter highly infested with *P. citricarpa* could be introduced from a commercial orchard with high disease pressure. This would remain a relatively cheap and easy approach for screening germplasm for CBS resistance. However, such a practice may not be in keeping with the thorough biosecurity policy of the research facility. The phytosanitary risks associated with introducing plant material from outside the research facility would need to be identified and mitigated. In any case, genetic solutions to CBS are unlikely to be found until effective screening methods can be developed.

Table 1.6.1 *Citrus* accessions, number of fruit sampled, number of suspected lesions of CBS, and the success of isolations for *Phyllosticta citricarpa*.

Accession	No. of fruit	Suspect lesions	*P. citricarpa* isolation
01C044	61	0	-
01C049	54	1	No
02C011	34	0	-
02C059	53	8	No
02C062	59	1	No
02C063	70	4	No
02C065	54	0	-
02C122	53	4	No
03C054	52	0	-
03C066	60	0	-
05C016	51	2	No
Baker's Sweet × *C. inodora*	7	1	Yes
Biji pommelo	14	0	-
Bos red × Chinotto A109	17	0	-
Cara	48	0	-
Chinotto hybrid (small red)	18	2	No
Chinotto × pommelo	22	0	-
C. australasica × Daisy	5	0	-
C. garrawayii	32	0	-
Daisy × *C. garrawayi*	21	3	No
Ellendale S3	52	0	-
IrM1	60	>5	No
IrM2	53	3	No
Murcott	61	0	-
Pomello	25	Speckled blotch?	No
Rough lemon	34	1	Yes

1.7 Inheritance of *E. fawcettii* resistance and susceptibility

Introduction

Citrus scab is a complex of various *Elsinoë* species and pathotypes. These various species and pathotypes cause citrus scab on particular cultivars and species of *Citrus*. Regardless of the host the symptoms of scab are generally observed as raised, corky pustules on the surface of fruit and/or leaves (Fig. 1.7.1) (Timmer, 2000). The presence of the pustules reduces the marketability of fruit. In Australia, citrus scab is mainly observed on lemons and some uncommon mandarins (Timmer *et al.*, 1996). Major commercial mandarin varieties such as Imperial are resistant to the pathotypes of the fungus occurring in Australia. However, hybridisation breeding aiming to develop improved varieties for Australian conditions has the potential to introduce susceptibility to scab into a mandarin background. It is therefore important to know if scab susceptibility is a segregating trait in hybrid populations, and if so know which parents used in hybridisation breeding are likely to produce high frequencies of susceptible progeny. Gaining a better understanding of sources of susceptibility to scab will help reduce the chances of commercially releasing new *Citrus* varieties susceptible to this disease.

Figure 1.7.1 Scab caused by *Elsinoë fawcettii* on a leaf of a *Citrus* hybrid.

Methods

In order to identify *Citrus* parents that produce high frequencies of progeny susceptible to citrus scab, the natural development of the disease in hybrid progeny blocks located at the Bundaberg Research Station was observed. Climatic conditions in the summer of 2010-2011 were ideal due to frequent rainfall. The progeny blocks contained young hybrid seedlings up to ~1m tall, planted at 10,000 trees per hectare. The high planting density and high proportion of susceptible vegetative growth and

proximity of this low growth to humidity from the constantly moist soil, was highly conducive to citrus scab development. All the plants were inspected in detail and the occurrence of symptomatic plants recorded. The resulting proportions of susceptible plants were then observed.

Results

A total of ~6,000 hybrid progeny resulting from ~80 crosses were inspected. The proportions of progeny with citrus scab symptoms from a single cross ranged from 0-100%. There were a number of parents that in most crosses produced progeny showing symptoms of scab. These included 02C061, 06Q007, 06Q035, and 06Q036. Similarly, there were a number of parents that tended to produce progeny not showing symptoms of scab. These included 03Q001, 06Q003, 06Q012, 07Q001, 07Q003, 07Q005, 07Q006, 'Encore' and 'Murcott'. The specific examples of 07Q005 and 06Q035 crossed with the same set of parents are provided in Table 1.7.1. The results in Table 1.7.1 show that crosses with 07Q005 produced mostly progeny that were free of scab symptoms, and the crosses with 06Q035 produced mostly progeny with scab symptoms.

Table 1.7.1 Numbers of hybrid seedlings of 07Q005 & 06Q035 crosses observed to be symptomatic and asymptomatic of citrus scab caused by *Elsinoë fawcettii* in evaluation blocks at the Bundaberg Research Station.

Crosses	No. symptomatic	No. asymptomatic
01C011 × 07Q005	0	30
'Aust Clem' ×07Q005	0	35
'Ellendale' × 07Q005	4	43
'Encore' × 07Q005	1	64
01C011 × 06Q035	70	1
'Aust Clem' × 06Q035	31	29
'Ellendale × 06Q035	47	13
'Encore' × 06Q035	70	63

Discussion

Observations of citrus scab symptoms in the progeny blocks at the Bundaberg Research Station strongly suggest that scab susceptibility is a heritable and segregating trait in *Citrus* hybrids. However, the overall set of crosses performed is not sufficient to determine the nature of the genetic control of this trait. More specific crosses and inoculations need to be performed to gain a more complete understanding of the genetics. Nevertheless a number of key parents have been identified for producing high or low frequencies of susceptible progeny. This knowledge will assist the breeder in selecting parents.

In this study the progeny were most likely exposed to the 'Tryon's' pathotype of *E. fawcettii*, but the 'Lemon' pathotype of *E. fawcettii* may also be present. To confirm no other species of *Elsinoë* were present, ten isolates were collected from the progeny blocks and their identity confirmed as *E. fawcettii* based on DNA sequence data (see full report provided to HAL). It is therefore only possible to make conclusions about the susceptibility of *Citrus* to the Tryon's and 'Lemon' pathotypes of *E. fawcettii*. Further work under controlled conditions using single isolates of the different pathotypes and species is required to understand how the genetics of scab susceptibility operates for all the different pathotypes.

This small study has shown that breeding for scab resistance in *Citrus* is possible, but it needs to be underpinned by a more thorough understanding of the species and pathotype variability in *Elsinoë* and the genetic control of susceptibility to these various *Elsinoë*. Furthermore, the development and use of a nursery seedling screening technique is essential to make significant progress as shown in the case of *Alternaria alternata* (see section 1.5). In addition to developing resistance to local pathotypes, it may also be possible to develop *Citrus* that are resistant to exotic pathotypes such as 'sweet orange scab'. Pre-emptive breeding of this nature would be a wise investment should an exotic pathotype become established in Australia.

1.8 Project outputs

The Germplasm component of this project proposed to deliver a number of project outputs for industry. The proposed outputs and how these have been individually addressed is provided:

1. Establish effective methods which allow screening of rootstock and scion germplasm with relevant pathogen strains to identify the level of resistance/tolerance in *Citrus* germplasm.

The most successful example of a CT07012 activity delivering this output has been the implementation of commercial-scale screening for resistance to EBS, which has already resulted in 20,000 resistance hybrids for commercial evaluation. The project has also shown that it may be possible to develop a similar process for screening for resistance to citrus scab.

2. Assessment of the tolerance/resistance to disease of potential new rootstocks and scions developed in the breeding program or imported.

Assessment of the tolerance/resistance to disease of potential new germplasm has focused on diseases of scions. The diseases assessed have been EBS, CBS, and scab. The activities of the project have contributed to rootstock assessments in a peripheral manner through assisting the breeder to obtain access to an insect-proof screenhouse which has enabled the screening of new rootstocks for resistance to *Citrus tristeza virus* (CTV). The CTV breeding activity is not funded through CT07012.

3. A better understanding of the inheritance of resistance to EBS in mandarins, which can be applied to the breeding program.

In order to gain a better understanding of the inheritance of resistance to EBS, while also delivering large numbers of resistant hybrids for horticultural evaluation, the segregation ratios arising from the various crosses were analysed. The outcomes of this analysis are reported in section 1.5.

4. Liaise with pathologists involved in budwood, seed scheme and nursery practices, and PEQ pathologist and help streamline procedures where required.

Throughout project CT07012, Andrew Miles has collaborated effectively with the citrus pathologist responsible for high health status germplasm, Nerida Donovan (NSW DPI). This collaboration is demonstrated through various publications and reports with the CT07012 report, and includes joint activities such as the inspection of the Auscitrus Budwood Multiplication Scheme.

1.9 Conclusions and future directions

Strong collaboration between pathology and breeding has the potential to significantly reduce the costs incurred by citrus growers for managing *Citrus* diseases, albeit with a long term focus. Project CT07012 has made significant progress in achieving this goal, with the most direct and concrete output being the production of large numbers of EBS resistant hybrids in a relatively short period of time. The likelihood of identifying an EBS resistant hybrid with commercial potential will be increased as the numbers of screened hybrids increases. The probability of finding varieties that are disease resistant and commercially acceptable has been maximised by developing screening methods suitable to testing very large numbers of seedlings. Project CT07012 has also investigated the potential for breeding for resistance to other diseases such as CBS and scab. Field deployment of disease resistance will not only reduce the overall usage of fungicides, but also reduce the risks associated with relying solely on fungicides for disease control. These risks include the withdrawal from use of fungicides, through to failures in disease control when fungicide applications cannot be made due to external forces such as adverse weather conditions. Future recommendations and actions are proposed below for each disease.

EBS (*Alternaria alternata*)

Recommendation: continue the routine screening of hybrids in the breeding program for resistance.

Action: a new project proposal has been prepared and submitted to HAL in November 2012 to allow this screening to continue.

Citrus tristeza virus

Recommendation: continue to support the maintenance of *Citrus* germplasm through Auscitrus and NSW DPI. Support the development of mild strain cross protection against severe strains of CTV causing orange stem pitting.

Action: a new project has been prepared and submitted to HAL by NSW DPI in November 2012.

Citrus scab (*Elsinoë* spp.)

Recommendation: specific research is needed to determine the genetic control of resistance to this disease, to both endemic and exotic pathotypes and species of the fungus. This study would include the development of a screening assay similar to that for screening for EBS resistance. The research required would be suitable for a PhD-level study.

Action: applications for funds to support a PhD study on citrus scab have so far been unsuccessful.

CBS (*Phyllosticta citricarpa*)

Recommendation: specific research is needed to determine if susceptibility to CBS is a heritable and segregating trait in *Citrus*. Determining this requires specific research and research tools, as unlike EBS, juvenile plants cannot be readily inoculated and assessed for susceptibility.

Action: a new project proposal has been prepared in collaboration with the University of Florida and submitted to the Citrus Research and Development Foundation and HAL in November 2012.

Chapter 2

Productivity

2.1 Introduction

The objectives of the activities outlined in this chapter are aimed to **increase profitability through more effective control of endemic diseases**. During project CT07012 the main impacts on orchard productivity have been fungal pathogens and serious floods in 2010 and 2013. In terms of fungal pathogens, the most obvious impacts on productivity have been from pathogens which reduce external fruit quality, such as Emperor brown spot (EBS) (*Alternaria alternata*) and citrus black spot (CBS) (*Phyllosticta citricarpa*). A major focus of the CT07012 project team was fungicide access and usage, and fungicide evaluation, to provide growers with tools to protect fruit. Specific activities have included:

- technical input into emergency use permit applications;
- extension material for growers on the use of fungicides; and
- glasshouse and field trials for fungicide evaluation.

The project team's involvement in flood recovery has focussed on management of pathogens such as *Phytophthora* sp. which can be associated with orchard inundation and extreme wet weather. The project team has also been involved with flood damage assessment and direct assistance to impacted growers in 2010 and 2013.

The activities described in this chapter address the more immediate, short term needs of the industry such as fungicide access. These activities are complementary to the longer term objectives such as breeding for disease resistance (see Chapter 1). Providing disease management solutions for both the short and long term will greatly reduce the negative impact of *Citrus* diseases in Australia.

2.2 Fungicide access and usage

During this project a number of fungicide access and use issues were addressed. These issues included background work to facilitate emergency use permits, achieve national registrations, and commence investigations into new fungicides. The key benefit of activities in this area was to provide growers with the tools to reduce losses in wetter than usual production seasons with higher disease pressure.

Emergency use permit for prochloraz

In March 2008, fruit incubations conducted by Andrew Miles indicated a high risk for anthracnose outbreaks in Imperial mandarins that season. To prepare growers, Andrew Miles and Sandra Hardy (NSW DPI) produced a brief extension note (below). In response, Qld citrus growers sought an emergency use permit for the fungicide prochloraz, through Peter Dal Santo (AgAware Consulting). Andrew Miles provided technical inputs into the permit application, including published background information to support the permit.

Anthracnose – a potential problem this season

Andrew Miles (Tree Pathology Centre – DPI&F & UQ) and Sandra Hardy (NSW DPI)

Miles AK, Hardy S (2008) Anthracnose - a potential problem this season. Coastal Fruitgrowers' Newsletter 68 (Autumn):8

A number of early indicators have suggested that postharvest anthracnose may be a problem this season, particularly for Imperial mandarins.

Symptoms of anthracnose appear as sunken, black lesions or a superficial, reddish brown discolouration on the fruit rind (Fig. 2.2.1). Initially only the rind is affected, and is sunken with a definite line of demarcation between healthy and diseased tissues. In the advanced stages, anthracnose penetrates deeply into the flesh and develops into an actual rot. Anthracnose is caused by the fungus *Colletotrichum gloeosporoides*. Fungal spores produced on dead foliage and twigs of trees can invade the fruit rind throughout the season, but symptoms generally do not appear until after harvest.

The fungal spores are spread onto fruit by rain and water splash, so in rainy seasons such as those that occurred in December-January the risk of anthracnose problems developing is potentially high. However, infection by the fungus is generally only a problem when fruit are subjected to some form of stress, including physical injury, high temperatures, overripe fruit and most importantly the process of degreening - which increases the sensitivity of fruit to anthracnose.

Figure 2.2.1 Symptoms of anthracnose in a batch of recently picked immature Imperial mandarins.

What can be done?

At this late stage of the season most of the fungal infection of fruit will have already occurred. Therefore, reducing the risk of symptom development after harvest is dependent on good postharvest handling of fruit.

To help reduce the risk of anthracnose developing, **remember to:**
- Harvest fruit at prime maturity if possible.
-Avoid picking immature fruit that will require lengthy degreening periods.
-Handle fruit carefully.
-Avoid exposing harvested fruit to long periods of temperatures above 25°C e.g. leaving full bins in the field after picking.

-Storage at less than 10°C is reported to help control anthracnose (but be wary of the risks of chilling injury).
-When degreening fruit carefully monitor the duration, temperature and ethylene concentration of the degreening room.
-Avoid unduly long storage.
-Do not pick, pack or degreen wet fruit.
-In the long term, keeping trees free of dead wood will help reduce the number of spores.

Fungicide registration application review

In 2009, Andrew Miles undertook a review of a new fungicide formulation for *Citrus* for the APVMA. The purpose of the review was to ensure the data provided in the registration application supports the efficacy claims on the proposed product label. The details of the product reviewed are confidential, but providing pathology expertise for these purposes is a requirement for product registrations.

Emergency use permits for iprodione and azoxystrobin

The 2010-11 production season had periods of extreme rainfall leading to widespread flooding in Queensland and elsewhere. The excess rainfall also increased pressure from the fungal diseases EBS (*Alternaria alternata*) and CBS (*Phyllosticta citricarpa*). To mitigate the impact of these diseases, Citrus Australia Limited and their collaborators sought to obtain emergency use permits for the fungicides iprodione (e.g. Rovral Aquaflo) and azoxystrobin (e.g. Amistar). Iprodione is effective against EBS, and azoxystrobin is effective against both diseases. Andrew Miles provided technical input into the permit applications, providing supporting data and publications. Much of the efficacy data supporting the application was generated under the past HAL project CT00021 *Screening New Products for Citrus Disease Control*. In order to assist growers in using these fungicides under the permits, Andrew Miles and Andrew Harty prepared the following report that was made available to growers through the Citrus Australia Limited website and summarised in Australian Citrus News.

Tips for the emergency use of iprodione and Amistar

Andrew Miles (andrew.miles@deedi.qld.gov.au**, ph 32554345)**
(Tree Pathology Centre, The University of Queensland and DEEDI)

Miles AK, Harty A (2011) Fungicides in Queensland. Australian Citrus News 87, 20-21.

http://www.citrusaustralia.com.au/latest-news/emergency-use-permits-achieved-for-two-new-fungicides

To combat the higher than normal impact of fungal diseases in citrus this season, emergency use permits have been obtained for iprodione (e.g. Rovral) and azoxystrobin (Amistar). This article aims to assist growers to make the most of these additional fungicides in their orchards.

Summary

- **Use iprodione (e.g. Rovral) and Amistar sparingly, in a protective strategy, and alternated with other fungicide activity codes to minimise resistance.**
- **Remember to consider the impact of fungicide residues on potential export markets.**
- **Avoid using dithiocarbamate fungicides such as mancozeb late in the season to keep residues below 0.2 mg/kg.**
- **Use postharvest fruit washes to reduce mancozeb residues in blocks where residues may be a problem.**

There is little doubt that the 2010-11 citrus production season has been favourable to fungal diseases. The largest economic impact is likely to be in Queensland, where mandarins that are susceptible to EBS, caused by the fungus *Alternaria alternata*, are already showing 30% fruit infection according to local pest scouts. Some blocks have been reported to be carrying up to 80% fruit infection! It is also likely that harvest will reveal the prevailing conditions in Queensland have favoured infection by the fungus *Phyllosticta citricarpa*, which causes CBS. Infection by the fungus *Colletotrichum gloeosporioides*, which causes postharvest anthracnose, is also likely to have been favoured in most citrus production regions around Australia.

Wherever possible, integrated disease management approaches are encouraged to reduce the impact of fungal diseases in citrus orchards. Examples include:

- selective limb removal and skirting to reduce leaf wetness and humidity in the canopy,
- mulch applications to suppress spores released from leaf litter (e.g. CBS and greasy spot), and
- responsible use of registered fungicides (i.e. copper and dithiocarbamates).

However, the reality is that in some seasons implementing the ideal integrated disease management strategy is simply not possible, with soils being too wet to allow equipment access, or trees even being under water! In seasons like this, there will unfortunately be many cases where the pathogens will win the battle. However, to help win the war at the citrus disease frontline, Citrus Australia Limited with the assistance of Bayer and Syngenta have coordinated attaining emergency use permits for two additional fungicides: iprodione (e.g. Rovral) and azoxystrobin (Amistar).

Whilst access to these fungicides doubles the chemical options available, and will greatly assist in disease control, it is important that growers have a good understanding of the potential residue and resistance issues that can arise. This article profiles the fungicides available, how they may best be used together, and provides advice on how resistance and residues might best be managed to ensure these important disease management tools are useful to growers for as long as possible.

Copper fungicide profile

Product name: Several (e.g. Kocide, Coppox, Norshield etc.)

Active ingredient: Several (e.g. copper hydroxide, copper oxychloride, cuprous oxide)

Use type: Surface protectant – copper fungicides only work if the fruit/foliage are thoroughly covered *before* a fungal spore lands on the fruit or leaf and tries to infect it.

Typical use pattern: Full-rate application during petal fall, or a half-rate application at petal fall and two to four weeks later. Copper fungicides are generally not used during the summer months to avoid copper causing fruit stippling and darkening of rind blemishes.

Use pattern will contribute to the control of: CBS (*Phyllosticta citricarpa*), EBS (*Alternaria alternata*), scab (*Elsinoë fawcettii*), melanose (*Diaporthe citri*), and anthracnose (*Colletotrichum gloeosporioides*).

Australian Maximum Residue Limit (MRL): 10 mg/kg

Withholding period (WHP): 1 day

Residue management: Copper residues are generally not considered of great concern in citrus due primarily to its early-season use, and comfortable MRL. Copper is not systemic or mobile in plants, therefore residue persistence is primarily effected by rainfall and dilution from increases in fruit surface area during growth.

Fungicide resistance code: (new) “M1”, (old) “Y” (fungicides of different codes should be alternated throughout the season to prevent resistance development).

Resistance management: The likelihood of fungal pathogens developing resistance to copper is considered low, because the active ingredient “poisons” the fungi in multiple ways. Testament to this is copper fungicides remaining in use despite being first used over 100 years ago.

Dithiocarbamate fungicide profile

Product name: Several (e.g. Dithane, Antracol, Zineb etc.)

Active ingredient: Several (e.g. mancozeb, propineb, zineb)

Use type: Surface protectant – fungicides such as mancozeb only work if the fruit/foliage are thoroughly covered *before* a fungal spore lands on the fruit or leaf and tries to infect it.

Typical use pattern: Application at 6 and 12 weeks after the copper application/s at petal fall. Subsequent applications for the control of mites occur depending on the activity of the pest.

Use pattern will contribute to the control of: CBS (*Phyllosticta citricarpa*) – applications within 20-24 weeks of flowering, EBS (*Alternaria alternata*) and anthracnose (*Colletotrichum gloeosporioides*).

Australian Maximum Residue Limit (MRL): 0.2 mg/kg

Withholding period (WHP): 14 days or "withholding period not required when used as directed".

WARNING: it is unlikely that this nominated withholding period will be sufficient to avoid MRL breaches i.e. do not spray mancozeb late in the season.

The use of mancozeb within even three months of harvest may be risky – see 'Dealing with mancozeb residues'.

Residue management: Dithiocarbamate residues in mandarins are of significant concern due to an extremely low MRL of only 0.2 mg/kg. Mancozeb does not readily move within the plant, and should only be present on the surface of fruit. In the field, residues should be reduced over time by rainfall and fruit expansion, therefore residues will be minimised by using mancozeb only early in the season.

Fungicide resistance code: (new) "M3", (old) "Y" (fungicides of different codes should be alternated throughout the season to prevent resistance development)

Resistance management: As with copper the likelihood of fungal pathogens developing resistance to dithiocarbamates is considered low, due to the fungicide "poisoning" fungi in multiple ways.

Dicarboximide fungicide profile

Product name: Several (e.g. Rovral, Corvette, Ippon etc.)

Active ingredient: Iprodione

Use type: Surface protectant and eradicant – fungicides such as iprodione will prevent fungal spores infecting fruit and leaves that have already been sprayed, as well as killing existing fungal infections that the fungicide comes into contact with. The fungicide also has systemic activity in some plants, but this is unconfirmed for citrus. **However, always use iprodione to protect fruit from infection, because using it to "cure" existing fungal infections greatly increases the chance of resistance developing.**

Typical use pattern: (according to emergency use permit for EBS – *Alternaria alternata*) Three applications, with each application at least 60 days apart. From the permit instructions:
"Time applications to coincide with
(i) Spring flush – fruit set (less than 5 mm) during September/October,
(ii) Following thinning (fruit 20 to 30 mm) during January, and
(iii) Autumn flush (fruit 30 to 40 mm) during April."

Use pattern will contribute to the control of: EBS (*Alternaria alternata*).

Australian Maximum Residue Limit (MRL): (emergency use permit) 5 mg/kg

Withholding period (WHP): (emergency use permit) 56 days

Residue management: Iprodione residues should not be of concern in citrus if the use pattern of the permit is adhered to. The domestic MRL has been set at 5 mg/kg based on data from the USA, Israel, Italy, New Zealand and South Africa. Be aware that some export markets have a nil, or lower MRL than Australia. A table of the MRLs for various export markets has been provided (Table 2.2.1).

Fungicide resistance code: (new) "2", (old) "B" (fungicides of different codes should be alternated throughout the season to prevent resistance development)

Resistance management: The likelihood of fungal pathogens developing resistance to iprodione is considered **medium to high risk**. Resistance of *Alternaria alternata* causing EBS of mandarins was confirmed in 1989 in a southeast Queensland citrus orchard after four consecutive years of eight applications per season. Similarly, resistance was confirmed in 1994 in an Israeli orchard after three consecutive years of three applications per season. **Responsible use of iprodione will be essential for prolonging the usefulness of this fungicide**. Resistance is best managed by using iprodione to protect, rather than "cure" fruit, and alternating iprodione applications with fungicides of other chemical codes i.e. copper, mancozeb or azoxystrobin.

Strobilurin fungicide profile

Product name: Amistar

Active ingredient: Azoxystrobin.

Use type: Surface protectant – strobilurin fungicides work best if the fruit/foliage are thoroughly covered *before* a fungal spore lands on the fruit or leaf and tries to infect it. These fungicides also have the ability to move within leaves, but do not move within the entire plant.

Typical use pattern: (according to emergency use permit for EBS – *Alternaria alternata* and CBS – *Phyllosticta citricarpa*) Two applications at least 14 days apart, following copper applications

Use pattern will contribute to the control of: CBS (*Phyllosticta citricarpa*), EBS (*Alternaria alternata*), and anthracnose (*Colletotrichum gloeosporioides*).

Australian Maximum Residue Limit (MRL): 2 mg/kg

Withholding period (WHP): 28 days

Residue management: Azoxystrobin residues should not be of concern in citrus if the emergency use permit use pattern is adhered to. The domestic MRL has been set at 2 mg/kg based on data from Australia, South Africa, and Brazil. Be aware that some export markets have a nil MRL. A table of the MRLs for various export markets has been provided (Table 2.2.1).

Fungicide resistance code: (new) "11", (old) "K" (fungicides of different codes should be alternated throughout the season to prevent resistance development)

Resistance management: The likelihood of fungal pathogens developing resistance to azoxystrobin is considered **high risk**, due to fungicide "poisoning" the fungi in a very specific manner. For example, reduced sensitivity to strobilurins has been reported for *Alternaria* species in other crops within just a few years of use. **Responsible use of azoxystrobin will be essential for prolonging the usefulness of this fungicide**. Resistance is best managed by alternating azoxystrobin applications with fungicides of other chemical codes i.e. copper, mancozeb or iprodione.

Making the most of the available fungicides

Copper, mancozeb, iprodione and Amistar are best used in protective strategies to ensure good disease control and minimise the risk of the various fungi becoming resistant (in particular to iprodione and Amistar). Once the fungi in the orchard become resistant, the fungicide becomes useless! **It cannot be stressed enough that iprodione and Amistar need to be used sparingly (i.e. as few sprays as possible, alternated with other fungicide activity groups) to minimise resistance development e.g. iprodione applications could be alternated with Amistar to avoid repeated exposure of the pathogens to a single fungicide mode of action. Using these fungicides "curatively" further promotes resistance development.** The development of resistance to these products would return the industry to relying only on copper and mancozeb. Therefore, growers should try to use these fungicides only when infection periods are likely; in the case of EBS, the disease will be most severe whenever leaves and fruit remain wet and temperatures average 25°C (temperatures below 20°C and above 30°C are less favourable).

In addition to targeting infection periods, other factors needing consideration for fungicide timing include adhering to withholding periods (WHP's) and maximum residue limits (MRL's), and accounting for the length of time after spraying that a fungicide is effective. Adhering to WHP's and MRL's in most cases just requires following the label instructions, however this is not likely to be the case for the dithiocarbamates such as mancozeb, for which residue data collected by the FAO suggests that residues exceeding the Australian MRL may be detected as long as 13 weeks after application – see 'Dealing with mancozeb residues' below for more detail.

After spraying a fungicide it is often not well understood how long the protection against disease will last. However it is widely accepted that fruit expansion and rainfall have a big impact on how long fungicides continue to effectively prevent infection after spraying. In the case of fruit expansion, experiments conducted in Florida have shown that copper residues on fruit can be reduced by about 90%, simply because the fruit increased in diameter by about 50%. Residue reduction because of fruit expansion will be a big issue early in the season when fruit are growing most rapidly. Experiments conducted in Spain have shown that rainfall can significantly reduce the length of time after spraying that a fungicide can provide high levels of protection against EBS. The experiments showed that most of the fungicides tested (copper oxychloride - wettable powder, mancozeb, iprodione, and pyraclostrobin – similar to Amistar) provided two to four weeks of protection against EBS when it did not rain, and fruit were not rapidly expanding. However, 70mm rainfall over a period of 6 days typically halved the number of weeks for which the fungicides provided high levels of protection against the disease. Interestingly, the effectiveness of copper formulations (suspended copper oxychloride, and wettable powder cuprous oxide) in controlling EBS was not significantly decreased by rainfall. Considering the effects of fruit expansion and rainfall, it may be necessary to apply fungicides more often when fruit are rapidly growing and/or wet weather is common.

Based on the label guidelines for the four available fungicides, their WHPs, MRLs, the demonstrated level of protection against EBS over time (under dry conditions, and low fruit expansion), and the need for an anti-resistance strategy, it is possible to provide an example spray program for EBS susceptible varieties (primarily Murcott) this season (Fig. 2.2.2). This example program shows the MAXIMUM allowable number of iprodione and Amistar applications, used according to the label requirements. It aims to avoid mancozeb residues by avoiding late-season mancozeb sprays. The lengths of the withholding periods are also displayed. It is advised that the program is taken as an *example* only; the exact frequency and

timing of applications would need to be adjusted for different varieties and weather conditions.

The example spray program (Fig. 2.2.2) aims to:

1. **Adhere to the label use patterns (see fungicide profiles above)**
2. **Alternate fungicide resistance codes to minimise resistance development**
3. **Adhere to domestic withholding patterns and maximum residue limits**

The different colours in Fig. 2.2.2 indicate the level of protection a particular fungicide offers. So, in the example of copper (top row of the figure), one spray can offer 100-80% protection against EBS for up to four weeks (i.e. 4 green boxes), reducing to 79-40% protection in the fifth week after spraying (the yellow box), and reducing to less than 39% protection in the sixth week after spraying (the red box). In other words, the copper fungicide should protect nearly as well at 4 weeks after spraying as it does in the first week after spraying. The level of protection over time can be interpreted for the other fungicides in the same way. It should be noted that rainfall and rapid fruit expansion will significantly reduce the number of weeks for which a fungicide can provide high levels of infection against EBS, so the data in Fig. 2.2.2 should be considered the best case scenario.

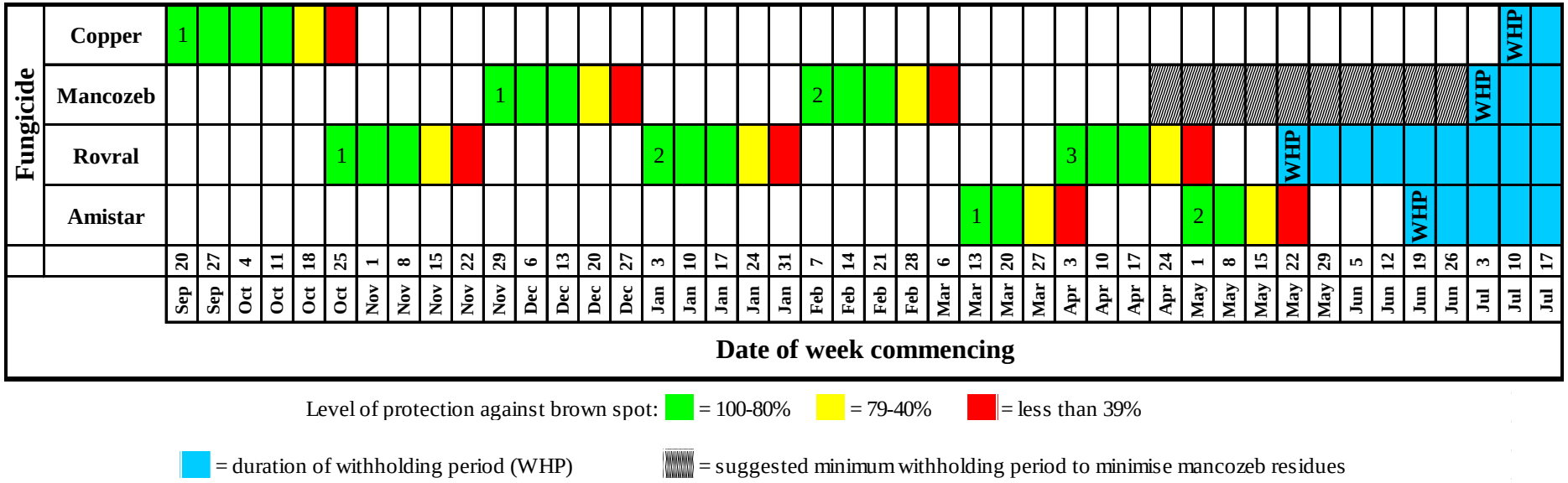


Figure 2.2.2 An *example* fungicide program for protection against EBS on Murcotts incorporating the emergency use guidelines for iprodione and Amistar for the 2010-11 citrus production season. The level of protection against EBS, and the number of weeks of protection, is based on dry conditions (no rainfall) and low rates of fruit expansion. NOTE: the exact frequency and timing of applications would need to be adjusted for different varieties and weather conditions.

Fungicides and export fruit

Be sure to keep the MRLs of any export destinations in mind when using fungicides. Table 2.2.1 provides the MRLs for mancozeb, iprodione, and Amistar. Some markets will not be accessible to fruit treated with certain fungicides unless it can be demonstrated that the fruit complies with the export Country's MRL, or does not contain any detectable residues where a nil tolerance is applicable. As more markets have higher MRLs for Amistar than iprodione, it may be preferable to finish the spray program with Amistar to increase the time between the last iprodione application and harvest (e.g. Fig. 2.2.2).

Table 2.2.1 Maximum residue limits (MRL) for various export destinations for mandarins. A nil tolerance to residues (i.e. not detectable in fruit) applies where no MRL value is listed.

Country	**Maximum Residue Limit (MRL) for MANDARINS by Country**		
	Iprodione (e.g. Rovral)	**Azoxystrobin (Amistar)**	**Mancozeb**
Codex	-	15	10
Indonesia	-	-	-
United States	-	10	4 (Ferbam)
Hong Kong	-	Codex	Codex
New Zealand	-	Codex	7
United Arab Emirates	-	Codex	Codex
Taiwan	0.05	1	2
Japan	10	2	2
Russia	-	-	0.1
Singapore	-	Codex	Codex
China	-	-	-
Canada	-	10	-
Netherlands	1	15	5
French Polynesia	1	15	5
United Kingdom	1	15	5
Malaysia	10	Codex	10
Sri Lanka			
Italy	1	15	5
Kuwait			
Thailand	-	Codex	2
Saudi Arabia			
Papua New Guinea			
Oman			
Qatar			
Maldives			
France	1	15	5
Bahrain			
Mauritius			
Reunion			

Country	Maximum Residue Limit (MRL) for MANDARINS by Country		
	Iprodione (e.g. Rovral)	**Azoxystrobin (Amistar)**	**Mancozeb**
Romania	1	15	5
Guam			
New Caledonia	1	15	5
Brunei Darussalam			
India	-	-	3
Seychelles			
Fiji			
East Timor, Dem Rep of			
Vietnam	-	15	-

Dealing with mancozeb residues

The MRL for mancozeb in citrus in Australia is very low at 0.2 mg/kg. To put the Australian MRL into perspective, the international CODEX MRL for mandarins is 50 times higher at 10 mg/kg. The low Australian MRL is probably the result of the original registration in the 1970's being based on only a few sprays very early in the season. Regardless of the reason, the low MRL leaves little margin for error. It is unfortunately very difficult to predict ahead of time if mancozeb residues will be a problem in any particular block, but to best assist growers Table 2.2.2 lists examples of different use patterns and the resulting residues after a given period of time, based on data collected from around the world by the FAO.

Table 2.2.2. Examples of use patterns and their resulting residue levels, based on residue trial data from citrus around the world collected by the FAO, are as follows:

Country, cultivar	Use pattern	Days after spraying	Residues (mg/kg CS_2)
Spain, Navel orange	1 spray of 44g/100L at 2000L/ha	24	0.12
Spain, Havelina orange	1 spray of 250g/100L at 6000L/ha	28	0.19
Japan, Amanatsu orange	2 sprays of 130g/100L at 3800L/ha	91	0.32
Australia, Valencia orange	2 sprays of 150g/100L (volume unspecified)	28	0.5
Japan, Okitsuwase mandarin	2 sprays of 190g/100L at 2500L/ha	60	1.8
Australia, Valencia orange	2 sprays of 300g/100L (volume unspecified)	28	1.6
Japan, Okitsuwase mandarin	4 sprays of 190g/100L at 2500L/ha	60	2.1
Florida, Valencia orange	4 sprays of 200g/100L at 9000L/ha	28	0.93
Florida, Bearss lemon	5 sprays of 190g/100L at 4700L/ha	27	0.82
Australian MRL			**0.20**

For more data see:
http://www.fao.org/ag/AGP/AGPP/Pesticid/JMPR/Download/93_eva/mancoz.pdf

Packers also have a role in dealing with chemical residues. The packing process involves washing, which provides a means to remove mancozeb residues on the fruit surface. The proportion removed can be quite significant, as seen in Table 2.2.3. High pressure washing systems are likely to remove surface chemical residues and, thereby, provide greater confidence that MRL's are not exceeded. However, it would be prudent for packers to test fruit for mancozeb residues before and after high pressure washing to ensure their system is effective for this purpose. High pressure washing has many additional benefits, including the removal of sooty mould, dirt and pathogens. This increases pack-out into higher value grades by removing cosmetic 'defects', and improves shelf life by removing decay-causing organisms.

Table 2.2.3 Examples of the effectiveness of postharvest removal of mancozeb residues from citrus and other fruit and vegetables.

Crop	Residue removal method	Residue reductions of:
Citrus - Satsuma	Washing (details unavailable)	52-93%,
Citrus - Clementine	Washing (details unavailable)	93-99%
Citrus - Newhall	Washing (details unavailable)	89-97%
Various vegetables	Washing with tap water for 2 minutes	20-52%
Apples	Fruit dips at various concentrations of chlorine, chlorine dioxide, ozone and hydrogen peroxyacetic acid (HPA), for varying durations	56-99% (chlorine) 36-87% (chlorine dioxide) 56-97% (ozone) 44-99% (HPA)
Apricots	Agitation in distilled water for 1 minute	35-70%

In the long term, fungicide residues in citrus susceptible to EBS will be best alleviated by the introduction of varieties resistant to the disease. The citrus breeding program based in Bundaberg has recently made the selection of resistant varieties routine, with resistant germplasm already identified in early and advanced selections (see the Aug/Sept 2010 issue of Australian Citrus News).

Summary

- **Use iprodione (e.g. Rovral) and Amistar sparingly, in a protective strategy, and alternated with other fungicide activity codes to minimise resistance.**
- **Remember to consider the impact of fungicide residues on potential export markets.**
- **Avoid using dithiocarbamate fungicides such as mancozeb late in the season to keep residues below 0.2 mg/kg.**
- **Use postharvest fruit washes to reduce mancozeb residues in blocks where residues may be a problem.**

Acknowledgements:
Andrew Harty, Kevin Bodnaruk, Peter Dal Santo, Peter Taverner, Nancy Cunningham, Dan Papacek, Malcolm Smith, Garry Fullelove and Andre Drenth.

Strategic Agrichemical Review Process

On the 10th November 2011, Andrew Miles participated in the Citrus Strategic Agrichemical Review Process (SARP). The goal of the process was to identify the pesticide requirements for the citrus industry, and identify how these needs were or were not being met. The SARP then provided recommendations for dealing with shortfalls in access to pesticides. A component of the SARP was fungicides for fungal disease management. Based on the findings of the SARP, suggested fungicides for evaluation included azoxystrobin (e.g. Amistar), iprodione (e.g. Rovral) boscalid (e.g. Filan), chlorothalonil (e.g. Elect 720), and difenoconazole (e.g. Score). Registration of azoxystrobin was highly likely, with a trade advice issued by the APVMA in January 2013. Residue trials and additional efficacy trials were also conducted for iprodione in the 2011-12 and 2012-13 seasons. Boscalid was included in the 2012-13 field trials (see Field evaluation of fungicides against CBS and EBS (2012-13)). Chlorothalonil was included in a pot trial for evaluation against EBS, but efficacy was poor. Difenoconazole was ruled out of further evaluation due to the potential resistance risk for using a Group 3 fungicide both preharvest AND postharvest (i.e. imazalil). In section 2.3 below, additional fungicides have also been identified for evaluation.

2.3 Fungicide evaluation

Efficacy of iprodione against EBS

Introduction

In order to progress the emergency use permit for iprodione to a full registration, the APVMA indicated that fruit residue trials were required to ensure an appropriate maximum residue limit (MRL) could be established. It was less certain, but a possibility, that up to date efficacy data for iprodione against EBS would be needed to support registration with the APVMA, and/or the interest of a potential registrant for the fungicide. As the need for residue trials was clear, the necessary trials were commenced through a VC project using funds from the Gayndah and Mundubbera Fruit Growers associations. To obtain up to date efficacy data and add value to these trials, Andrew Miles, Dan Papacek and Malcolm Smith conducted disease assessments on the trees used for the residue trials in collaboration with residue trial project leader Dale Griffin (Crop Protection Research Pty Ltd).

Methods

In order to assess the efficacy of iprodione against *Alternaria alternata*, the amount of EBS was assessed on fruit following treatment with the fungicide. The three treatments were Farmoz Chief Aquaflo (500g/L iprodione) at 1 mL/L ("1×") and 2 mL/L ("2×") and a no iprodione control. In total three trials were conducted over the 2011-2012 season: 1) Murcott tangor at Wallaville; 2) Murcott tangor at Gayndah; and 3) Daisy mandarin at Mundubbera. At each trial the three treatments were applied to four replicate trees each in a randomised design. Each tree was sprayed to before run-off using a motorised hand lance. Three applications of each treatment were made over the season (Table 2.3.1) at each site. Disease was assessed on 20 fruit on each data tree. The number of EBS lesions (active and dormant) was counted on each fruit. Active lesions were sunken, leathery, brown spots, whereas dormant lesions were

corky, light coloured spots that are often raised. At all sites copper and mancozeb fungicides were also applied in the grower's standard fungicide regime. Therefore disease assessments were made within 2-8 weeks of each treatment application to avoid any effect of these other fungicides. At the Daisy mandarin site, an additional assessment was made on trees treated with the grower's standard fungicide program. Data were analysed using ANOVA with a $\log_e$ transformation to improve the underlying assumptions of the ANOVA.

Table 2.3.1 Iprodione treatment application dates

Site	Application 1	Application 2	Application 3
Daisy mandarin (Mundubbera)	01/12/11	08/2/12	10/4/12
Murcott tangor (Wallaville)	01/12/11	08/2/12	09/5/12
Murcott tangor (Gayndah)	01/12/11	08/2/12	09/5/12

Results

At the 13th December 2011 and 21st February 2012 assessments, disease was only observed at the Wallaville trial site. Disease incidence and severity was highest at the Daisy mandarin site in Mundubbera based on all the assessment times (Tables 2.3.2-2.3.4). Disease pressure was lowest at the Murcott site at Gayndah. The application of iprodione typically reduced the severity and incidence of EBS. When the reduction was statistically significant, the incidence of active EBS lesions was always significantly lower than the control for the 2× treatment. The 1× treatment did not always have significantly lower amounts of EBS than the control.

Discussion

The key finding from these trials is that the 2× rate of iprodione appears to provide a higher level of disease control for a longer period of time than the 1× rate. This result was unexpected, and indicates a need to further investigate iprodione rates for EBS control. In general though, iprodione treated fruit had less disease, consistent with previous studies (Miles *et al.*, 2005; Mayers and Hutton, 1986). The highest incidence and severity of EBS being in the Daisy mandarins was not surprising, as this variety is known anecdotally by growers for its high susceptibility to the disease.

The possible explanations for not consistently detecting a significant reduction in disease at the 1× rate are: 1) detection of differences in efficacy will only be possible if a treatment is followed closely by an infection event in the field; 2) infection events need to occur within approximately 14 to 21 days of treatment with iprodione; and 3) a history of iprodione use in nurseries has led to fungicide resistance in orchards. The first explanation is logical; if an infection event does not occur after treatment with the fungicide, no differences in disease are likely to be detected. Under the constraints of the residue trial design it was not possible to always have the fungicide applied at the optimal timing for disease.

The second explanation is based on research that has shown that iprodione provides approximately 14 to 21 days of reliable protection from infection depending on the amount of rainfall (Vicent *et al.*, 2007). Therefore, if the infection event or disease assessment is made outside of this 14 to21 day period, the ability to observe treatment effects will be reduced. Interestingly, the findings of Vicent et al. (2007) are based on a rate equivalent to 1.5× in our experiments. It is likely that the period of efficacy at 1x would be less than at 1.5×. Furthermore, it is likely the period of protection will be longer at 2×. This is evident in the assessments made 50 days after the most recent iprodione application, whereby the 2× rate commonly had

significantly less EBS than the untreated control, whilst the 1× was not significantly different to the control.

The issue of iprodione resistance (explanation 3) is dealt with in another section of this report ('Sensitivity of *Alternaria alternata* to iprodione'). In summary, iprodione resistance was not shown to be highly prevalent in commercial orchards at this time.

As the rates of iprodione appear to need further investigation, specific iprodione efficacy trials for the 2012-13 season have been commenced under project CT09055 - Co-ordinating a market development program for the Australian citrus value chain. The assessment and reporting of these trials will be completed under a new project. An increase in the rate of iprodione is currently considered feasible, with the 2011-12 season residue trials showing low residues in the 2× treatment. The cost of iprodione has also significantly dropped since the product has become generic. At this stage it is neither residue- or cost-prohibitive to increase the iprodione rate for EBS control in mandarins if further trials indicate an increased efficacy at the 2× rate.

Table 2.3.2 Incidence[A] and severity[B] of active dormant, active, and total EBS lesions on Daisy mandarin fruit in Mundubbera after treatment with different rates of iprodione[C].

		Dormant lesions		**Active lesions**		**Total lesions**	
Date of assessment[D]	**Treatment**	**Severity**	**Incidence**	**Severity**	**Incidence**	**Severity**	**Incidence**
29 Mar 2012	Control	0.5	8	1.9	**20 a**	2.4	22
(50 days)	1×	0.5	6	0.8	**13 ab**	1.4	16
	2×	0.5	7	0.5	**7 b**	1.0	12
	Grower	0.1	3	0.6	**9 b**	0.8	12
	P value	N.S.	N.S.	N.S.	**0.026**	N.S.	N.S.
31 May 2012	Control	1.0	17	**2.0 a**	**20 a**	3.1	25
(22 days)	1×	0.6	10	**0.6 b**	**12 ab**	1.2	18
	2×	0.5	8	**0.5 b**	**9 b**	1.0	15
	P value	N.S.	N.S.	**0.009**	**0.034**	N.S.	N.S.

[A]Incidence is the proportion of fruit with one or more lesions.
[B]Severity is the mean number of lesions per fruit.
[C]Mean values followed by the same letter are not significantly different.
[D]Number of days in parenthesis indicates the number of days after the most recent iprodione application.

Table 2.3.3 Incidence[A] and severity[B] of dormant, active and total EBS lesions on Murcott tangor fruit in Wallaville after treatment with different rates of iprodione[C].

Date of assessment[D]	Treatment	Dormant lesions Severity	Dormant lesions Incidence	Active lesions Severity	Active lesions Incidence	Total lesions Severity	Total lesions Incidence
13 Dec 2011	Control			0.95	15		
(12 days)	1×			0.15	7		
	2×			0.33	9		
	P value			N.S.	N.S		
21 Feb 2012	Control	**0.62 a**	**14 a**	0.19	5	**0.91 a**	**16 a**
(13 days)	1×	**0.22 b**	**7 b**	0.05	2	**0.32 b**	**8 b**
	2×	**0.27 b**	**6 b**	0.04	2	**0.26 b**	**8 b**
	P value	**0.013**	**0.009**	N.S.	N.S.	**0.004**	**0.007**
29 Mar 2012	Control	**0.5 a**	10	0.10	2.0	**0.60 a**	**11 a**
(50 days)	1×	**0.4 ab**	10	0.05	2.0	**0.44 a**	**11 a**
	2×	**0.2 b**	6	0.01	0.5	**0.19 b**	**6 b**
	P value	**0.045**	N.S.	N.S.	N.S.	**0.011**	**0.008**
31 May 2012	Control	**0.64 a**	**12 a**	**0.04 a**	**2.3 a**	**0.71 a**	**12 a**
(22 days)	1×	**0.22 b**	**6 b**	**0.03 a**	**1.5 ab**	**0.26 b**	**7 b**
	2×	**0.13 b**	**4 b**	**0.00 b**	**0.0 b**	**0.13 c**	**4 c**
	P value	**0.001**	**<0.001**	**0.049**	**0.022**	**<0.001**	**<0.001**

[A]Incidence is the proportion of fruit with one or more lesions.
[B]Severity is the mean number of lesions per fruit.
[C]Mean values followed by the same letter are not significantly different.
[D]Number of days in parenthesis indicates the number of days after the most recent iprodione application.

Table 2.3.4 Incidence[A] and severity[B] of dormant, active and total EBS lesions on Murcott tangor fruit in Gayndah after treatment with different rates of iprodione[C].

Date of assessment[D]	Treatment	Dormant lesions Severity	Dormant lesions Incidence	Active lesions Severity	Active lesions Incidence	Total lesions Severity	Total lesions Incidence
29 Mar 2012	Control	0.08	1.4	0.3	6.2	0.5	6.5
(50 days)	1×	0.01	0.4	0.1	2.4	0.1	2.7
	2×	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	P value	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
31 May 2012	Control	0.28	2.8	**0.38 a**	**10 a**	0.7	**12 a**
(22 days)	1×	0.02	1.3	**0.18 ab**	**3.5 ab**	0.2	**4 b**
	2×	0.02	0.8	**0.01 b**	**1.3 b**	0.1	**2 b**
	P value	N.S.	N.S.	**0.041**	**0.032**	N.S.	**0.017**

[A]Incidence is the proportion of fruit with one or more lesions.
[B]Severity is the mean number of lesions per fruit.
[C]Mean values followed by the same letter are not significantly different.
[D]Number of days in parenthesis indicates the number of days after the most recent iprodione application.

Business case for the financial support of registration of iprodione in citrus

The fungicide iprodione is no longer under patent with the original registrant Bayer. The fungicide is now sold under a number of different generic brand names. Expanding the existing generic label to include *Citrus* fruit will require one of the generic manufacturers to be the registrant in the APVMA process. The following business case was prepared to assist in attracting a registrant for iprodione in *Citrus*.

Case for financial support to achieve full registration of iprodione for the control EBS (*Alternaria alternata*) in mandarin fruit

Prepared by:
Andrew Miles (Citrus Pathologist, DEEDI Qld)
Peter Dal Santo (Consultant, AgAware Consulting Pty. Ltd)
Andrew Harty (Market Development Manager, Citrus Australia Ltd)

Background: The fungus *Alternaria alternata* causes EBS, a disease causing severe fruit blemish and reduced tree performance of susceptible mandarin and tangelo varieties (Timmer et al. 2000). The disease is most prominent in Queensland, where there are ~1600 ha of susceptible varieties planted; Murcott (~1300 ha), Taylor Lee (~110 ha), Nova (90 ha) and several others planted in smaller numbers (Sette et al. 2011). Susceptible varieties are planted in other states, however the vast majority are not grown in a climate conducive of the EBS disease, or they are only planted in small numbers. Therefore Queensland is where the vast majority of commercial use will occur.

The nature of EBS is such that disease control relies heavily on fungicide applications to protect fruit and young foliage from damage. However, only copper fungicides are currently registered for EBS control in Queensland. To assist Queensland growers to manage EBS an emergency use permit for the efficacious fungicide iprodione (Miles et al. 2005; Solel et al. 1997) was issued in March 2011 (EUP PER12582). However, this fungicide will be required for EBS control beyond the life of the permit, which expires in July 2012. Full registration is therefore desirable.

Maximum allowable iprodione usage in Queensland:

Area of susceptible varieties = 1600 ha
Maximum number of applications according to EUP use pattern = 3
Rate of iprodione = 50g active ingredient / 100L
Typical application rate per hectare = 10,000L / ha via high pressure oscillating boom

Maximum allowable annual usage = 24,000 kg a.i. per annum, which is equivalent to 48,000 L of Rovral Aquaflo or equivalent 500g/L iprodione commercial product.

References:

Miles AK, Willingham SL, Cooke AW (2005) Field Evaluation of a Plant Activator, Captan, Chlorothalonil, Copper Hydroxide, Iprodione, Mancozeb and Strobilurins for the Control of Citrus Brown Spot of Mandarin. Australasian Plant Pathology 34, 63-71.

Sette F, Hawtin J, Argus S (2011) '2011 National Citrus Plantings Database CT10035.' Horticulture Australia Limited, Sydney, Australia.

Solel Z, Oren Y, Kimchi M (1997) Control of Alternaria brown spot of Minneola tangelo with fungicides. Crop Protection 16, 659-664.

Timmer LW, Garnsey SM, Graham JH (2000) 'Compendium of Citrus Diseases Second Edition.' (APS Press: St. Paul, Minnesota).

Sensitivity of *Alternaria alternata* to iprodione

The fungicide iprodione has never been registered in Australia for use on *Citrus* fruit. It has however been registered for use on "non-bearing citrus". The major use of iprodione has therefore been in nurseries. A significant concern over expanding the registration of iprodione to include bearing trees (i.e. use in nurseries AND orchards) is the potential for resistance having developed in nurseries, and then plants carrying resistant populations of *A. alternata* being planted in orchards. To address this concern, fungicide sensitivity testing was undertaken as a graduate research project at The University of Queensland by Essam AL-Quaraishi under the supervision of Elizabeth Aitken, David Armour and Andrew Miles. The research project was carried out in conjunction with the iprodione residue/efficacy trials outlined in the section above. The work was not a direct milestone of project CT07012, but the findings are directly relevant to several CT07012 activities. Therefore, a summary of the work is provided.

The first experiment conducted was to test the sensitivity of an existing library of *A. alternata* cultures from *Citrus* held by Andrew Miles and the Brisbane Plant Pathology Herbarium. Of the 20 isolates tested, only 4 were found to be insensitive to iprodione at 50 µL/L iprodione. Of these isolates, two were from Palmwoods where iprodione resistance has been reported in the past (Hutton, 1989).

The second experiment involved the strategic sampling of the residue/efficacy trial conducted on Daisy mandarins, as described in the section Efficacy of iprodione against EBS. The Daisy mandarin site had the highest level of EBS and was considered the most likely to harbor resistant isolates. At the end of the season, leaves with EBS symptoms were collected by Andrew Miles for each replicate data tree of each treatment (control, 1× iprodione, 2× iprodione). Single spore isolates of *A. alternata* were then obtained from 10 isolates from each of four replicate trees from each of three treatments, giving a total of 120 isolates. The 120 isolates were then tested for sensitivity to iprodione. In total only 5 isolates of the 120 tested showed resistance to iprodione at 50 µL/L iprodione. Of the 5 isolates there was no clear correlation between the number of resistant isolates and the particular treatment from which they were sampled.

The low overall occurrence of resistant isolates in these experiments is encouraging, as it suggests fungicide resistance is not dominant in the population of *A. alternata*. However, the existence of resistant isolates, albeit at a low level, indicates that resistance management strategies will remain essential. Therefore, new fungicides with different resistance activity need to be incorporated into fungicide programs in citrus orchards. The following sections in the report describe how this will be achieved.

Report reference:
AL-Quraishi E (2012) Resistance to Iprodione Fungicide in *Alternaria alternata* Isolates from Citrus in Queensland Mandarin's Orchards. Graduate Research Project III (AGRC7618) Thesis, The University of Queensland, Brisbane.

Development of a project proposal for evaluating new fungicides for brown and CBS

Pursuit of new fungicides for the control of EBS and CBS of *Citrus* has been identified by Citrus Australia Limited (CAL) as a research priority through local consultation with Queensland growers. As such, a revised milestone (milestone 11) of project CT07012 was for Andrew Miles to prepare a project proposal for conducting fungicide efficacy research. A draft proposal document was prepared and appended to the relevant milestone report. In its appended form the proposal was not submitted to HAL directly. Instead, the proposal was used as a protocol for undertaking the work required to generate new registrations for *Citrus*. This has occurred in three ways. Firstly, the methods of the proposal were followed to identify a range of potential fungicides (see the section below Identification of new fungicides for control of EBS and CBS). Secondly, in addition to the Queensland industry raising fungicides as a research priority, the industry noted that an Industry Development Officer role (under project CT09055 *Coordinating a market development program for the Australian citrus value chain*) was not a priority. CAL sought a milestone variation from HAL to CT09055 for funds to be used to fast track the commencement of fungicide trials in the 2012-13 season (see section below Field evaluation of fungicides for the control of EBS and CBS). Thirdly, the on-going fungicide research needs identified in the draft proposal for the 2013-14 and 2014-15 seasons have been incorporated into a project proposal (CT13020 *Increasing market access, profitability and sustainability through integrated approaches to fungal disease control*) submitted to HAL on the 9th November 2012.

Identification of new fungicides for control of EBS and CBS

Management of EBS and CBS relies significantly on fungicide use. Fungicide effectiveness is improved by integrating fungicide sprays with cultural practices such as canopy management to improve penetration and air flow, as well as inoculum management such as mulching over leaf litter to suppress spore production (Miles *et al.*, 2008). Despite these integrated approaches to disease management, fungal diseases remain a production challenge. EBS in particular caused extensive damage in the 2010-11 and 2012-13 seasons because of above average rainfall. While breeding for resistance was a long term solution to EBS, effective solutions were required in the short term.

The most practical short term solution for fungal disease control is fungicide application. At present only copper- and dithiocarbamate-based fungicides have full registration for use in citrus production. More recently, emergency use permits have been sought for the fungicides iprodione and azoxystrobin. Full registration for azoxystrobin, based on efficacy data generated by HAL project CT00021, is currently being processed by the Australian Pesticides and Veterinary Medicines Authority (APVMA). Residue trials to support an application to the APVMA for registration of iprodione were commenced in the 2011-12 season. Whilst attaining full registration for four fungicide active ingredients will be a significant step forward for disease control in *Citrus*, limitations will still exist. For example, the absolute best case number of weeks of protection achievable with the four existing fungicides and their label use patterns is approximately 23 weeks at 100-80% efficacy (Miles, 2011b; Vicent *et al.*, 2007), out of an approximately 40-week-long production season. This best case duration of protection assumes no coverage decline due to rainfall or fruit expansion, so the real-world duration of protection is likely to be far less than 23 weeks, leaving fruit exposed to infection for much longer than 20 weeks. Furthermore, copper fungicides can cause rind stippling (Schutte *et al.*, 1997) and accentuate marks on the rind, mancozeb can be disruptive to the IPM predator *Amblyseius victoriensis* (Smith and Papacek, 1991), and the development of resistance in *Alternaria* spp. is a risk for iprodione in *Citrus* (Hutton, 1989) and azoxystrobin in other crops (Luo *et al.*, 2007; Rosenzweig *et al.*, 2008). Therefore, it is essential to seek effective additional fungicide options to improve fruit protection and minimise resistance development by alternating different fungicide resistance activity groups.

In addition to identifying fungicides that are effective and able to be integrated into anti-resistance use patterns, it is important that fungicide maximum residue limits (MRLs) are achievable for key export markets such as Japan. Therefore, the MRL profiles of potential fungicides need to be considered to ensure that fruit treated with these fungicides can still be safely exported. Furthermore, it is essential that any new fungicides do not induce rind blemishes or other forms of phytotoxicity.

The overall aim of this section was to identify potential fungicide candidates for further screening of efficacy in field trials. This was achieved by: 1) searching published literature for existing efficacy data; 2) contacting citrus researchers overseas where the same diseases occur; 3) directly contacting ten fungicide manufacturers; and 4) placing an open request with the fungicide user group formed by Citrus Australia Limited for suggested actives. After compiling a long list of fungicides, the list was scrutinised based on: 1) efficacy potential; 2) fungicide resistance activity group (no new fungicides could be of the same resistance activity group as any already registered fungicide); 3) and compatibility with export MRL's using Japan as the benchmark. The list of fungicides identified for evaluation is shown in Table 2.3.5.

Using the above approaches, the following products were RULED OUT as likely candidates:

- **All fungicides in activity group 2** (i.e. the same group as iprodione);
- **All fungicides in activity group 3** (old group C): preharvest use of these fungicides presents too great a risk for developing resistance to imazalil;
- **All fungicides in activity group 9** (old group I): as the postharvest registration of pyrimethanil (group 9) is likely, preharvest use of any group 9 fungicide would be risky. E.g. cyprodinil is group 9, and was removed from the list;
- **All fungicides in activity group 11** (i.e. same Amistar) e.g. famoxadone;

- **All fungicides in activity group 12** (i.e. same as fludioxonil);
- **Any additional dithiocarbamate** (e.g. mancozeb): the dithiocarbamate MRL is only 0.2ppm, and no additional residues would be tolerated. E.g. ferbam;
- Propiconazole had poor efficacy against EBS in Florida (Timmer and Zitko, 1997), and also has low MRL in Japan of only 0.05ppm;
- Thiophanate methyl had poor efficacy was found against EBS in Florida (Timmer and Zitko, 1997);
- Fluazinam had poor efficacy against EBS in Florida (Timmer and Zitko, 1997) and Israel (Solel *et al.*, 1997);
- Dodine is in the same resistance group as guazatine (albeit multi-site, group M), and also has a low Japan MRL;
- Spiroxamine – Bayer was unlikely to support registration.

The following products may have suitable MRLs and resistance activity groups, but lack sufficient background efficacy data to support the expense of full field trials. Preliminary screening in a pot trial was recommended prior to undertaking field evaluation:

- Hydrogen peroxide / peroxyacetic acid (Peratec)
- Didecyl dimethyl ammonium chloride (Sporekill)
- Captafol (e.g. Difolatan)
- Chlorothalonil (e.g. Elect 700)
- Dithianon (e.g. Delan 700)
- Isopyrazam
- Tea tree oil (e.g. Timorex Gold)
- Chitosan defence activator (e.g. Aminogro)
- Acibenzolar-S-methyl defence activator (e.g. Bion)

The following products were recommended for field evaluation. Field trials on products including these have commenced in the 2012-13 season:

- Boscalid (e.g. Filan)
- Fluxapyroxad (e.g. Xemium)
- Penthiopyrad (e.g. Fontelis)
- Bayer coded molecule DC-104
- Bayer coded molecule DC-105
- Captan

Table 2.3.5 Specific notes and details of candidate fungicides.

Active (product name)	**Type**	**Activity code**	**Australian labels e.g.**	**Efficacy evidence**	**Notes**	**Manufacturer notes**
Boscalid (e.g. Filan)	anilide fungicides; pyridine fungicides	7 (new) G (old)	*Alternaria* in potatoes and tomatoes etc	Registered in Florida for EBS in a formulation including pyraclostrobin (Pristine, BASF).	Japan MRL 10ppm **<u>Recommendation:</u> field trials**	BASF (verbal interest received, priority for Pristine, but would still support boscalid on its own)
Fluxapyroxad (e.g. Imbrex/Xemium)	pyrazole-carboxamides	7 (new) G (old)	Applications for various fungal diseases of barley under the name Imbrex	Not yet any specific for citrus. However new chemistry is likely to receive manufacturer support for registration.	Unknown Japan MRL (new fungicide so an MRL is yet to be set) **<u>Recommendation:</u> field trials**	BASF (verbal interest received, priority for Priaxor, but would still support fluxapyroxad on its own)
Penthiopyrad (e.g. Velista/Fontelis)	pyrazole	7 (new) G (old)	Applications for various fungal diseases in various vegetable, fruit and nut crops	Several USA labels. Not yet any specific for citrus. However new chemistry is likely to receive manufacturer support for registration.	Unknown Japan MRL (new fungicide so an MRL is yet to be set) **<u>Recommendation:</u> field trials**	Dupont

Active (product name)	Type	Activity code	Australian labels e.g.	Efficacy evidence	Notes	Manufacturer notes
Captan	phthalimide fungicides	M4 (new) Y (old)	Several, widely used general fungicide. Local evidence for efficacy against EBS (single trial)	Some local supporting data available – screened in Qld trials with some success. Solel et al. (1997) considers ineffective for EBS. Timmer & Zitko 1997, not very effective.	The EPA in the USA are allowing the re-registration of captan, following review. Japan MRL 5ppm APVMA residue advice from Jason Lutze July 2012: shouldn't be problems with dietary intake, but does have an "acute reference dose" set. This means registration would need a lot of residue data (basically the same as iprodione) including dried pomace (waste product of juice). APVMA review advice – nothing formally on the radar. Suggests initially pursuing a permit rather than a full label, aiming for small and minimal changes in use. Avoid trying to go for a big new registration across all citrus in Australia. Product has potential to be a late-season equivalent to mancozeb – i.e. a cheap, multisite product for resistance management. **<u>Recommendation:</u> field trials**	Several manufacturers. Melpat International has shown interest in supporting Captan. Melpat are regular sponsor of the CAL conferences and are keen to collaborate.
DC-104 DC-105	Confidential	New to citrus	None yet	Bayer/HAL VC project funded efficacy trials over the 2011-12 season. Promising results were found for CBS and EBS.	Unknown Japan MRL (new fungicide so an MRL is yet to be set) **<u>Recommendation:</u> field trials**	Bayer
Didecyl dimethyl ammonium chloride (Sporekill)	quaternary ammonium compound	Unspecified, but it is broad spectrum and non-systemic	General sanitiser, ornamentals	Registered in South Africa with reduced rates of mancozeb or copper for EBS and CBS.	Could be an MRL issue if its use is registered postharvest. **<u>Recommendation:</u> glasshouse evaluation**	Ekko

Active (product name)	Type	Activity code	Australian labels e.g.	Efficacy evidence	Notes	Manufacturer notes
Hydrogen peroxide / peroxyacetic acid (Peratec)	Inorganic	M	None yet	Possible postharvest sanitiser.	Sanitisers don't offer residual activity i.e. probably won't offer any long term (weeks) protection **<u>Recommendation:</u> glasshouse evaluation**	Jaegar Australia Pty Ltd
Captafol (e.g. Difolatan)	phthalimide fungicides	M4 (new) Y (old)	None – possibly due to registrations of captan in grapes?	Used to be registered in the USA Also is listed under the ROTTERDAM CONVENTION and may be problematic for exports.	Japan MRL unknown **<u>Recommendation:</u> glasshouse evaluation**	Farmoz
Chlorothalonil (e.g. Elect 700)	Chloronitriles	M5 (new) Y (old)	Several	Some local supporting data available – screened in Qld trials. "Walabi" (chlorothalonil + pyrimethanil) was effective against EBS in a single Qld trial. Solel et al. (1997) considers ineffective for EBS.	Japan MRL 0.01ppm. Multisite activity good for resistance management. **Currently listed as a "priority 2" chemical nominated for review by the APVMA for human health, environmental and residue reasons: note that the dithiocarbamates (e.g. mancozeb) are also priority 2.** **<u>Recommendation:</u> glasshouse evaluation**	Several (Bayer wouldn't support application)
Dithianon (e.g. Delan 700)	Quinone	M9 (new), Y (old)	Various fruits	Better than copper against citrus scab (Whiteside, 1991) Efficacy against melanose (Tsay and Chuang, 1986)	High Japan MRL of 5ppm **<u>Recommendation:</u> glasshouse evaluation**	Crop Care
Isopyrazam	SDHI (succinate dehydrogenase inhibitors)	7	Septoria in cereals	None	Unknown Japan MRL **<u>Recommendation:</u> glasshouse evaluation**	Syngenta
Tea tree oil (e.g. Timorex Gold)	Oil-plant extract	Unspecified	Powdery mildew in cucurbits, tomato and capsicum	Nothing specific for citrus.	None? **<u>Recommendation:</u> glasshouse evaluation**	Biomor Australia

Glasshouse evaluation of fungicides against EBS in Murcott seedlings

Introduction

In the section above a number of compounds were recommended for glasshouse evaluation prior to being considered for full-scale field trials. A glasshouse trial was carried out with these compounds on seedlings of 'Murcott' tangor (*Citrus* × *aurantium*) inoculated with a highly pathogenic strain of *Alternaria alternata* (AKM 452). Typical commercial fungicide applications are made in a protective strategy, therefore most compounds were applied 24 hours prior to infection. Also included were two products, Sporekill and Peratec. The efficacy of these products pre- and post-infection is not well established for EBS of *Citrus*. The products therefore were applied at 24 hours before infection and 24 hours post infection to better define their fungicidal activity. In addition, the trial also included mixtures of Sporekill with reduced rates of mancozeb. The addition of Sporekill to a ½ rate of mancozeb has resulted in comparable levels of disease control to that achieved using the full rate of mancozeb alone (Schutte, 2008). As the fungicide captan was to be included in commercial-scale field trials, a mixture of reduced rates of captan with Sporekill were included in the glasshouse trial to test for a similar synergistic effect. Finally, mancozeb with and without Sporekill was applied 24 hours after infection to test if the addition of Sporekill improved the post-infection efficacy of mancozeb.

Methods

In order to evaluate the efficacy of various fungicidal compounds against EBS on a small scale, 'Murcott' tangor (*Citrus* × *aurantium*) seedlings were treated with various fungicides and challenged pre- and post-inoculation. Seeds of 'Murcott' were sown and grown to approximately 30 cm tall. All leaves were then removed to stimulate a synchronized flush of young susceptible leaves. The pre-infection treatments in Table 2.3.6 were applied to seven replicate seedlings per treatment using a hand operated mister. Leaves were wet to just before the point of run-off. The seedlings were allowed to dry for 24 hrs. All the seedlings were then transferred to a plant growth room operating in darkness at 25 °C and 80% relative humidity, then inoculated using a hand operated mister to apply 1×10^4 conidia per mL of a highly pathogenic isolate (AKM 452) of *A. alternata*. Conidia were produced directly from agar using the same methods for producing inoculum for resistance screening in the breeding program (see section 1.4). The seedlings were then incubated for 3 days in the controlled environment room and transferred to a standard shadehouse for a further 7 days before the plants were observed for any signs of disease or phytotoxicity. After 21 days the plants were again completely defoliated to stimulate a synchronised flush. The pre-infection treatments in Table 2.3.6 were reapplied to the seven replicate seedlings per treatment using a hand operated mister. Leaves were wet to just before the point of run-off. The seedlings were allowed to dry for 24 hrs. All the seedlings were then transferred to a controlled environment room operating at 25 °C and 98% relative humidity, then inoculated using a hand operated mister to apply 3×10^4 conidia per mL of a highly pathogenic isolate (AKM 452) of *A. alternata*. Conidia were produced as above. The inoculated plants were then incubated for 24 hrs. Seedlings receiving the post-infection treatments according to Table 2.3.6 were removed from the room and then treated and allowed to dry for 24 hours before returning to 25 °C and 98% relative humidity. The seedlings were incubated for a further 3 days. After this time the plants were transferred to a shadehouse for 7 days

prior to assessing the seedlings for disease. The severity of EBS in the seedlings was assessed on a rating scale of 1 to 7, whereby: rating 1 = asymptomatic; rating 2 = <10% of the plant necrotic and very few symptoms; rating 3 = 34-10% of the plant necrotic; rating 4 = 35-50% of the plant necrotic with remaining leaves healthy; rating 5 = 51-75% of the plant necrotic with a few turgid leaves remaining; rating 6 = >75% of the plant necrotic with wilted green leaves; and rating 7 = dead plant. Data were analysed using ANOVA.

Table 2.3.6 Details of the treatments applied to 'Murcott' seedlings pre- and post-infection by *A. alternata*.

Treatments	Details
24 hrs pre-infection	
Control	Untreated
Mancozeb	Dithane 2.00 g/L
Hydrogen peroxide / peroxyacetic acid	Peratec 10.00 mL/L
DDAC	Sporekill 1.00 mL/L
DDAC + ½ mancozeb	Sporekill 1.00 mL/L + Dithane 1.00 g/L
DDAC + ½ captan	Sporekill 1.00 mL/L + Captan 1.25 g/L
Captan	Captan 2.50 g/L
Isopyrazam	Sequris Flexi 1.20 mL/L
Dithianon	Delan 700 1.00 g/L
Chlorothalonil	Elect 720 1 mL/L
Iprodione + ½ captan	Rovral Aquaflo 1 mL/L + captan 1.25 g/L
Tea tree oil	Tea tree oil 1.9 mL/L
24 hrs post-infection	
Hydrogen peroxide / peroxyacetic acid	Peratec 10.00 mL/L
DDAC	Sporekill 1.00 mL/L
DDAC + ½ mancozeb	Sporekill 1.00 mL/L + Dithane 1.00 g/L
Mancozeb	Dithane 2.00 g/L
Control	Untreated

DDAC = didecyl dimethyl ammonium chloride

Results

After the first round of treatment application and inoculation no symptoms of disease were observed in the control or any other treatment, suggesting that the incubation conditions were not suitable for infection and disease development. Similarly, no symptoms of phytotoxicity were observed in any of the control or treated seedlings. In the second round of treatment application and inoculation, defoliation and twig dieback were the major symptoms of EBS that were observed. Fig. 2.3.1 shows the severity of disease symptoms that were observed.

The most effective pre-infection fungicide treatments were DDAC + ½ captan, captan, isopyrazam, dithianon, and mancozeb (Table 2.3.7). Fig. 2.3.2 shows the DDAC + ½ captan plants compared with the untreated control plants. DDAC + ½ mancozeb, Tea tree oil, peratec, iprodione + ½ captan, chlorothalonil and DDAC were not significantly different from the untreated control. DDAC on its own showed very poor efficacy, but did show possible synergistic effects with both captan and mancozeb, whereby the ½ rate mixtures of these fungicides with DDAC performed as well as the full rates of these fungicides on their own. There were no statistically significant differences in efficacy between any of the post-infection treatments and the untreated control.

Figure 2.3.1 Examples of seedlings showing different levels of *Alternaria alternata* infection. Plants are rated from left to right as: rating 1 = asymptomatic; rating 2 = <10% of the plant necrotic and very few symptoms; rating 3 = 34-10% of the plant necrotic; rating 4 = 35-50% of the plant necrotic with remaining leave healthy; rating 5 = 51-75% of the plant necrotic with a few turgid leaves remaining; rating 6 = >75% of the plant necrotic with wilted green leaves; and rating 7 = dead plant.

Table 2.3.7 Mean severity rating of EBS in 'Murcott' seedlings treated with various anti-fungal compounds either 24 hrs pre- or post-infection by *A. alternata*.

	Mean severity[A,B]	
Treatment	*24 hrs pre-infection*	*24 hrs post-infection*
DDAC + ½ captan	2.7 a	
Captan	2.9 a	
Isopyrazam	3.0 ab	
Dithianon	3.7 abc	
Mancozeb	4.3 abcd	5.6
DDAC + ½ mancozeb	4.7 bcde	5.4
Tea tree oil	5.1 cdef	
Hydrogen peroxide / peroxyacetic acid	5.8 def	5.4
Iprodione + ½ captan	5.9 def	
Chlorothalonil	5.9 def	
Control	6.4 ef	4.3
DDAC	6.6 f	3.8
P	<0.001	>0.05

[A]Severity of disease was rated on a 1-7 scale where: 1 = asymptomatic; rating 2 = <10% of the plant necrotic and very few symptoms; rating 3 = 34-10% of the plant necrotic; rating 4 = 35-50% of the plant necrotic with remaining leave healthy; rating 5 = 51-75% of the plant necrotic with a few turgid leaves remaining; rating 6 = >75% of the plant necrotic with wilted green leaves; and rating 7 = dead plant.
[B]Mean values followed by the same letter are not significantly different.

Figure 2.3.2 Comparison of EBS disease in 'Murcott' seedlings treated with DDAC + ½ captan (left) and untreated control seedlings (right).

Discussion

The glasshouse trial identified four treatments with potential efficacy against EBS in the field: DDAC + ½ captan; captan; isopyrazam; and dithianon. The other treatments were not recommended for further field evaluation based on the trial results. The efficacy of isopyrazam against a citrus disease was shown here for the first time, however the result is not surprising considering the efficacy of other succinate dehydrogenase inhibiting (SDHI) fungicides such as boscalid (see Field evaluation of fungicides against CBS and EBS (2012-13)). Dithianon has efficacy against scab (Whiteside, 1990) and melanose (Jwu-guh and Tsai-young, 1989), but efficacy against EBS has not been demonstrated. The multisite mode of action of dithianon is particularly desirable for fungicide resistance management in EBS fungicide programs. It was surprising that the combination of captan + iprodione had limited efficacy, as both fungicides are known to be efficacious individually. This result may warrant further investigation.

To our knowledge this is the first time a possible synergistic effect between DDAC and captan has been demonstrated against *A. alternata*. Use of this combination of products has however been reported in Argentina for control of *Phytophthora* trunk canker, but with no mention of any synergistic effect (CRI, 2012). The possible synergistic effect needs further investigation, including additional comparisons to other rates of captan with and without different rates of DDAC. The mode of action of the synergy should also be investigated, as the mode of action could range from DDAC being fungicidal (CRI, 2012), through to the DDAC simply acting as a surfactant (Juergensen *et al.*, 2000). If the latter is the case, there may be cheaper ways to increase the efficacy of reduced rates of fungicides such as mancozeb or captan than tank mixing with DDAC.

The efficacy and multisite mode of action of the tank mixture of captan and DDAC is potentially very useful for the control of EBS in orchards. Captan alone was expected to give good results based on previous studies (Miles *et al.*, 2005) and results observed in the field trials detailed in later sections of this report (see 'Field evaluation of fungicides against CBS and EBS (2012-13)'). Sporekill used alone was not effective, as has been observed in South Africa (Schutte, 2008). Field trials investigating any synergy may be worthwhile, but it should only be considered if the tank mixture approach is cost beneficial to growers. A later section investigates the costs associated with the use of DDAC tank mixtures with mancozeb or captan.

Whilst the first round of treatment application and inoculation did not result in any disease development, this did provide an opportunity to make observations for any foliar phytotoxicity in the absence of any confounding effects of disease symptoms. In this trial none of the treatments applied induced symptoms of phytotoxicity. This is particularly important in the case of the previously un-tested combination of DDAC and captan.

Detached fruit evaluation of captan and mancozeb in combinations with Sporekill

Introduction

The possibility of a synergistic effect between didecyl-dimethylammonium chloride (DDAC) and fungicides such as mancozeb was shown in South Africa (Schutte, 2008) and in the glasshouse evaluation detailed above. Any synergism is of interest as the potential to reduce the rates of mancozeb used against fungal diseases could help growers adhere to the low maximum residue limit for mancozeb in Australia (see 'Dealing with mancozeb residues').

DDAC is a quaternary ammonium compound, which is in a group of compounds known to be strong surfactants and are used as commercial disinfectants (Juergensen *et al.*, 2000). As well as being referred to as a "disinfectant", DDAC is often referred to as a "fungicide", "biocide", or "sanitiser" (Dubois *et al.*, 2000). With respect to fungi, the likely mode of action is to disrupt the cell membrane and cause leakage of the cell contents, as well having some impact on respiration (Xiao and Kreber, 2005). From the few studies carried out regarding the response of fungi to DDAC, it has been observed that at low concentrations DDAC can be "sporostatic"; in which case spore germination is prevented in the presence of DDAC, but will resume upon removal of the DDAC (Xiao and Kreber, 2005). Furthermore, it has then been observed that higher concentrations of DDAC are required to prevent vegetative growth of fungi. For example, the fungi *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* were found to have far lower EC_{50} values for DDAC for spore germination than for mycelial growth (Gramaje *et al.*, 2009). Inhibition of spore germination has been observed at <2.5 ppm (Bhuiyan *et al.*, 2012), and inhibition of mycelial growth has been observed as requiring as much as 1000 ppm (Dubois *et al.*, 2000). These sporostatic to fungicidal effects of DDAC are likely to contribute to any synergistic effect when combined with fungicides such as mancozeb or captan. In addition to being fungicidal, DDAC is a cationic surfactant (Juergensen *et al.*, 2000). It may also be possible that the surfactant properties of the DDAC can enhance the deposition of a fungicide such as mancozeb on the plant surface. For example, control of CBS using lower rates of mancozeb combined with mineral oil as

a surfactant has been observed to be superior to the full rate of mancozeb applied on its own (Schutte *et al.*, 2003).

As combinations of the fungicides mancozeb or captan with DDAC could provide good fungal disease control, all while reducing the applied rates of the fungicides and providing a multi-site mode of action, these combination treatments could prove very useful to citrus growers. However, the necessary field trials to make these treatments a commercial reality will be expensive and time consuming. It is therefore necessary to gather further evidence for any synergistic effect before investing in field assessments.

Methods

In order to determine if a synergistic effect exists between DDAC (Sporekill) and the fungicides mancozeb and captan, a detached fruit assay was conducted on fruit of 'Murcott' tangor (*Citrus* × *aurantium*). Fruit without any signs of EBS were harvested approximately 8 weeks prior to commercial maturity from an orchard in Mundubbera by Malcolm Wallis (Citricare). The fruit were then washed in soapy water to remove any fungicide residues, and allowed to air dry. Once dry the fruit were treated with the various treatments detailed in Table 2.3.8. Treatments were applied to a minimum of three replicates consisting of 16 fruit each using a hand held mister. Treatments were applied to just before run-off, then left at ambient conditions for 48 hours to dry. After 48 hours all the fruit were inoculated with a spore suspension containing 1×10^5 conidia per mL of *Alternaria alternata* isolate AKM452. The suspension of conidia was prepared as described previously (see section 1.4). The suspension was applied using a hand held mister to just before the point of run-off. The fruit were immediately incubated at 25°C and 100% relative humidity for 13 days before being assessed for disease. Disease severity was assessed by counting the number of EBS lesions on each fruit. Disease severity was compared between treatments using a $\log_{10}$ transformation of the data and Analysis of Variance (ANOVA). To account for any zero values in the transformation, 0.1 was added to the plot means. To qualitatively confirm the presence of the pathogen after incubation, any superficial fungal material on the surface of the fruit was removed using sticky tape. The sticky tape was then placed over a drop of lacto fuchsin on a glass slide and observed under a microscope.

Table 2.3.8 Treatments applied to 'Murcott' fruit.

Treatment	Details
Water control	Distilled water only
Mz 2.00 g	Mancozeb (Penncozeb) 2.00 g/L
Mz 1.00 g	Mancozeb (Penncozeb) 1.00 g/L
Mz 1.00 g + DDAC	Mancozeb (Penncozeb) 1.00 g/L + Sporekill 1 mL/L
Captan 2.50 g	Captan WG 2.50 g/L
Captan 1.25 g	Captan WG 1.25 g/L
Captan 1.25 g + DDAC	Captan WG 1.25 g/L + Sporekill 1 mL/L
DDAC	Sporekill 1 mL/L

Results

EBS symptoms were significantly more severe on fruit in the water control (Fig. 2.3.3 left) than in any other treatment ($p\leq0.001$, Fig. 2.3.3 right & 2.3.4). The DDAC only treatment had significantly less EBS than the water control (Fig. 2.3.4). Mz 1.00 g had significantly more disease than Mz 2.00 g, but the combination treatment of Mz 1.00 g + DDAC had the same lower amount of disease as the Mz 2.00 g treatment.

Furthermore, the severity of EBS in the DDAC only treatment was not significantly different from the Mz 1.00 g treatment. No differences were observed between the treatments containing captan. Light microscopy showed extensive mycelial growth arising from conidia consistent in appearance with *A. alternata* (Fig. 2.3.5 top). On fruit treated with mancozeb or captan, germinating conidia were rarely observed. On fruit treated with DDAC, healthy, ungerminated conidia were observed (Fig. 2.3.5 bottom).

Figure 2.3.3 Development of EBS symptoms in fruit treated with water (left) compared with fruit treated with fungicide (right).

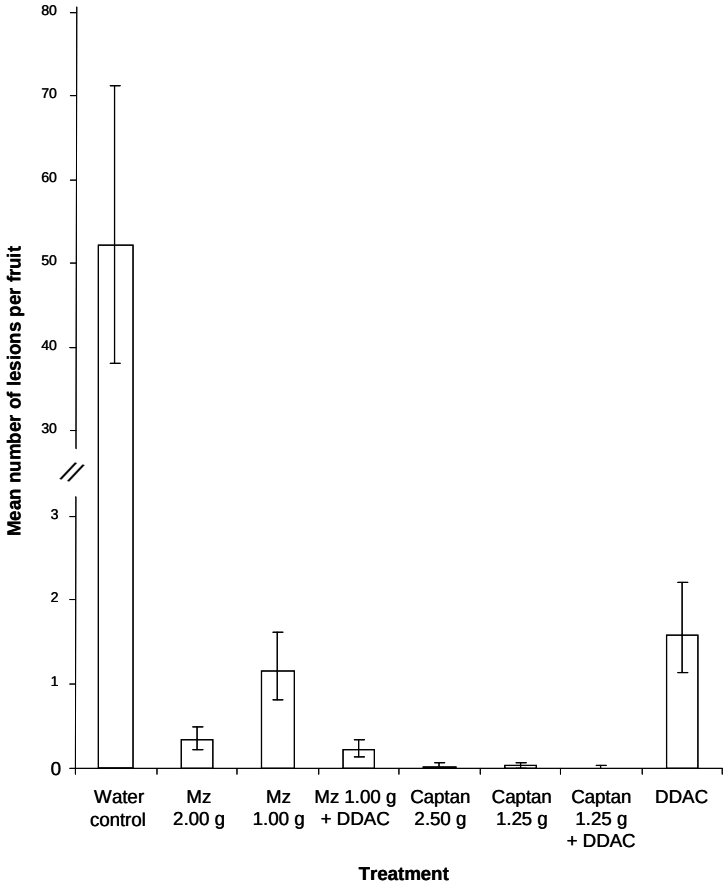


Figure 2.3.4 Severity of EBS in fruit treated with various rates of the fungicides mancozeb (Mz) and captan, and combinations with DDAC (Sporekill). Error bars indicate least significant difference.

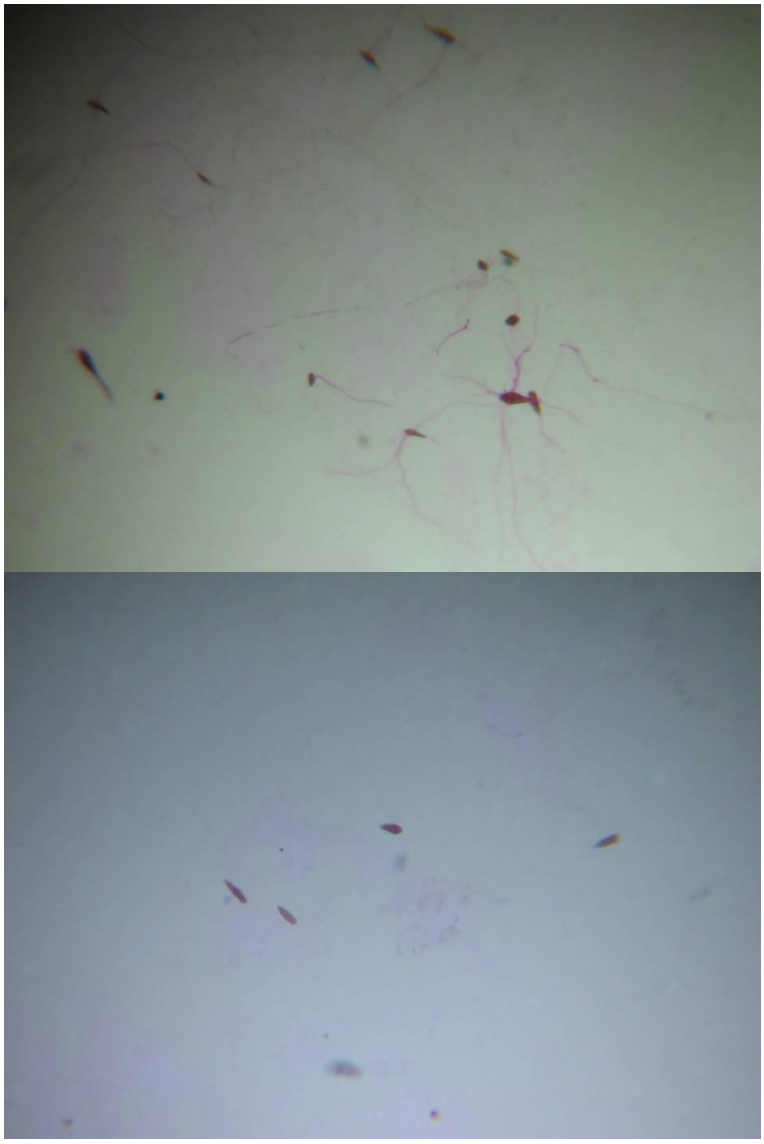


Figure 2.3.5 Germinating conidia and formation of mycelium of *Alternaria alternata* on the surface of 'Murcott' fruit treated with water (top), and conidia failing to germinate on the surface of fruit treated with Sporekill (bottom).

Discussion

The detached fruit experiment supports findings from South Africa (Schutte, 2008) that indicated the label rate of mancozeb (Penncozeb) of 2.00 g/L could be reduced to 1.00 g/L when combined with 1.00 mL/L Sporekill (DDAC). However, our experiment also showed a reduction in efficacy associated with reducing the rate of mancozeb alone to 1.00 g/L. Furthermore, our experiment showed that DDAC used alone provided a very significant reduction in disease compared with the water control. Apparently this was not observed in the field in the South African study.

The efficacy of DDAC on its own in our experiment shows that DDAC can provide some level of prophylactic activity for at least 48 hrs after treatment. While some evidence for the direct efficacy of DDAC against *A. alternata* has been shown in a previous study (Daus *et al.*, 2011), no studies investigating the residual efficacy of DDAC at different times after application could be found for this pathogen or any

others. Regardless, it is likely that the ability of DDAC to prevent spore germination, as shown in Fig. 2.3.5 and in other studies (Xiao and Kreber, 2005), contributes to the efficacy of the combination treatment of DDAC and mancozeb.

In the case of the treatments including Captan, all these treatments were highly effective in preventing disease and showed no significant differences between each other. This may indicate that testing for synergistic effects between DDAC and captan would require much lower rates of captan. Based on the field evaluations against EBS there have not been statistically significant differences in efficacy between the 2.50 g/L and 1.25 g/L captan treatments; though there has been a trend towards less disease in the 2.50 g/L treatment. The full assessment of this field trial at harvest will provide a better indication of the captan rate that is required to achieve maximum disease control. Once this rate is established it will be possible to investigate any synergistic effects between DDAC and captan.

The potential efficacy against EBS for treatments of DDAC in combination with mancozeb has been shown in the field in South Africa (Schutte, 2008), in 'Murcott' seedlings, and now detached fruit. DDAC in combination with captan is also likely to be efficacious, but there is less evidence than for mancozeb. It is highly likely that combination treatments with DDAC could provide commercial control of EBS in Australian orchards, but other factors still need to be considered. For example: are combination treatments with DDAC cost-effective; do they reduce fungicide residue levels at harvest; and are they likely to induce any forms of blemish on fruit? While the last two questions require field trials to answer them, the potential cost-benefits of combination treatments with DDAC are shown below.

Potential cost-benefit of using tank mixtures of reduced rates of fungicide with Sporekill

In South Africa it has been shown that rates of mancozeb and copper fungicide products can be halved when tank mixed with Sporekill (didecyl dimethyl ammonium chloride) without any reduction in fungicide efficacy (Schutte, 2008). As Sporekill used on its own had limited effect on disease, it was concluded that there is a synergistic effect between the fungicides and Sporekill. Further work is needed to confirm any synergistic effects of Sporekill, but assuming the synergy exists, any potential cost saving associated with the use of these tank mixtures is of particular interest. The sum of any cost saving needs to be determined before considering investing in field trials and registration of any tank mixes. Table 2.3.9 shows the comparative costs associated with the different fungicide treatments. Based on the cost estimates shown in Table 2.3.9, a significant saving to industry exists if 1.25 g/L Captan WG + 1 mL/L Sporekill can be used as effectively as 2.5 g/L Captan WG. In the case of tank mixing with Dithane and Sporekill, the Sporekill is more expensive than Dithane and therefore tank mixing does not present a cost saving. However, if the mixture of Dithane + Sporekill can be used to reduce dithiocarbamate residues from mancozeb (see 'Dealing with mancozeb residues') there still may be some incentive for pursuing the tank mix option.

Table 2.3.9 Comparison of the costs of a single application using standard rates of the fungicides Dithane and Captan WG, with the costs of using reduced rates of the fungicides tank mixed with Sporekill.

		Dithane[a] 200 g/100 L		**Dithane 100 g/100 L + Sporekill[b] 100 mL/100**					
	Application volume[c] (L)	Dithane applied (kg)	**Cost of Dithane**	Dithane applied (kg)	Cost of Dithane	Sporekill applied (L)	Cost of Sporekill	**Cost of tank mix**	**Saving from tank mix**
All of Qld:	14,485,000	28,970	**$202,790**	14,485	$101,395	14,485	$133,986	**$235,381**	**-32,591**
Per hectare:	9,000	18	**$126**	9	$63	9	$83	**$146**	**-$20**
	4,000	8	**$56**	4	$28	4	$37	**$65**	**-$9**
		Captan[d] 250 g/100 L		**Captan 125 g/100 L + Sporekill 100 mL/100 L**					
		Captan applied (kg)	**Cost of Captan**	Captan applied (kg)	Cost of Captan	Sporekill applied (L)	Cost of Sporekill	**Cost of tank mix**	**Saving from tank mix**
All of Qld:	14,485,000	36,212	**$470,763**	18,106	$235,381	14,485	$133,986	**$369,368**	**$101,395**
Per hectare:	9,000	23	**$293**	11	$146	9	$83	**$230**	**$63**
	4,000	10	**$130**	5	$65	4	$37	**$102**	**$28**

[a]Dithane Rainshield fungicide (750 g/L mancozeb)

[b]Sporekill agricultural disinfectant (120 g/L didecyl-dimethylammonium chloride)

[c]Estimations of the application volumes based on a) a survey of 22 Murcott blocks in the Central Burnett indicated that 17/22 blocks were sprayed at >6,000 L/ha, and 5/22 blocks were sprayed at <6,000 L/ha (Miles et al. 2010), b) based on the opinion of local consultants, the median volume applied in the ">6,000 L/ha" category would be 9,000 L/ha, and 4,000 L/ha in the "<6,000 L/ha" category, c) points a) and b) are extrapolated to 77% of the production area being sprayed at 9,000 L/ha and 23 % at 4,000 L/ha and d) there are 1,845 ha of bearing mandarins in Qld (Sette *et al.*, 2011), equating to 1,421 ha sprayed at 9,000 L/ha and 424 ha at 4,000 L/ha.

[d]Captan WG fungicide (800 g/kg captan)

Efficacy of Bayer fungicides against CBS (2011-12)

Introduction

Through Bayer / HAL VC project CT11004 *Fungicide screen for the control of brown spot in citrus*, a range of new Bayer fungicides were screened against EBS in Queensland in the 2011-12 season. To add significant value to these trials, Andrew Miles through CT07012, Rob Vitelli (Bayer) and Chris Themsen (Peracto) collaborated to collect, incubate and assess fruit from the field trials for CBS.

Methods

In order to determine the efficacy of new Bayer fungicides against CBS, fruit were harvested from two field trials conducted under CT11004 for the evaluation of EBS efficacy (see CT11004 final report for full trial details). Two trials were conducted on Murcott tangor; one at Wallaville and one at Gayndah. The treatments applied were DC-104 and DC-099 at three rates each, DC-105 at one rate, Amistar and Dithane as positive controls, as well as a nil treatment as a control. Each treatment was applied to four single tree replicates in a randomised complete block. Treatment applications at each site commenced at petal fall, followed by another four applications at approximately 21 day intervals. At commercial harvest, approximately 20 fruit per replicate tree were sampled from the Gayndah trial. Only very low numbers of fruit could be obtained from the Wallaville trial site, with typically only 5 fruit able to be assessed. Sampled fruit were incubated at the Maroochy Research Facility at 27°C, 80% RH and constant light for 3 weeks to break the latency of CBS symptoms. The number of CBS lesions per fruit (severity) was counted, and the proportion of fruit with one or more lesions (incidence) was derived from these counts. Severity and incidence of CBS in the different treatments were compared using Analysis of Variance (ANOVA) and a Generalised Linear Model (GLM), respectively.

Results

At Gayndah, all the fungicide treatments significantly reduced the severity of CBS compared with the untreated control, with the lowest severity of CBS observed in the DC-099 30mL and 40 mL, DC-105, Amistar and Dithane treatments (Table 2.3.10). The incidence of CBS at Gayndah was significantly reduced compared to the untreated control by all treatments except DC-104 at 10 mL and 20 mL. The incidence of disease was lowest in the DC-099 at 30 mL and 40 mL, DC-105, Amistar and Dithane treatments (Table 2.3.11). There were no significant differences in CBS in the treatments applied at the Wallaville site.

Table 2.3.10 Severity of CBS in Murcott tangor treated with various fungicides.

Treatment		Severity of CBS[A] (mean number of lesions per fruit)	
		Gayndah	**Wallaville**
DC-104	0.1 mL/L	0.39 (2.46) b	0.31 (2.03)
	0.2 mL/L	0.34 (2.19) b	0.45 (2.80)
	0.4 mL/L	0.26 (1.84) b	0.49 (3.09)
DC-099	0.2 mL/L	0.24 (1.75) b	
	0.3 mL/L	0.08 (1.20) c	0.21 (1.61)
	0.4 mL/L	0.03 (1.07) c	0.28 (1.89)
DC-105	1.5 g/L	0.02 (1.05) c	0.32 (2.07)
Amistar	0.4 mL/L	0.07 (1.18) c	0.25 (1.77)
Dithane DF	2.0 g/L	0.04 (1.09) c	0.40 (2.53)
Untreated		0.68 (4.76) a	0.64 (4.37)
p-value		<0.001	0.198
SED		0.08	0.16
95% LSD		0.17	0.32

[A]Mean values followed by the same letter are not significantly different. Back-transformed means are shown in parentheses.

Table 2.3.11 Incidence of CBS in Murcott tangor treated with various fungicides.

Treatment		Incidence of CBS[A] (proportion of fruit with one of more lesions)	
		Gayndah	**Wallaville**
DC-104	0.1 mL/L	0.658 (0.0911) ab	0.546 (0.1803)
	0.2 mL/L	0.646 (0.0901) ab	0.679 (0.1500)
	0.4 mL/L	0.442 (0.0947) bc	0.710 (0.1386)
DC-099	0.2 mL/L	0.342 (0.0911) cd	
	0.3 mL/L	0.120 (0.0628) de	0.600 (0.2632)
	0.4 mL/L	0.067 (0.0482) e	0.281 (0.1345)
DC-105	1.5 g/L	0.052 (0.0423) e	0.615 (0.1622)
Amistar	0.4 mL/L	0.141 (0.0660) de	0.375 (0.1454)
Dithane DF	2.0 g/L	0.091 (0.0548) e	0.594 (0.1476)
Untreated		0.787 (0.0792) a	0.760 (0.1453)
p-value		<0.001	0.434

[A]Mean values followed by the same letter are not significantly different. Standard errors of the means are shown in parenthesis.

Discussion

The results of the CBS assessments from the Gayndah site have shown strong efficacy for DC-105 and DC-099, which both significantly reduced the incidence and severity of CBS at the rates that were tested. DC-104 showed less promise, but this may have occurred because the tested rates were too low; higher rates of the product may prove successful. At the Wallaville site no significant treatment effects were observed. A difference in disease pressure between the two sites is unlikely to be the explanation because incidence and severity of CBS in the untreated controls were similar at both sites. It is more likely that the small sample sizes available from the Wallaville site led to increased variation and an inability to discern treatment effects. Further trial work with these fungicides is recommended.

Field evaluation of fungicides against CBS and EBS (2012-13)

Introduction

In previous sections of this report it has been shown that new fungicides are required to improve the control of CBS and EBS, and a number of potential fungicides have been identified for evaluation in field trials. In order to undertake trials to assess the efficacy of these fungicides, a collaboration between Bugs for Bugs and the CT07012 project team was possible through project CT09055 *Coordinating a market development program for the Australian citrus value chain*. Two field trials were established; one for CBS in Imperial mandarin, and one for EBS in Murcott tangor. At both sites various fungicides were applied throughout the periods of fruit susceptibility to each disease, and then the amount of disease in the various treatments was assessed. For CBS, the level of disease was assessed at the end of the season and after incubating fruit. For EBS, fruit assessment could be made during the season, with final end-of-season assessments made and reported through a project to follow on from CT07012. The aim was to identify the best candidate fungicides for registration.

Methods

CBS

In the 2012-13 season the efficacy of various fungicides against CBS was evaluated in a field trial located in a high disease pressure area near Mundubbera, Qld. The trial comprised of 'Imperial' mandarin (*C. reticulata*) trees on 'Cleopatra' mandarin rootstock (*C. reticulata*), planted in 1992 at a 7.3 m × 5.5 m spacing. Each treatment was applied to four individual replicate trees, arranged in randomized blocks spread over four rows of trees. All the treatments in Table 2.3.12 were applied five times at approximately monthly intervals (27/9/12, 1/11/12, 29/11/12, 2/1/13, 6/2/13) during the first 20-24 weeks of fruit growth when fruit are susceptible to *P. citricarpa* (Wager, 1952; Baldassari *et al.*, 2006; Kotze, 1981). All treatments were applied at the standard and 2× standard rates, with the exception of mancozeb only being applied at the standard rate. Treatment applications were made at 20 L per tree using a custom built hand lance sprayer with dual D4 hollow cone nozzles, operating at 50 psi delivered by a 6.0 horsepower Subaru Robin EX17 gas engine-driven pressure pump (Subaru, Japan). Also included were four replicate trees that were not treated with fungicide as a control. On the 28th January 2013 the trial site was subject to inundation by flood waters resulting from ex-tropical cyclone Oswald. The trial trees were inundated for approximately 24 hours, to a depth approximately 75% the height of the trees. At commercial maturity (8th May 2013), approximately 60 fruit were harvested from each row-side (east and west) of each data tree. The fruit were then incubated for 3 weeks at 27°C, 80% relative humidity, and permanent light to break the latency of all *P. citricarpa* infections (Fig. 2.3.5 left) (Brodrick and Rabie, 1970). Any fruit showing postharvest mould or breakdown during incubation were recorded, and then discarded to prevent further decay of surrounding fruit. After incubation CBS symptoms on each fruit were quantified by inspecting each fruit by eye and light microscopy (Fig. 2.3.5 right). In the case of hard spot and freckle spot, the numbers of lesions of each type on each fruit were counted. For virulent CBS lesions and speckled blotch, estimates of the percentage of the fruit surface area affected were made. Disease incidence was defined as the proportion of fruit with one or more

lesions. Disease severity was defined as the number of lesions per fruit. A rating of fruit presentation was made by grading the overall appearance of the fruit from each side of each data tree. Fruit were rated on a 1 to 4 scale, where 1 was poorest in appearance, and 4 was the best in appearance.

Disease incidence data were analysed using a generalised linear model, assuming a binomial distribution and complementary log-log link function. Disease severity and fruit presentation data were analysed using analysis of variance (ANOVA) and a $\log_{10}$ transformation where required to improve the underlying assumptions of the model. To be able to analyse the effect of the various treatments on the combined severity of all the observed forms of CBS, the estimates of fruit surface area affected by virulent spot and speckled blotch were converted to an equivalent number of lesions; assuming 1% of surface area was equivalent to 10 spots of 3 mm diameter.

Table 2.3.12 Product names, active ingredients, suppliers and standard rates of chemicals used in CBS chemical control experiments carried out in Queensland.

Product name	Active ingredient	Supplier	Standard rate of product	Standard rate of active ingredient
Penncozeb 750DF	75% mancozeb	NuFarm	2.00 g/L	1.500 g/L
Captan 800WG	80% captan	Farmoz	1.25 g/L	1.000 g/L
DC-104	Confidential	Bayer	0.20 mL/L	-
DC-105	Confidential	Bayer	0.50 g/L	-
Fontelis	20% penthiopyrad	DuPont	0.75 mL/L	0.150 mL/L
Filan	50% boscalid	BASF	0.30 g/L	0.150 g/L
Xemium	30% fluxapyroxad	BASF	0.25 mL/L	0.075 mL/L

Figure 2.3.5 Incubation of imperial fruit (left) and assessment of fruit after incubation (right).

EBS

In the 2012-13 season the efficacy of various fungicides against EBS was evaluated in a field trial located in a high disease pressure area near Wallaville, Qld. The trial comprised of 'Murcott' tangor (*Citrus* × *aurantium*) trees on 'Benton' rootstock (*C.* × *aurantium* × *C. trifoliata*), planted in 2009 at a 7 m × 4 m spacing. Each treatment was applied to four individual replicate trees, arranged in randomized blocks arranged over two rows of trees. All the treatments (Table 2.3.13) were applied eight times at approximately monthly intervals (28/9/12, 2/11/12, 30/11/12, 3/1/13, 6/2/13, 7/3/13, 10/4/13, 8/5/13). Fruit are susceptible to EBS throughout the entire season (Miles *et*

al., 2005). With the exception of mancozeb and phosphorous acid, all treatments were applied at the standard and 2× standard rates. Treatment applications were made at 10 L per tree using a custom built hand lance sprayer with dual D4 hollow cone nozzles, operating at 50 psi delivered by a 6.0 horsepower Subaru Robin EX17 gas engine-driven pressure pump (Subaru, Japan). Also included were four replicate trees that were not treated with fungicide as a control.

Table 2.3.13 Product names, active ingredients, suppliers and standard rates of chemicals used in EBS chemical control experiments carried out in Queensland.

Product name	Active ingredient	Supplier	Standard rate of product	Standard rate of active ingredient
Penncozeb 750DF	75% mancozeb	NuFarm	2.00 g/L	1.500 g/L
Captan 800WG	80% captan	Farmoz	1.25 g/L	1.000 g/L
DC-104	Confidential	Bayer	0.20 mL/L	-
DC-105	Confidential	Bayer	0.50 g/L	-
Fontelis	20% penthiopyrad	DuPont	0.75 mL/L	0.150 mL/L
Filan	50% boscalid	BASF	0.30 g/L	0.150 g/L
Xemium/Imbrex	30% fluxapyroxad	BASF	0.25 mL/L	0.075 mL/L
Chief	50% iprodione	Farmoz	1.00 mL/L	0.500 mL/L
SprayPhos	62% phosphorus acid	Spraygro	2.25 mL/L	1.364 mL/L

Disease was assessed twice throughout the season on the 11th December 2012 and 14th March 2013, after two and six applications had been applied, respectively. At each assessment 25 fruit were arbitrarily selected from around the canopy of each data tree and categorised as either clean (no EBS lesions) or diseased (1 or more EBS lesions). The incidence of EBS in each data tree was then calculated. Comparison of the incidence of EBS between treatments was performed using Analysis of Variance (ANOVA).

Results

CBS

An incidence of 50% of fruit, and a severity of 18.1 equivalent lesions per fruit, was observed for total CBS in the untreated control fruit (Table 2.3.14 & Fig. 2.3.6a,b). The most common symptom type observed was freckle spot, followed by hard spot and virulent spot (Fig. 2.3.6). Speckled blotch occurred at too low a level to be reported separately, but the occurrence of speckled blotch was included in the total CBS data. Freckle spot was characterised as slightly depressed, orange to brick red spots. Hard spot was characterised as red to black-rimmed depressed lesions with a light grey or brown centre containing pycnidia. Virulent spot was characterised as a coalescence of freckle spots, and speckled blotch as areas of many minute black spots on the fruit surface. The incidence and severity of CBS was generally highest or equal highest in the untreated control, whilst the incidence and severity of CBS was generally lowest or equal lowest in fruit treated with the industry standard fungicide, mancozeb. In terms of total CBS incidence, the treatments with significantly lower CBS incidence than the untreated control were mancozeb, captan 1.00 g/L, both rates of DC-105 and penthiopyrad, and the high rates of boscalid and fluxapyroxad (Table 2.3.14 & Fig. 2.3.6a). Of the treatments with significantly lower incidence of CBS than the control, the high rates of DC-105 and boscalid were not significantly different from mancozeb. In terms of total CBS severity, all the fungicide treatments resulted in significantly less severe CBS than the untreated control.

The incidence of freckle spot was lowest at 10% in the mancozeb treatment, closely followed by the high rates of DC-105 and boscalid at 12% and 17%, respectively (Table 2.3.14 & Fig. 2.3.6c). The incidence of freckle spot in the low rates of captan and DC-105, and the high rate of penthiopyrad were significantly higher than that of mancozeb, but were still significantly lower than the untreated control. The severity of freckle spot was significantly reduced by all fungicides except the high rate of captan, and the low rates of boscalid and fluxapyroxad.

The incidence and severity of hard spot were significantly lower than the untreated control in fruit treated with mancozeb, or the high rates of captan, DC-105, penthiopyrad, boscalid, and fluxapyroxad. None of these treatments were significantly different from mancozeb (Table 2.3.14 & Fig. 2.3.6e,f). The incidence of virulent spot was lowest (1.3%) at the high rate of penthiopyrad, but this was not significantly lower than mancozeb, the low rate of captan, the high rates of DC-104, DC-105, and fluxapyroxad, or both rates of penthiopyrad and boscalid (Table 2.3.14 & Fig. 2.3.6g). Virulent spot severity was significantly reduced compared to the untreated control by all the fungicide treatments except the low rate of fluxapyroxad (Table 2.3.14 & Fig. 2.3.6h). The lowest severity of virulent spot (2%) was observed in the high rates of DC-105 and penthiopyrad, but most other treatments were not significantly different from these treatments except for the captan treatments, the low rate of fluxapyroxad, and the untreated control.

CBS was typically worse on the western side of the canopy (Table 2.3.14). However, the trend was only significant in the case of total CBS severity, hard spot, and virulent spot severity. In the case of the incidence of virulent spot, the factor of canopy side was excluded from the analysis as no fruit treated with Penncozeb on the east side of the trees showed virulent spot. This zero proportion for this treatment would lead to over-inflated standard errors.

Treatment effects on fruit presentation were not significant (Table 2.3.14), but presentation of fruit from the west side of the canopy was better than fruit from the east side.

Table 2.3.14 Incidence and severity of CBS and its various symptom types, and visual presentation, in fruit treated in a commercial orchard with various fungicides[a].

		Total CBS[b]		Freckle spot		Hard spot		Virulent spot		Presentation
Treatment		**Incidence (%)[c]**	**Severity[d]**	**Incidence (%)**	**Severity**	**Incidence (%)**	**Severity**	**Incidence (%)**	**Severity**	**(1-4 scale)[e]**
Mancozeb	1.500 g/L	-2.06 (12) e	2.6 cd	-2.253 (10) e	1.1 efg	-4.1 (1.6) d	-1.6 (0.02) d	-3.75 (2.3) bcde	-1.3 (4) cd	2.3
Captan	2.000 g/L	-0.67 (40) abc	7.9 bc	-0.781 (37) abc	2.96 abcd	-3.2 (4.0) bcd	-1.4 (0.03) cd	-3.01 (4.8) abcd	-0.8 (14) bc	2.7
	1.000 g/L	-0.87 (34) bc	8.5 b	-1.156 (27) bcd	2.1 cdef	-2.7 (6.6) abc	-1.0 (0.09) abc	-3.08 (4.5) bcde	-0. 7 (20) bc	2.8
DC-104	0.400 mL/L	-0.76 (38) abc	2.9 cd	-0.960 (32) abc	1.4 defg	-2.6 (7.5) abc	-1.0 (0.09) abc	-3.67 (2.6) bcde	-1.2 (5) cd	2.4
	0.200 mL/L	-0.61 (42) abc	6.5 bcd	-0.816 (36) abc	2.5 bcde	-2.2 (10.1) a	-0.9 (0.12) ab	-2.97 (5.0) abc	-0.9 (12) bcd	2.4
DC-105	1.000 g/L	-1.91 (14) e	3.8 bcd	-2.101 (12) e	0.7 g	-3.6 (2.8) d	-1.4 (0.03) cd	-4.19 (1.5) de	-1.5 (2) d	3.3
	0.500 g/L	-0.96 (32) bcd	5. 6 bcd	-1.238 (25) cd	1.9 cdefg	-2.7 (6.6) abc	-0.8 (0.15) a	-3.05 (4.6) abcd	-0.9 (11) bcd	2.9
Penthiopyrad	0.300 mL/L	-0.98 (31) cd	2.2 d	-1.160 (27) bcd	1.5 defg	-3.2 (3.8) bcd	-1.3 (0.05) bcd	-4.32 (1.3) e	-1.5 (2) d	2.6
	0.150 mL/L	-0.85 (35) bc	5.2 bcd	-1.088 (29) abc	2.4 bcde	-2.5 (8.0) ab	-0.9 (0.12) ab	-3.56 (2.8) bcde	-0.9 (11) bcd	2.8
Boscalid	0.300 g/L	-1.42 (22) de	2.6 cd	-1.704 (17) de	0.9 fg	-3.2 (4.1) bcd	-1.3 (0.04) bcd	-3.96 (1.9) cde	-1.1 (7) bcd	3.3
	0.150 g/L	-0.49 (46) ab	6.2 bcd	-0.639 (41) ab	3.1 abc	-2.2 (10.1) a	-0.8 (0.17) a	-3.30 (3.6) bcde	-1.1 (7) bcd	2.9
Fluxapyroxad	0.150 mL/L	-0.98 (31) cd	2.7 cd	-1.124 (28) abcd	1.6 cdefg	-3.3 (3.5) cd	-1.3 (0.04) bcd	-3.75 (2.3) bcde	-1.2 (5) cd	2.6
	0.075 mL/L	-0.58 (43) abc	7.1 bcd	-0.788 (37) abc	3.8 ab	-2.5 (7.9) ab	-0.9 (0.12) ab	-2.83 (5.8) ab	-0.5 (30) ab	2.4
Control		-0.34 (50) a	18.1 a	-0.612 (42) a	4.2 a	-2.2 (10.1) a	-0.8 (0.18) a	-2.35 (9.1) a	-0.1 (88) a	2.2
LSD		0.52	5.4	0.577	1.5	0.82	0.4	1.06	0.6	0.7
p-value		<0.001	<0.001	<0.001	<0.001	0.003	0.003	0.007	0.003	0.067
Canopy side										
West		-0.92 (33)	6.9 a	-1.201 (26)	2.0	-2.6 (6.9) a	-1.0 (0.09) a	n.a.	-0.8 (14) a	2.8 a
East		-1.00 (31)	4.8 b	-1.145 (27)	2.3	-3.1 (4.4) b	-1.2 (0.06) b	n.a.	-1.1 (6) b	2.5 b
LSD		0.16	1.8	0.179	0.5	0.3	0.17	n.a.	0.3	0.2
p-value		0.81	0.031			0.003	0.013	n.a.	0.012	0.001

[a]Mean values within columns followed by the same letter are not significantly different at $p \leq 0.005$. Numbers in parentheses are back transformed means.
[b]Total CBS is the total of all lesion types; freckle spot, hard spot, virulent spot and speckled blotch (data not shown).
[c]Incidence refers to the proportion of fruit with symptoms of CBS.
[d]Severity refers to the number of spots per fruit. In the case of Total CBS, the measures of fruit surface area were converted to a number of spots based on the assumption that 1% of fruit surface area was equivalent to 10 spots of 3mm diameter.
[e]Fruit presentation was assessed on a 1 to 4 scale where 1 = the poorest presentation and 4 = the best presentation.

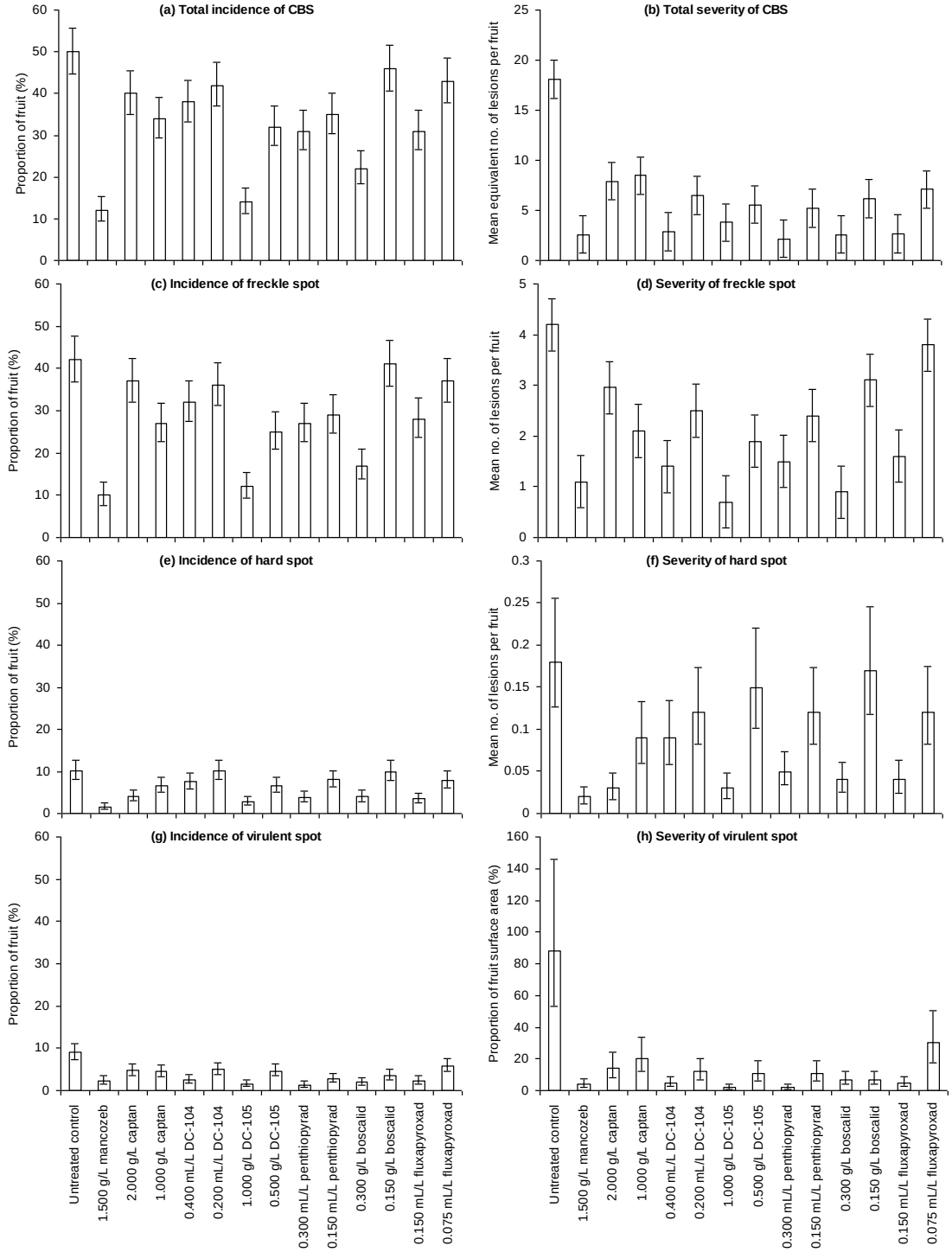


Figure 2.3.6 Histograms showing the effect of various fungicide treatments on the incidence and severity of total CBS (a & b), and the various symptoms types of freckle spot (c & d), hard spot (e & f), and virulent spot (g & h). Incidence refers to the proportion of fruit with 1 or more symptoms of CBS. Severity refers to the number of spots per fruit or the proportion of surface area affected. In the case of Total CBS severity (b), the measures of fruit surface area were converted to a number of spots based on the assumption that 1% of fruit surface area was equivalent to 10 spots of 3mm diameter.

EBS

The incidence of EBS in the untreated control ranged from 71 to 82% depending on assessment date (Table 2.3.15). All the fungicide treatments except phosphorous acid significantly reduced the incidence of disease compared to the untreated control. Treatments with significantly less diseased fruit than the industry standard fungicide, mancozeb, at both assessment dates were the high rates of DC-105, boscalid, fluxapyroxad, and iprodione. Treatments that were not significantly different to mancozeb at both assessment dates were both rates of captan, DC-104, and penthiopyrad, while only the low rates of boscalid and iprodione were equivalent to mancozeb. When comparing the three SDHI fungicides boscalid, penthiopyrad, and fluxapyroxad at the equivalent rate of 0.15 mL/L, fluxapyroxad resulted in significantly less disease than the other two fungicides. Treatment with phosphorous acid was equivalent to the untreated control.

Table 2.3.15 Incidence of EBS in fruit treated in a commercial orchard with various fungicides[a].

		Incidence[b]	
Treatment		**11th December 2012**	**14th March 2013**
Mancozeb	1.500 g/L	53.5 cde	45 efg
Captan	2.000 g/L	36.0 abcd	40 cdefg
	1.000 g/L	50.0 bcde	41 defg
DC-104	0.400 mL/L	35.5 abc	47 fg
	0.200 mL/L	54.0 de	55 g
DC-105	1.000 g/L	24.5 a	16 ab
	0.500 g/L	39.0 abcde	25 abcd
Penthiopyrad	0.300 mL/L	36.5 abcde	36 cdef
	0.150 mL/L	54.5 e	50 fg
Boscalid	0.300 g/L	32.5 ab	23 abc
	0.150 g/L	40.0 abcde	47 fg
Fluxapyroxad	0.150 mL/L	24.5 a	14 a
	0.075 ml/L	26.0 a	28 abcde
Iprodione	1.000 mL/L	28.0 a	17 ab
	0.500 mL/L	37.5 abcde	32 bcdef
Phosphorous acid	1.364 mL/L	85.5 f	84 h
Control		82.4 f	71 h
LSD		15.8	16
P		<0.001	<0.001

[a]Mean values within columns followed by the same letter are not significantly different at $p \leq 0.005$.
[b]Incidence refers to the proportion of fruit with symptoms of EBS.

Discussion

The field trials conducted in the 2012-13 season have identified a range of fungicides as potential alternatives to the current industry standard, mancozeb. The efficacy of mancozeb against CBS and EBS is well established (Miles *et al.*, 2004; Miles *et al.*, 2005), and has been re-affirmed in this trial and the trials conducted by Bayer in the 2011-12 season. The promising alternative fungicides to mancozeb for CBS control include the coded Bayer fungicides DC-105, and the succinate dehydrogenase inhibitor (SDHI) fungicides fluxapyroxad, boscalid and penthiopyrad. When comparing the SDHI fungicides boscalid, penthiopyrad and fluxapyroxad at equal rates of active ingredient, it appears that *P. citricarpa* is more sensitive to fluxapyroxad (Xemium) than boscalid. Fluxapyroxad may therefore provide higher levels of efficacy if applied at a rate of active ingredient comparable to the higher rate

of boscalid. DC-105 and the SDHI fungicides all show promise for the control of both CBS and EBS. For EBS alone, the fungicides DC-104, captan and iprodione also look promising. Less promising fungicide-disease combinations were phosphorous acid for EBS, and captan and DC-104 for CBS.

The SDHI fungicides disrupt the function of the enzyme succinate dehydrogenase involved in electron transfer in fungal respiration (Hewitt, 1998). No previous reports of SDHI fungicides being trialled against *P. citricarpa* could be found, suggesting that our results are the first to do so, and to show a significant reduction in CBS incidence and severity in the field. In general, reports regarding the efficacy of SDHI fungicides against fungi closely related to *P. citricarpa* are rare. In contrast, SDHI fungicides are known to be effective against various genera including *Alternaria*, *Monilinia*, *Sclerotinia* and *Botrytis* (Avenot and Michailides, 2007). Boscalid, for example, is registered in a formulation including the strobilurin fungicide pyraclostrobin for the control of *A. alternata* in *Citrus* in Florida. It is not surprising then, that the SDHI fungicides showed efficacy against EBS in our experiment. As the active ingredients of DC-104 and DC-105 are not known, it is not possible to make comparisons of our results with other studies.

The Fungicide Resistance Action Committee (FRAC) considers the SDHIs to have a medium to high risk of developing resistance, therefore anti-resistance strategies are necessary. The reality of this risk has been demonstrated for many examples including resistance to boscalid of *A. alternata* in pistachio (Avenot and Michailides, 2007), *Didymella bryoniae* in watermelon (Stevenson *et al.*, 2008), and *B. cinerea* in strawberry (Fernandez-Ortuno *et al.*, 2012). In the case of managing this risk in Australia, any registration of an SDHI (FRAC resistance group 7) must be rotated with the existing fungicides mancozeb (group M3), copper (group M1) and azoxystrobin (group 11). The requirements for anti-resistance strategies for DC-105 are unknown at this stage.

The efficacy of the fungicide iprodione against EBS is well known (Hutton, 1989; Solel *et al.*, 1997; Miles *et al.*, 2005). However, in our trial the efficacy of iprodione was marginally improved when applied at twice the current label rate of 0.5 mL active ingredient/L. A similar observation was made in the 2011-12 field trials investigating the efficacy of iprodione against EBS. Surprisingly, no previous studies could be found that compared the efficacy of rates of iprodione higher than the current label rate for efficacy against EBS in *Citrus*. Depending on the outcome of the full assessment of the EBS trial, it may be worthwhile pursuing registration of iprodione at the higher rate for the control of EBS in orchards. The ability to register this use will depend largely on the results of residue studies being conducted by Dale Griffin (Crop Protection Research).

The difference in efficacy of captan to CBS and EBS was evident in past trials, where captan at 1 g/L was ineffective against CBS (Miles *et al.*, 2004), but showed good efficacy for EBS (Miles *et al.*, 2005). However, it was hoped that increasing the rate of captan may sufficiently increase efficacy against both diseases. Our trials only showed the increase in the captan rate to improve the efficacy against EBS, with no improvement in efficacy observed for CBS. Future efforts will therefore focus on the efficacy of captan against EBS. The registration of a multisite mode of action fungicide like captan will be very useful for managing *A. alternata* resistance to fungicides such as the SDHIs, azoxystrobin and iprodione. A multisite fungicide is still needed for managing *P. citricarpa* resistance.

Canopy side had significant effects on some of the CBS symptom types and fruit presentation. In the case of CBS being worse on the western side of the tree, this

has been observed in past trials (Miles *et al.*, 2004; Kiely, 1948). The poorer visual presentation of fruit on the eastern side is believed to be related to the deposition of silt during the inundation of the trees by flood waters. During the flooding the flow of water was from west to east. Therefore, the eastern side of the canopy was the eddy side of the flow. The reduced velocity of the water on the eastern side is hypothesised to have resulted in high deposits of silt on the fruit on the eastern side, and therefore a poorer visual presentation. Visual presentation was not influenced by treatment, indicating that no fungicides induced readily detectable levels blemish.

The trials conducted in the 2012-13 season have identified several promising new fungicides for managing citrus diseases. Based on the results of these trials, and other trials detailed in this report, the treatments in Table 2.3.16 are suggested for further trials to be conducted in the 2013-14 season.

Table 2.3.16 Suggested fungicide treatments (rates of active ingredient) for efficacy trials to be conducted in the 2013-14 season.

Treatment	CBS	EBS
Untreated control		
Mancozeb standard	1.50 g/L	1.50 g/L
Boscalid alone	0.15 g/L	0.15 g/L
	0.30 g/L	0.30 g/L
	0.60 g/L	0.60 g/L
Boscalid in use pattern	0.30 g/L	0.30 g/L
Captan alone		1.00 g/L
		2.00 g/L
		3.00 g/L
Captan in use pattern		2.00 g/L
DC-104 alone		0.20 ml/L
		0.40 ml/L
		0.80 ml/L
DC-104 in use pattern		0.40 ml/L
DC-105 alone	0.50 g/L	0.25 g/L
	1.00 g/L	0.50 g/L
	2.00 g/L	1.00 g/L
DC-105 in use pattern	1.00 g/L	0.50 g/L
Dithianon alone	0.70 g/L	0.70 g/L
Fluxapyroxad alone	0.15 g/L	0.04 g/L
	0.30 g/L	0.08 g/L
	0.60 g/L	0.15 g/L
Fluxapyroxad in use pattern	0.30 g/L	0.08 g/L

In vitro screening of postharvest fungicides for control of *Phyllosticta citricarpa*

Introduction

Controlling CBS caused by *Phyllosticta citricarpa* using a postharvest fungicide has proven notoriously difficult, with field control remaining the most successful approach to controlling the disease (Agostini *et al.*, 2006). However, a postharvest treatment for CBS remains a highly desirable goal. In 2013, a voluntary contribution from Syngenta to CT07012 was used to screen four fungicides *in vitro* to determine which fungicides may have the greatest potential for postharvest efficacy against *P. citricarpa*. Three of these fungicides were coded products, and the fourth was the strobilurin fungicide Amistar 250SC (250g/L azoxystrobin). Azoxystrobin was

included as a control, as postharvest treatment of infected fruit with this fungicide has shown some ability to reduce CBS incidence, formation of pycnidia in lesions, and ability to recover the pathogen from lesions (Wyatt *et al.*, 2008; Korf, 1998). However, a more reliable treatment than azoxystrobin is still needed for commercial application. A fungicide that *P. citricarpa* is more sensitive to than azoxystrobin *in vitro* may provide higher efficacy in fruit than azoxystrobin. Conversely, poor *in vitro* sensitivity could suggest limited efficacy in fruit, and rule out further experiments with that fungicide.

Methods

In order to determine the *in vitro* sensitivity of *P. citricarpa* to potential postharvest fungicides, the growth of three isolates of *P. citricarpa* (BRIP 52614, 53714, 53717) on ½ strength potato dextrose agar (PDA) amended with various rates of fungicides was determined. The PDA was amended to final concentrations of 0.001, 0.01, 0.1, 1 and 10 ppm of each fungicide. The fungicides of interest were coded products SYN PHT 3, SYN PHT 4, and SYN CUF 10, as well as the fungicide azoxystrobin. PDA without any fungicide was included as a control. PDA plates were inoculated in the centre with 3 mm diameter plugs of mycelium from 2-week-old colonies of the isolates. All plates were incubated in the dark at 25°C. Colony growth of three replicate colonies was measured after 7 days as the mean of two perpendicular diameters of the colony. Growth inhibition was expressed as a proportion of the colony diameter relative to the control. Curves of the $\log_{10}$ concentration versus percent growth inhibition were generated and tested for fit to various models (simple linear, exponential, Gompertz, 3 and 4 parameter logistic curves). The concentration to inhibit growth by 50% (EC_{50}) was then determined. To qualitatively determine the rates necessary to completely inhibit vegetative growth, the isolates were also grown on plates amended with 50, 125, 250, 500, 1000, 1500 and 2000 ppm. The amended plates were inoculated, incubated and growth measured as above.

Results

The $\log_{10}$ concentration versus percent growth inhibition curves for each chemical were found to each fit a different model (Fig. 2.3.7 A-D). The azoxystrobin, SYN PHT 3, SYN PHT 4, and SYN CUF 10 inhibition curves were best described by the exponential (adjusted R^2 = 65.1), Gompertz (adjusted R^2 = 71.0), 3 parameter logistic (adjusted R^2 = 81.3), and simple linear models (adjusted R^2 = 70.5), respectively. From the fitted models, EC_{50} values were determined to be 0.077 ppm for SYN PHT 3, 0.155 ppm for azoxystrobin, 0.476 ppm for SYN PHT 4 and 1.919 for SYN CUF 10.

In determining the concentration to completely inhibit growth, all isolates failed to grow on PDA amended with ≥50 ppm SYN PHT 3 and SYN PHT 4. Growth was completely inhibited by SYN CUF 10 at concentrations ≥250 ppm. Azoxystrobin failed to completely inhibit growth of the isolates at any of the tested concentrations. A maximum of only ~75% inhibition was achieved by azoxystrobin at 50 ppm and above. While growth of *P. citricarpa* continued at very high rates of azoxystrobin, colony morphology was not typical of the untreated control (Fig. 2.3.8). After transferring mycelium from the 1000 ppm azoxystrobin plate to a fresh control plate, the growth habit returned to a more typical appearance.

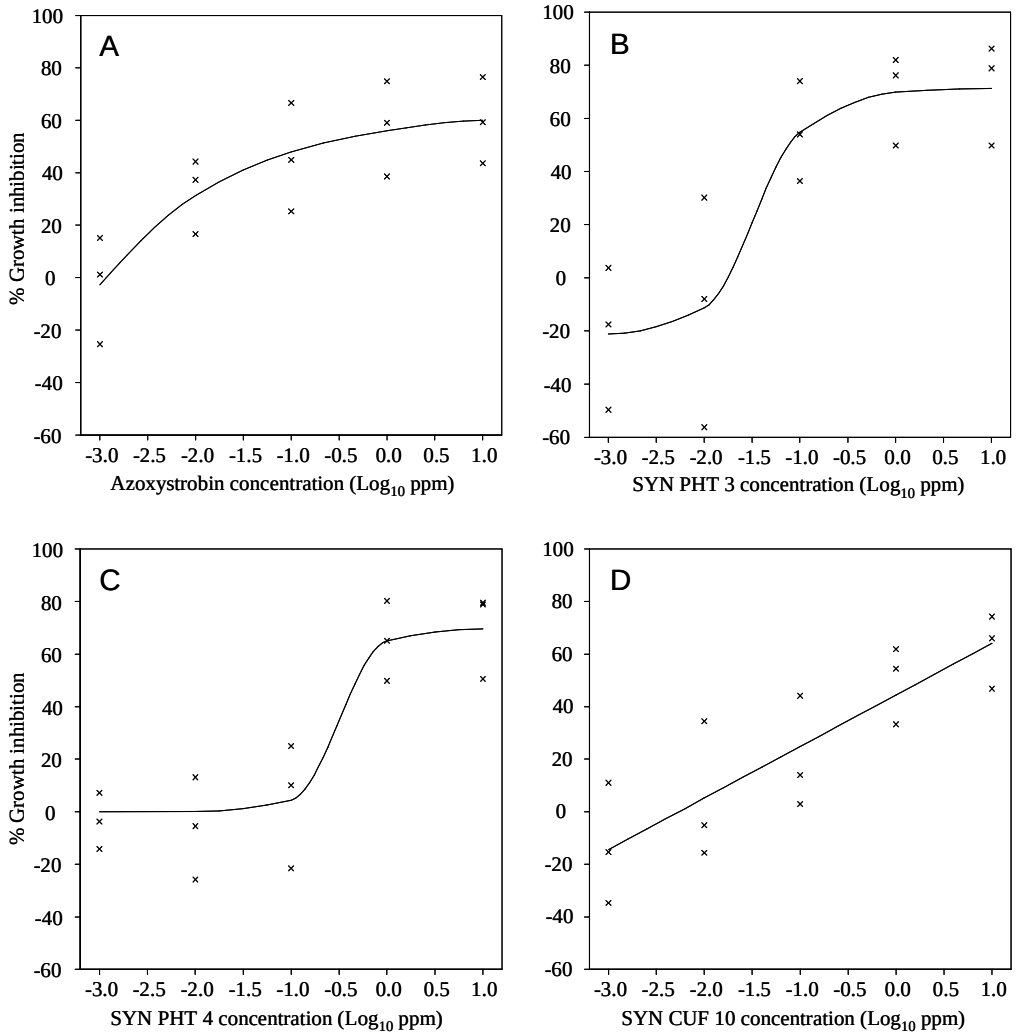


Figure 2.3.7 Percent of inhibition of vegetative growth of *Phyllosticta citricarpa* grown on various concentrations of the fungicides azoxystrobin (A), SYN PHT 3 (B), SYN PHT 4 (C), and SYN CUF 10 (D).

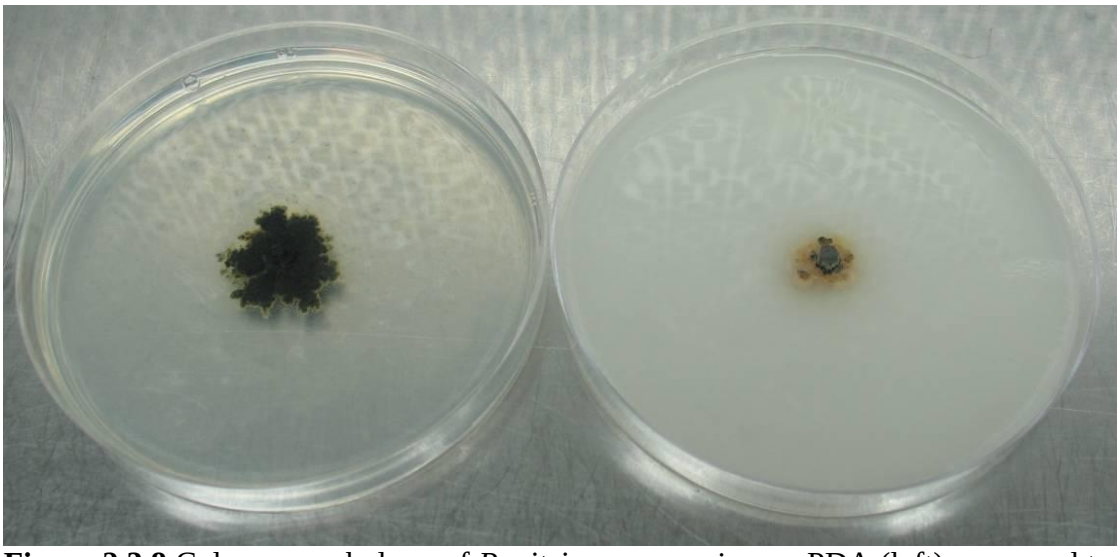


Figure 2.3.8 Colony morphology of *P. citricarpa* growing on PDA (left) compared to the morphology when grown on PDA + 1000 ppm azoxystrobin (right).

Discussion

In this experiment we set out to identify if any of the tested fungicides may have potential as a postharvest treatment for CBS control, using *in vitro* sensitivity as an indicator of possible efficacy. We found *P. citricarpa* to be most sensitive to SYN PHT 3, followed by azoxystrobin, SYN PHT 4 and SYN CUF 10. Considering azoxystrobin has shown some level postharvest efficacy against CBS in the past (Wyatt *et al.*, 2008; Korf, 1998), it stands to reason that any fungicide with a lower EC_{50} than azoxystrobin has potential to provide equivalent or better postharvest efficacy. In this case, SYN PHT 3 is therefore the most promising fungicide of those tested.

The three isolates used in our experiments are more sensitive to azoxystrobin than those in a study from Brazil (Possiede *et al.*, 2009), where the EC_{50} value appears to exceed 10 ppm compared to 0.155 ppm for our isolates. However, comparison to a study on a population of isolates from Florida, USA, found a mean EC_{50} of 0.021 ppm (Hincapie *et al.*, *Accepted*); several times lower a concentration than observed for our isolates. This disparity in results may be due to our low sample size, or alternatively may be due to *P. citricarpa* being a relatively recent introduction to the USA (Schubert *et al.*, 2012). In the latter case it may be possible that the younger *P. citricarpa* population in Florida has not been extensively exposed to fungicides such as azoxystrobin, or is a population derived from only one or a few very sensitive individuals of the fungus.

The inability of azoxystrobin to completely inhibit growth, even at concentrations of 10 ppm and above, has been observed by others (Hincapie *et al.*, *Accepted;* Possiede *et al.*, 2009). This, in addition to our isolates returning to normal growth after being transferred from 1000 ppm to 0 ppm, indicates that azoxystrobin might be better classified as a fungistat, rather than a fungicide. The fungistatic nature of azoxystrobin has also been observed by Rohel et al. (2001), whereby the growth of *Mycosphaerella graminicola* within wheat tissues was significantly slowed, but not ceased, when treated with azoxystrobin. The likely explanation put forward by Rohel et al. (2001) is that once azoxystrobin has disrupted respiration through inhibition of the bc1 complex in mitochondria, other less efficient pathways for respiration may be sufficient to maintain some level of growth.

2.4 Floods

Flood/rainfall damage to Central Burnett citrus orchards, June 7th 2011

In December 2010, flood waters affected the river systems of Gayndah (16.1m) and Mundubbera (18.25m). Some of the most severe direct effects of the flood waters were felt at three individual citrus orchards. The following text is based on field visits by Andrew Miles and Garry Fullelove (DAFF Qld) to the most severely impacted orchards in the Central Burnett.

Orchard 1:

Inundated trees are recovering well following complete submersion. Tree death is rare. Trees show a healthy new foliar flush, but several dead shoots give the trees a "twiggy" appearance (Fig. 2.4.1 left). The remaining dead twigs may provide inoculum sources for blemish diseases such as melanose and anthracnose in the future, but remedial pruning to remove dead twigs probably would not be cost effective. Also of note is that even after nearly 6 months, old foliage present during the flooding remains covered in a very persistent layer of silt (Fig. 2.4.1 right). The silt layer is likely to interfere with foliar spray application effectiveness (fungicides, nutrients etc.), however the clean new flush should allow effective uptake. Root inspection found very little evidence for *Phytophthora* activity at this stage. As the new flush hardens off, the orchardist will most likely apply a foliar phosphorous acid application to ensure new root growth is well protected from *Phytophthora*. Inundated, but not completely submerged blocks at Orchard 1 appear healthy with a good crop of marketable fruit (Fig. 2.4.2).

Figure 2.4.1 "Twiggy" appearance of inundated trees with new vegetative flush (left) and silt covering older leaves (right).

Figure 2.4.2 Partially submerged trees looking healthy with a good crop.

Orchard 2

Orchard 2 is probably the worst impacted orchard in terms of reduction in productive tree numbers. The orchardist has chosen to destroy nearly all inundated trees, mostly Murcott tangors, for two main reasons: i) direct loss of fruit and productive foliage following inundation, and ii) the fungal disease EBS (caused by *Alternaria alternata*) has been extremely challenging to manage under an organic regime. The combination of EBS and the additional tree stress from the inundation was highly likely to leave the trees unprofitable. Following the removal of these trees, the orchard has been reduced from 11 ha to 7 ha.

A few rows of inundated grapefruit have been left in the ground as they might return to full productivity. These trees are currently very twiggy, and showing some new flush growth (Fig. 2.4.3).

Figure 2.4.3 Inundated grapefruit.

Orchard 3

Unlike the two orchards above, Orchard 3 had a few rows of trees subject to rapid moving water. The direct result was for a few trees to be uprooted (Fig. 2.4.4 left), and these have subsequently been removed (Fig. 2.4.4 right). Some trees still have significant amounts of debris in their canopy (Fig. 2.4.5 left), and are slanted from the flowing water (Fig. 2.4.5 right). The fruit remaining on the trees is unsaleable (Fig. 2.4.6).

Figure 2.4.4 Uprooting of trees (left) followed by complete removal (right) by the grower.

Figure 2.4.5 Debris in trees (left) and slanting from water flow (right).

Figure 2.4.6 Direct blemish of fruit from flood waters.

Other issues:

Whilst the flood waters had direct impacts on a small number of orchards, far wider damage has resulted from the high amount of rainfall so far this season. The high rainfall has resulted in very high fungal disease pressure; primarily EBS (*Alternaria alternata*) and CBS (*Phyllosticta citricarpa*). The effects of EBS are already evident, with severe fruit and foliage damage (Fig. 2.4.7) in susceptible varieties (i.e. most mandarins except for the resistant Imperial). CBS damage was also evident in several cases (Fig. 2.4.8):

Figure 2.4.7 Damage caused by Alternaria alternata in foliage (left) and fruit (right).

Figure 2.4.8 Symptoms of CBS caused by *Phyllosticta citricarpa* on lemon fruit.

In response to the high disease pressure, some fungicide applications have resulted in serious blemishing of the fruit (Fig. 2.4.9); in particular the IRM1 and 2 low seed Murcotts (similar damage has also been observed in the regular Murcott). The precise cause of the damage is not clear, but losses will be very high in some blocks.

Figure 2.4.9 Spray injury on IRM1 and 2 fruit.

Flood damage to Central Burnett citrus orchards, January 2013

In January 2013 ex-tropical cyclone Oswald resulted in major flooding in the main citrus production regions in Queensland. On the 27th January Mundubbera recorded 315 mm, Gayndah 282 mm, and Wallaville 480 mm. In the Central Burnett the resulting flooding was estimated between 4 and 5 m above the flood height experienced in 2010. Flood levels were close to those experienced in the record flood of 1942.

Andrew Miles was based in the Central Burnett from the 4th-8th and 18th-22nd of February to contribute to the flood recovery effort. A summary of key issues is provided below.

It was observed that impacted orchards fell loosely into two categories:

1) Certainty of continuation in citrus production:

- Recovery efforts well underway;
- These orchards tended to have crop losses confined to a minor portion of the orchard, and some income from the remainder of the orchard is highly likely for the 2012-13 season.

2) Uncertainty of continuation in citrus production;

- Generally these orchards are smaller and/or sustained crop loss in most of the orchard;
- In some cases repair and recovery tasks had not commenced as of 22nd of February;
- Possible financial/technical reasons for not commencing repairs and recovery:
    - Unknown cost to recover;
    - Unsure how best to prioritise activities such tree recovery.

To a large extent the first category of orchards has provided insight into issues for the second category of growers. Consultation with the first group has assisted with the extension of knowledge to the second group.

Examples of some of the costs associated with recovery are shown below. These costs were able to be incorporated into a damage assessment spreadsheet developed primarily by Judy Shepherd (Gayndah Fruit Growers Association), with input from John Owen-Turner (QDAFF contractor), Andrew Mead (QDAFF) and Andrew Miles (QDAFF). This spreadsheet was used to capture damage impact data to support the case for category D financial assistance, as well as being provided to several growers to assist with their record keeping.

Direct orchard impacts

Damage	Incidence	Severity
Primary irrigation infrastructure (i.e. foot valves, pumps, motors, starters, etc.)	Nearly all growers in the district sourcing irrigation from the river system have completely lost, or sustained serious damage.	Complete loss estimated at $20-30k per pumping site.
Irrigation (i.e. sprinklers, poly pipe etc.)	Nearly all growers adjacent to any watercourse (river or creek) have had a level of inundation sufficient to damage irrigation.	Estimated costs per tree: Poly pipe ~$2.80 Sprinklers ~$3.50-$7.00 Labour ~$2.00-$5.00
Complete loss of trees	Most orchards where trees were completely submerged tended to completely lose 1-2 rows of trees on the outside of blocks due to high water velocity. Trees were either completely missing or unsalvageable.	Estimated replacement cost per tree: Private variety ~$25 Public variety ~$15 Labour ~$5
Trees leant over but salvageable, and debris (silt, grass etc.) deposited in trees.	Most growers have had some trees where the canopy is partially or fully submerged, leading to debris deposition. Outer rows of blocks have some trees leant over by the water velocity, but could be propped up.	Estimated cost per tree: Cleaning debris ~$12.50-$25.00 Propping trees ~$5.00-$10.00
Inundation of sheds (non-packing)	Moderate numbers of orchard sheds have been inundated.	Impacts are highly variable depending on contents of shed. Some examples are: • Basic tractor (no complex electronics) ~$300 to overhaul • Complex tractor ~$15,000 to overhaul • Picking bin (clean) ~$15 • Picking bin (replace) ~$200 • Picking bags ~$110 • Clippers ~$20
Inundation of packing sheds	Have only heard reports of 1 packing shed and packing line being inundated.	Packing line repair costs unknown.
General infrastructure	Most orchards have damage to fences and internal roads. Some orchards have damage to dams.	Fence ~$1.50 per m (materials only) Road base ~$30 m^3 Heavy machinery ~$75-175 per hour

Damage	Incidence	Severity
Riverbank erosion	Nearly all orchards adjacent to rivers have been exposed to erosion issues.	Orchards on outsides of bends have lost large volumes of soil. • Repair costs are largely unknown due to not knowing how to repair. • In a few cases washouts are close to homes. Orchards on the insides of bends have had large depositions of sand. • One orchard has reported 30 trees being completely buried.
Homes	Several orchard homes have been inundated.	Estimated costs ~$30,000 to $75,000 per home.
External roads	Moderate number of orchards had external roads damaged.	Major in the case of the Old Gayndah-Mundubbera Rd. This road is the only option for fruit transport for several orchards. Many minor road closures due to debris were cleared within several days of waters receding.

Industry impacts

As an industry on the whole, the most widely shared issues were observed to be:

1. Erosion of the riverbank and knowing how best to repair existing damage or mitigate further damage.
    a. A common complaint has been large washouts associated with toppling of large, old trees. Such trees are required to be maintained on the riverbank for bank stabilisation, but when these trees fall as a result of flood water it destabilises large areas of the riverbank. Many growers are of the opinion that small trees or grass would be preferable for stabilisation. Expert investigation of this issue is required.
2. Lost road infrastructure (e.g. Old Gayndah-Mundubbera Rd).
    a. Portions of this road are unsealed and very low lying. The road was also severely damaged in the 2010 flooding. A longer term solution would be beneficial.
3. Access to tradesman; mainly electricians and plumbers.
    a. Only 2-3 or three electricians reside in the Central Burnett, requiring tradesman to be sourced from other areas. A major need is electricians to repair starters for irrigation motors.

Financial assistance issues

Government assistance through the Commonwealth/State Government funded Natural Disaster Relief and Recovery Arrangements became available to orchards in the region at Category C, then Category D levels. Whilst gratefully received by orchardists, a number of issues were raised by orchardists:

1. The labour time of existing orchard staff diverted to flood clean up and repair duties was not a claimable cost for disaster relief.

2. Businesses that have multiple orchard locations, but operating under a single ABN, are only being eligible for a single relief grant.
3. Financial relief strategies based on increasing industry productivity may be preferable to existing one-off financial grants, or very low interest loans that are only accessible to businesses unable to seek financial assistance from commercial lenders. Suggested alternatives have included loans at interest rates ~2-3 percent lower than commercial lender rates, or tax reductions for businesses in disaster affected regions. These approaches would assist businesses to operate with better financial security for a longer period of time, whereas existing relief options may only partially subsidise recovery costs, or commit severely impacted operations to further debt. Another point raised was that under disaster circumstances it can be very difficult for businesses facing closure to exit the industry. Providing longer term financial relief strategies may assist to create exit strategies for marginal businesses, by providing an incentive for more secure businesses to purchase their orchard.

Managing citrus orchards affected by wet weather

To assist growers with orchards affected by the wet weather/flooding in December 2010, Malcolm Smith, Andrew Miles and Garry Fullelove prepared a brief factsheet for circulation to orchardists. The factsheet was updated for the January 2013 floods and is available online at:

http://www.business.qld.gov.au/__data/assets/pdf_file/0015/5343/managing-citrus-orchards-weather.pdf

2.5 Project outputs

The productivity component of this project proposed to deliver a number of project outputs for industry. The proposed outputs and how these have been individually addressed is provided:

1. Written assessment of the potential benefits in EBS control using the simulated 'Alter-Rater' system under Australian climatic conditions, including recommendations for a future project should the simulated outcomes look promising.

The simulated 'Alter-Rater' assessment was removed from the project activities in 2009 (milestone variation request December 2009) in order to allow resources to be used for higher priority activities such as the completion of HAL project CT03005 and an Asian Markets for Horticulture Initiative project following the resignation of the projects' leader. The other high priority activity to arise was the technical evaluation of import risk analyses.

2. Ad hoc pathology input into rind breakdown problems where a pathogen may be involved.

The major rind breakdown issue to arise during project CT07012 was the anthracnose outbreak in 2008, which led to the pursuit of an emergency use permit for prochloraz.

3. Co-supervision of a PhD student to work on a major disease of *Citrus* produced in Australia's south.

Funding to support a PhD student in citrus pathology could not be obtained during project CT07012.

4. *In vitro* efficacy data for five Syngenta fungicides against Guignardia citricarpa.

In vitro efficacy data was generated for the four fungicides provided by Syngenta. As one less fungicide was provided to the project team, the number of *P. citricarpa* isolates to be used was increased from one to three. The number of rates tested was also increased from six to eleven.

5. Other outputs

More generally, the aim of the pathology activities relating to productivity has been to reduce the negative impacts of citrus pathogens on orchard productivity. There have been a number of pathogens impacting on productivity during this project, and associated activities aiming to reduce their impact. The pathogens and the relevant project outputs are:

Anthracnose (*Colletotrichum gloeosporioides*)

- Technical input into an emergency use permit for the postharvest fungicide prochloraz.

CBS (*Phyllosticta citricarpa*)

- Technical input into an emergency use permit for the fungicide azoxystrobin.
- Two seasons of efficacy data generated for three novel Bayer fungicides.
- One season of efficacy data generated for three promising succinate-dehydrogenase inhibitor (SDHI) fungicides, and 1 promising multi-site activity fungicide.

EBS (*Alternaria alternata*)

- Technical input into an emergency use permit for the fungicides azoxystrobin and iprodione.
- Two seasons of efficacy data generated for iprodione.
- One season of efficacy data generated for three promising succinate-dehydrogenase inhibitor (SDHI) fungicides, and 1 promising multi-site activity fungicide.
- One glasshouse trial showing efficacy of additional SDHI and multi-site fungicides.

Through project CT07012 it has also been possible for the industry to access citrus pathology expertise and assistance following the floods in 2010 and 2013. The main pathology issues arising from the floods and high rainfall have been phytophthora diseases, CBS and EBS. The project team and collaborators contributed to the production of a flood–specific factsheet for growers, made available through Citrus Australia Limited. Access to fungicides, as covered above, was also a major contribution of expertise from the project team during the flood assistance.

2.6 Conclusions and future directions

During this project the major pathological limitations to productivity have been from the fungal diseases EBS and CBS. The floods in 2010 and 2013 were also extremely significant in their own right, as well as increasing the impact of fungal diseases on citrus productivity. A major activity of project CT07012 has therefore been to increase access to fungicides through contributions to emergency use permits for azoxystrobin and iprodione to provide short term access to additional fungicides. The progression from emergency use permit to full registration is looking positive for azoxystrobin, with the APVMA releasing a trade advice for its full registration in January 2013. In addition, the necessary residue and efficacy data for iprodione has been produced under collaborating projects and CT07012. However, azoxystrobin and iprodione are both at risk of resistance development. Based on the results of efficacy trials conducted under CT07012, the most promising new fungicides for managing this resistance risk are the multi-site fungicide captan, and succinate dehydrogenase inhibitor (SDHI) fungicides such as boscalid. The Bayer fungicides DC-104 and DC-105 may also be promising for resistance management, but their resistance activity groups are confidential. In order to deliver registrations of these fungicides additional seasons of efficacy data are required. Therefore, a new project proposal CT13020 *Increasing market access, profitability and sustainability through integrated approaches to fungal disease control* was submitted to HAL in November 2012. A major component of this proposal is the ongoing development of new fungicides for citrus disease control.

Identifying fungicides with efficacy against diseases such as CBS and EBS is the first step in registering new fungicides for citrus disease control. However, it is important to also determine the best use pattern for the new fungicides. A critical, yet often overlooked, aspect of developing a use pattern is knowing for how long an application protects fruit against infection. Therefore, the CT13020 proposal includes experiments to determine the duration of efficacy of the various fungicides.

The cost of new fungicides will also be an important consideration in their adoption by citrus growers to control disease. The existing fungicides registered for use in *Citrus* are older, very low cost products. It is highly unlikely that any new fungicides will be as cheap. For example, a single application of mancozeb to one hectare of *Citrus* at 10,000 L/ha costs approximately $110, whereas an equivalent application of the SDHI fungicide boscalid may cost as much as $780. This difference in price equates to approximately $1M per application across the 1600 ha of *Citrus* susceptible to EBS. It will therefore be necessary to determine ways to reduce the costs of new fungicides. For example, the synergistic effect between captan and sporekill (see section above) has the potential to save the Queensland industry ~$100,000 per application (Table 2.3.9).

SDHI fungicide

Recommendation: Pursue registration of a single SDHI fungicide, primarily for the management of EBS. The candidate fungicide should ideally be the most efficacious, have support from the manufacturer for registration, and/or potentially be the most cost effective for grower use.

Action: A new project proposal has been prepared and submitted to HAL in November 2012 to allow continued collection of efficacy data.

Captan

Recommendation: Continue collection of efficacy data for captan against EBS only. Reduced rates of captan in combination with Sporekill should also be investigated if resources are available. Captan has the potential to be a low-cost substitute for mancozeb applications required later in the season, when residue issues are likely.

Action: A new project proposal has been prepared and submitted to HAL in November 2012 to allow continue collection of efficacy data.

Bayer products DC-104 and DC-105

Recommendation: Continue to determine the use pattern and resistance management implications for these Bayer fungicides. While both fungicides look promising for the management of EBS, only DC-105 appears to be useful for CBS management.

Action: A new project proposal has been prepared and submitted to HAL in November 2012 to allow continue collection of efficacy data.

Chapter 3

Market Access and Biosecurity

3.1 Introduction

The objectives of the activities outlined in this chapter are aimed to **develop a national approach to surveillance and research to underpin market access and provide pathology input into policy documents e.g. import risk analyses, incursion management plans, target pest lists etc**. The areas of market access and biosecurity are priorities for the citrus industry. These two subjects are intrinsically linked, as robust biosecurity is needed to ensure introductions of pest and/or diseases do not occur and do not result in loss of market access. Market access and biosecurity are also issues to which plant pathology is very relevant. For example, *Phyllosticta citricarpa*, the cause of citrus black spot (CBS), is a quarantine pathogen preventing export market access to the USA, EU and NZ. While the bacteria *Candidatus* Liberibacter spp. causing huanglongbing (HLB), and *Xanthomonas citri* subsp. *citri* causing citrus canker are both major biosecurity threats to citrus production in Australia. The CT07012 project team and collaborators have therefore undertaken a number of activities aiming to overcome pathogen barriers to market access, and to increase the biosecurity of the citrus industry in Australia.

Biosecurity activities have included:

- preparation of a review of surveillance for exotic citrus diseases;
- contributions to various awareness and training exercises and materials;
- disease specific activities for huanglongbing, citrus canker and citrus scab.

Specific market access activities have included:

- peer-review of import risk analysis for fruit imports from Japan;
- identification of research priorities for fruit imports from Korea;
- technical input into gaining market access for fruit from CBS endemic regions of Australia.

Undertaking these various activities is necessary to ensure the viability and longevity of the Australian citrus industry. Maintaining and improving the phytosanitary status of the Australian citrus industry is one of the few competitive edges for the industry in the midst of a high Australian dollar and high labour costs. Therefore, the activities of CT07012 have been needed, and similar activities continued to be needed into the future.

3.2 Review of surveillance for exotic citrus diseases

In order to develop a national approach to surveillance for exotic citrus diseases in Australia, it was necessary to firstly review existing activities and consider different approaches to surveillance based on the findings of the review. Such a review was conducted by project CT07012, and recommendations made for improving the surveillance effort for exotic citrus diseases. The review document was presented to Citrus Australia Limited in 2011. The report summary**,** recommendations, and project

activities for the delivery of action items during project CT07012 are provided in this report.

Excerpt from: Miles AK (2011) 'Review of surveillance for exotic citrus disease threats to Australia ' The University of Queensland and AgriScience Queensland - a part of the Department of Employment, Economic Development and Innovation Brisbane, Australia.

The full report has been provided to HAL and is available upon request.

Summary

The Australian citrus industry is under continuous threat from the introduction of exotic pests and diseases. There are many ways in which the risk of introduction and establishment may be minimised, but an efficient approach to safeguarding the industry is essential. It is important that existing biosecurity activities are identified, and new activities sought to address any shortfalls. As plant pathologists have a role to play in providing the science to underpin plant-related biosecurity activities, it is important that their contribution be maximised to strengthen this role. Out of the overall biosecurity efforts in Australia, this report is only focussed on the current surveillance activities in Australia relevant to citrus production, identifying potential gaps in surveillance, and providing recommendations to the Australian citrus industry and citrus pathologists on how to improve current biosecurity surveillance arrangements for citrus producers.

From the findings of this report the following key recommendations and action items are made to the Australian citrus industry:

1. Existing biosecurity surveillance could be bolstered by the Australian citrus industry directly investing in exotic pest and disease surveys in commercial citrus. However, this should only be considered if the dedicated resources are enough to significantly improve the likelihood of detection.

Action: Industry determines their desired surveillance outcomes and engages research providers and regulatory bodies to quantify the resources needed to implement the required surveillance.

2. Develop and support projects that will increase the likelihood of incidental detection of exotic pests and diseases by training existing personnel such as pest scouts, industry development officers, horticulturalists, growers, nurserymen and the general public.

Action: Industry encourage the Office of the Chief Plant Protection Officer (OCCPO) to undertake citrus biosecurity training for surveillance staff, pest scouts and consultants (etc.), similar to that undertaken by Biosecurity Queensland in 2010. OCCPO has expressed interest in supporting this in the recent past.

3. Support the identification of key hazard sites and their optimal timing for surveillance.

Action: Citrus industry to engage with biosecurity agencies to identify key hazard sites (for example sharing plantings database information) and ensure surveillance is carried out by these agencies.

4. Continue to invest in the expansion and accuracy of the National Citrus Plantings Database, and utilise the data for surveillance purposes.

Action: Industry permits access, with appropriate privacy stipulations, to the National Citrus Plantings Database data for surveillance purposes.

5. Ensure proposed surveillance methodologies are based on sound epidemiological, biometry and logistic principals.

Action: If Industry is to invest in a surveillance program, the methods should be developed/reviewed by a multidisciplinary team with expertise in epidemiology, biometry, and surveillance logistics.

6. Invest in the maintenance and capture of expertise relevant to citrus production, which will also underpin biosecurity activities.

Action: Industry identifies, through the new Citrus Industry Strategic Plan for example, its future weaknesses in expertise relevant to citrus production (e.g. horticulture, entomology, breeding etc.). This same expertise is required to underpin production and biosecurity.

7. Invest in initiatives that provide training opportunities for industry personnel (e.g. pest scouts, growers, nurserymen, researchers etc.).

Action: Industry can support training to all levels of industry personnel; travel grants for growers (e.g. USA HLB study tour), to PhD scholarships for university graduates. Investments in training are likely to enhance the awareness of the relevance and importance of safeguarding the citrus industry from the incursion of exotic pests and diseases.

8. Endorse the importation of positive control specimens for use in diagnostic laboratories in Australia.

Action: Industry responds decisively and swiftly on cases where the importation of a positive control specimen is required to ensure diagnostic laboratories can determine quickly and accurately the causal agent of disease symptoms believed to be caused by exotic pests or diseases.

9. Support the collection and storage of diagnostic surveillance information in a format compatible with the emerging national database system.

Action: Industry supports disease survey initiatives to determine the distribution of pests and diseases, and endorses storage and access of data by professionals within the state and federal agencies.

Delivery of action items during project CT07012

During project CT07012 contributions have been made to the delivery of several of the action items listed above:

Recommendation 2, Action: Industry encourage the Office of the Chief Plant Protection Officer (OCCPO) to undertake citrus biosecurity training for surveillance staff, pest scouts and consultants (etc.), similar to that undertaken by Biosecurity Queensland in 2010. OCCPO has expressed interest in supporting this in the recent past.

The major way CT07012 contributed to this action item was through the participation and joint funding of the biosecurity awareness and training program delivered in Queensland. Full details of this activity can be found in section 3.3 below.

Recommendation 6, Action: Industry identifies, through the new Citrus Industry Strategic Plan for example, its future weaknesses in expertise relevant to citrus production (e.g. horticulture, entomology, breeding etc.). This same expertise is required to underpin production and biosecurity.

Project CT07012 is itself an example of industry investing in the capture and development of expertise relevant to biosecurity, as well as citrus production in general. As government agencies continue to disinvest in expertise, projects such as CT07012 will become critical for industry to maintain access to expertise.

Recommendation 9, Action: Industry responds decisively and swiftly on cases where the importation of a positive control specimen is required to ensure diagnostic laboratories can determine quickly and accurately the causal agent of disease symptoms believed to be caused by exotic pests or diseases.

During CT07012 it was necessary to seek permits for importation from overseas and use of living specimens of *Elsinoë* spp. for use in the studies detailed in section 3.7. In this case Citrus Australia Limited supported the importation of the specimens in order to facilitate the required research.

3.3 Biosecurity training and awareness

A major conclusion of the 'Review of surveillance for exotic citrus disease threats to Australia' (above) was that training of industry personnel is a highly cost-effective means of improving the probability of early detection and successful eradication. Therefore activities to address this conclusion within project CT07012 were undertaken. These activities have included co-organising and co-funding a major training and awareness program with Ceri Pearce and Biosecurity Queensland.

CT07012 has also provided input into various awareness materials, and also co-authored and presented an exotic disease awareness seminar targeted to researchers.

Engaging industry personnel in biosecurity surveillance: Biosecurity Queensland's exotic citrus pest training

In order to greatly improve the probability of detecting major biosecurity threats to the citrus industry as soon as possible, an exotic pest and disease training initiative was undertaken by Ceri Pearce and Andrew Miles. The training package in its delivered form initially came about through the independent actions of Andrew Miles and Ceri Pearce. Through project CT07012, Andrew Miles had been undertaking a review of surveillance activities for exotic pests and diseases of *Citrus* (above). Through undertaking the review it became clear that the pest scouts employed by citrus growers in Queensland could make a very significant contribution to surveillance. This is largely due to the fact that the network of scouts monitors endemic pests in nearly all commercial orchards in Queensland on a monthly basis. If given the necessary training, these pest scouts would likely be the first to discover an exotic pest or disease outbreak occurring in a commercial orchard in Queensland. To provide the necessary training, Andrew Miles was organising for expert Andrew Beattie (University of Western Sydney) to deliver a seminar to pest scouts in the Central Burnett. At the same time, Ceri Pearce (Biosecurity Queensland) was organising a training series on exotic pests and diseases of *Citrus* for Biosecurity Queensland surveillance staff. Collaboration between Ceri Pearce and Andrew Miles was logical, and their combined efforts resulted in the expansion of the two training exercises into a single, very comprehensive initiative which brought industry pest scouts and Biosecurity Queensland staff together for training.

Full details of the training package are contained in a full report, available from Ceri Pearce (ceri.pearce@daff.qld.gov.au) on request:

Pearce CA (2010) 'Evaluation of Biosecurity Queensland's Exotic Citrus Pest Surveillance Training.' Department of Employment, Economic Development and Innovation, Cairns, Queensland.

In summary, training sessions were conducted in Mareeba, Mundubbera and Brisbane, with 18, 19, and 8 participants at each workshop, respectively. The training package comprised a booklet containing:

- Introduction to the workshop, course facilitators and evaluation forms
- Training program outline
- A series of fact sheets (also available online: http://www.daff.qld.gov.au/4790_6460.htm) including:
    - Asiatic citrus psyllid
    - African citrus psyllid
    - Huanglongbing
    - Conditions that can be confused with huanglongbing
    - Citrus fruit borer
    - Navel orangeworm
    - Citrus canker
    - Sweet orange scab
    - Mal secco
    - Citrus tristeza virus (mandarin stem pitting strains)
    - Citrus powdery mildew

- Good orchard hygiene
- Reporting an Emergency Plant Pest: a guide for industry pest scouts

Additional information distributed with the training package included:

- A list of additional reference material (books, internet) for further reading
- Orchard Biosecurity Manual for the Citrus Industry (by Plant Health Australia and Citrus Australia Limited)
- Asiatic citrus psyllid bookmark (by ACG, UWS, NSW Primary Industries and HAL)
- Brochure: Vital information for plant producers (DAFF)
- Brochure: Vital information for travelling farm workers (DAFF)

The training was supported by a series of PowerPoint presentations which were delivered to underpin the fact sheets listed above, with the addition of a presentation entitled 'Psyllid Surveillance' that specifically outlined surveillance methodologies that can be used to assist psyllid detection.

To derive value to industry beyond the training sessions themselves, the full set of fact sheets has been provided by Ceri Pearce to Citrus Australia Limited for hosting on their website. To promote the training activity being undertaking in other states, and the training concept to other crops, details of the training have been presented at various national forums:

Pearce CA, Miles AK (2011) Engaging industry personnel in biosecurity surveillance: Biosecurity Queensland's exotic citrus pest training. In 'ACPP APPS Darwin 2011, New Frontiers in Plant Pathology for Asia and Oceania'. Darwin Convention Centre, Darwin, NT p. 139.

Pearce CA, Miles AK (2011) Engaging industry personnel in biosecurity surveillance: Biosecurity Queensland's exotic citrus pest training. In 'Citrus Australia National Conference'. Wolf Blass Visitor Centre, Barossa Valley, South Australia.

Biosecurity awareness/extension material

Throughout the project, the project team and collaborators have contributed expertise and images for various biosecurity factsheets and awareness materials. Specific examples include images for the 'Orchard biosecurity manual for the citrus industry' produced by Plant Health Australia in 2009, and factsheets on citrus canker, powdery mildew and huanglongbing produced by Ceri Pearce of Biosecurity Queensland. Images captured by Andrew Miles during the 2004 outbreak of citrus canker in Emerald have also been extensively used in printed materials produced by the National Citrus Canker Eradication Program.

Key exotic disease threats to the Australian citrus industry

Promoting awareness of the exotic citrus disease threats is critical to improving the likelihood that any incursions are detected as quickly as possible. The activities above have focused on providing awareness to pest scouts, consultants, biosecurity staff and growers. However, increasing the awareness of other horticultural professionals is also of value; particularly those professionals not specialising in *Citrus*. To address

this need citrus pathologists Andrew Miles and Nerida Donovan (NSW DPI) prepared a presentation for delivery to researchers. The presentation was delivered at:

Miles AK, Donovan N (2007) Key exotic disease threats to the Australian citrus industry. In 'Australasian Plant Pathology Society Seminar Series'. (Indooroopilly Research Centre, Brisbane. 30th October).

Miles AK, Donovan N (2011) Key exotic disease threats to the Australian citrus industry. In 'Staff Seminar'. (Berrimah Research Farm, North Territory. 16th September).

3.4 Import risk analyses

To ensure the Australian citrus industry is not subject to increased risk of introduction of exotic pests and diseases as a result of fresh fruit imports, it is essential that market access decisions for imports are made with the highest level of scientific rigour possible. This project has aimed to elevate the levels of rigour applied to specific import risk analyses (IRA) by undertaking thorough reviews of draft IRAs when they are made available for public comment. The project team has then provided the authors of the IRAs and industry with detailed written feedback to provide the opportunity to increase the rigour of the documents. The process adopted in this project aims to replicate the peer-review process applied by scientific journals to ensure rigour in published manuscripts.

Fresh fruit from Japan

In 2008, Biosecurity Australia (now Department of Agriculture, Fisheries and Forestry) released the draft IRA for the importation of fresh *Citrus* fruit from Japan. The primary phytosanitary risk associated with the IRA was citrus canker. Given the serious impact citrus canker could have, and has had, in Australia it was necessary to ensure that the IRA was undertaken with the highest level of rigour possible, to ensure the Australian citrus industry is not subject to increased risk of importation of the disease. Project CT07012 reviewed the IRA and submitted its findings through the public comment process. The changes made to the IRA following public comment were assessed and outstanding issues raised through an appeal to the Secretariat, Import Risk Analysis Appeals Panel. The appeal document prepared by the Tree Pathology Centre outlines both the original review findings and the subsequent changes made to the IRA.

The full report has been provided to HAL and is available upon request.

Fresh fruit from Korea

Following market access to Australia being granted to fresh fruit from Japan under a systems approach for mitigating the risk of citrus canker, Korea also proposed a systems approach to attain market access to Australia. Given the necessity to thoroughly mitigate the risk of introducing citrus canker to Australia, Korea indicated interest in working with Australian researchers to address gaps in existing research. Citrus Australia Limited nominated Pat Barkley and Andrew Miles as a research team to work with Korea. Pat Barkley and Andrew Miles prepared a list of research

questions to address the current shortfalls in the literature. These questions are listed below. The eventual outcome after bilateral talks between Korea and Australia for Australian market access was that Korea de-prioritised the issue in favour for more pressing issues for Korea.

Research Questions for Korea

We understand that considerable research has been conducted in South Korea on citrus canker, but some of it is not easily accessible to us. So we would appreciate any information that can be provided by way of answers to the questions below, if possible substantiated by published papers and internal reports. In this way Australia could be provided with Korean data to substantiate the systems approach Korea is proposing.
The questions below are listed under 5 major headings, representing the key points in the import risk pathway:

1. Risk that harvested fruit are infected?
Suitability of host for infection:

- What clones of Unshiu will be exported and what is their susceptibility to canker (fruit and leaves)?
- Unshiu mandarin is often referred to as being "resistant" to Xcc. What is the nature of the resistance to Xcc of the Unshui clones to be exported? For example:

  *Do Unshiu mandarin fruits show classic canker lesions, but less of them?

  *Are lesions of reduced size or different appearance to those on susceptible hosts?

  *Are infections asymptomatic, but the pathogen still survives/multiplies?

  *Are infections asymptomatic, but the pathogen cannot survive/multiply?

  *How do these various factors change with different clones, e.g. early versus late maturing?
- When are fruits and leaves most susceptible in Korea? Does infection of mature fruits occur? If so, do typical canker symptoms develop or are symptoms "non-erumpent or pin point greenish spots" as described by Koizumi (1972)?
- Have Korean scientists studied the role of leafminer in canker infections?

Suitability of environmental conditions for infection:

- Have Korean scientists studied the role of climatic conditions, such as cyclonic winds in Xcc infection?
- How do climatic conditions and leafminer interact in canker infection?
- Have Korean scientists studied seasonal changes in Xcc populations in orchards in Jeju?
- When does canker infection occur? What months? When is leafminer problematic?

- Have dispersal distances with wind-driven rain been studied on Jeju Island?

Impact of production system:

- Have studies been conducted to determine the typical incidence of Unshiu mandarin fruit infected with Xcc at harvest (symptomatic and asymptomatic and associated with injuries) on Jeju Island? How do standard orchard practices compare with any proposed systems approaches?
- Can epiphytic populations of Xcc be detected at harvest? How rapidly do they decline after harvest?
- Have spray trials been conducted to determine the % reduction in disease which can be achieved?
- What spray programme is used for canker control? Is streptomycin used? Does each orchard keep individual spray records?
- Has any canker disease forecasting been done on Jeju Island to predict canker infection periods and hence advise growers on when to spray?
- Are there mixed varieties in the orchards (in particular, those other than Unshui)?
- Will Unshiu fruit be from orchard trees or grown in polyhouses?
- What other advice is given to growers regarding canker and leafminer controls?
- What orchard inspections occur for citrus canker? Are infected trees removed immediately?
- Are picking boxes treated with disinfectant before return to an orchard?
- Has Xcc resistance to copper been detected in South Korea?

2. Risk that pathogen survives packing and post harvest treatment?

- Have any studies been conducted on irradiation, or other postharvest disinfestation technologies, as a phytosanitary treatment for citrus fruit with citrus canker? An effective postharvest disinfestation strategy would be highly desirable in a systems approach.
- Have any studies been conducted in Korea on the effects of chlorine dips and SOPP on recovery of Xcc from citrus fruit?
- Are fruit washed prior to dipping in bleach or SOPP?
- Do authorities inspect fruit at packing? Is there a sampling rate for canker detection? If canker is found, what happens?
- Have any machine vision systems been used in South Korea for blemish detection?
- What training is given to pickers and packing shed staff to cull fruit with lesions?

- How are culled fruit disposed?

3. Risk that pathogen survives shipment?

- Has Korea undertaken any studies on the effect of shipping conditions (e.g. cold treatment) on Xcc survival?
- What strategies will Korea implement to ensure infectious leaves or other citrus residues are not included in shipments?

4. Risk that fruit are moved to suitable habitat in Australia?

- What months will export occur from (a) field trees and (b) polyhouses?
- Does Korea intend to export fruit to particular markets/states/cities in Australia?

5. Pest find suitable host and establish or incite disease?

- Has any 'fruit to tree' transmission of canker been observed or studied in S. Korea either (a) within tree or (b) from fallen infected fruit to tree?

3.5 Huanglongbing (citrus greening)

Huanglongbing (HLB) or "citrus greening" is a devastating disease of *Citrus* caused by non-culturable bacteria of the genus *Candidatus* Liberibacter spp. (Garnier *et al.*, 2000). The presence of the bacteria in the phloem of *Citrus* trees results in disruption of nutrient movement within the plant, resulting in a range of nutrient deficiency symptoms, yield loss and eventually tree death (Garnier and Bove, 2000). The seriousness of the disease is illustrated by the estimated US$3.6 billion in lost juice processing revenue over 4 years due to the presence of HLB in Florida (Hodges and Spreen, 2012). Given the impact HLB has on commercial citrus production it is considered the most important exotic disease threat to the Australian citrus industry. Therefore project CT07012 has contributed to HLB-related activities where possible. HLB itself is not a research area within CT07012, but it is crucial for the CT07012 project team and collaborators to accumulate and share knowledge in this area. The following section outlines details of the CT07012 activities in this area.

HLB-ACP Taskforce

In 2009, the huanglongbing-Asian citrus psyllid (HLB-ACP) Taskforce was established to coordinate Australian HLB-related efforts. The task force included representatives of the various state and commonwealth agencies. These representatives included CT07012 team member Andrew Miles (DAFF Qld) and collaborators including Pat Barkley (CAL), Richard Davis (DAFF), Jo Luck (Vic DPI), Nerida Donovan (NSW DPI), Andrew Beattie and Paul Holford (UWS). The inaugural teleconference established the terms of reference of the task force as:

1. Independently analyse the validity of the post-incursion pre-endemic delimiting survey methodology suggested in the Pest Specific Contingency Plan for HLB and its vectors.
2. Provide advice on how to establish protocols for testing seed, budwood source trees and *Citrus* nursery plants for HLB, and protocols for destroying those testing positive.

3. Identify risks, maintain currency of information including emerging technology, report on emerging characteristics of both HLB and ACP and track its global distribution.
4. Advise on maintenance of diagnostic capabilities.
5. Advise on regional trends relating to numbers of abandoned orchards.
6. Advise on the development and coordination of the production and dissemination of resource material on HLB and its vectors between the states, OCPPO, PHA and industry.
7. Provide findings in report form to PHC twice a year.

During project CT07012 HLB-ACP Taskforce teleconferences were held three times (30th Sept 2009, 23 March 2010, 30 July 2010). Andrew Miles and other project collaborators participated in these teleconferences and were assigned and completed various actions items. In the case of Andrew Miles the following action items were addressed:

Teleconference 1:

- Andrew Miles to follow up decisions made at the ACIAR meeting to be held on 3 November on the proposed project to partly-fund salary for officer to work on HLB-ACP using a Qld DPI budget surplus.
    - An update on the ACIAR meeting and Qld DPI investment in HLB was provided to Fiona Macbeth by Andrew Miles on the 21st November 2009.
- Andrew Miles to look into adding Pat Barkley's email address to distribution group for journals.
    - Andrew Miles arranged for journal alerts to be sent to Pat Barkley, with this being maintained indefinitely.

Teleconference 2:

- Andrew Miles to send details from study on benefits and costs of surveillance for circulation to Taskforce.
    - See appended report from section 3.2 above.

HLB incursion management plan workshop

In February 2009, Andrew Miles attended the HLB incursion management plan workshop in Melbourne arranged by the Office of the Chief Plant Protection Officer. The workshop provided opportunity for an analysis of the practical implementation of the draft plan. A key recommendation of the workshop was to develop a more basic working plan, underpinned by the extensive review contained in the draft plan. The project team is aware of two working plans being developed. One is underway for the nursery industry and Andrew Miles has reviewed a draft. The second is to be produced by PHA under a project proposal submitted to HAL in November 2012.

Investigating the potential of in-field starch accumulation tests for targeted citrus pathogen surveillance in Australia

At the incursion management plan workshop outlined above, a question that was raised was would in-field tests for starch accumulation be useful for HLB surveillance in Australia. In order to address this question, Andrew Miles led a collaborative field trip and laboratory experiment as part of the 2009 citrus pathology workshop.

Collaborators in the exercise included Nerida Donovan (NSW DPI), Paul Holford (UWS), Richard Davis (NAQS), Kathy Grice, Malcolm Smith (QPI&F), and Andre Drenth. A joint poster describing the work and findings was prepared for presentation at the Australian Plant Pathology Society Conference in October 2009 (Fig. 3.5.1). What the work demonstrated was that starch accumulation can result from many factors other than HLB. Therefore its usefulness in pre-incursion surveillance may be limited. However, post-incursion the test may prove more useful.

Miles AK, Donovan N, Holford P, Davis R, Grice K, Smith M, Drenth A (2009) Investigating the potential of in-field starch accumulation tests for targeted citrus pathogen surveillance in Australia. In '17th Australasian Plant Pathology Conference, Plant Health Management'. Newcastle, NSW p. 180.

ACIAR HLB: Research and Development Priorities

In November 2009 the Australian Centre for International Agricultural Research (ACIAR) held a meeting of various citrus/HLB experts to identify HLB research priorities that could be met through the ACIAR program. Andrew Miles was in attendance and co-authored and co-presented two presentations:

Persley D, Young A, Miles AK (2009) Past, present, and potential future of HLB research based in Queensland In 'ACIAR HLB research prioritisation meeting'. Sydney International Airport Hotel, 3rd November. (Australian Centre for International Agricultural Research).

Smith MW, Weinert MP, Miles AK (2009) HLB aspects of the Sikkim/Australia citrus project. In 'ACIAR HLB research prioritisation meeting'. Sydney International Airport Hotel, 3rd November. (Australian Centre for International Agricultural Research).

A full report of the prioritisation meeting was prepared by Brian Stynes:

Stynes B (2009) 'ACIAR. Huanglongbing: Research and Development Priorities. A workshop report (DRAFT).' Australian Centre for International Agricultural Research, Sydney International Airport Hotel, 3rd November.

The CT07012 project team is not aware of any call for projects having arisen from this meeting.

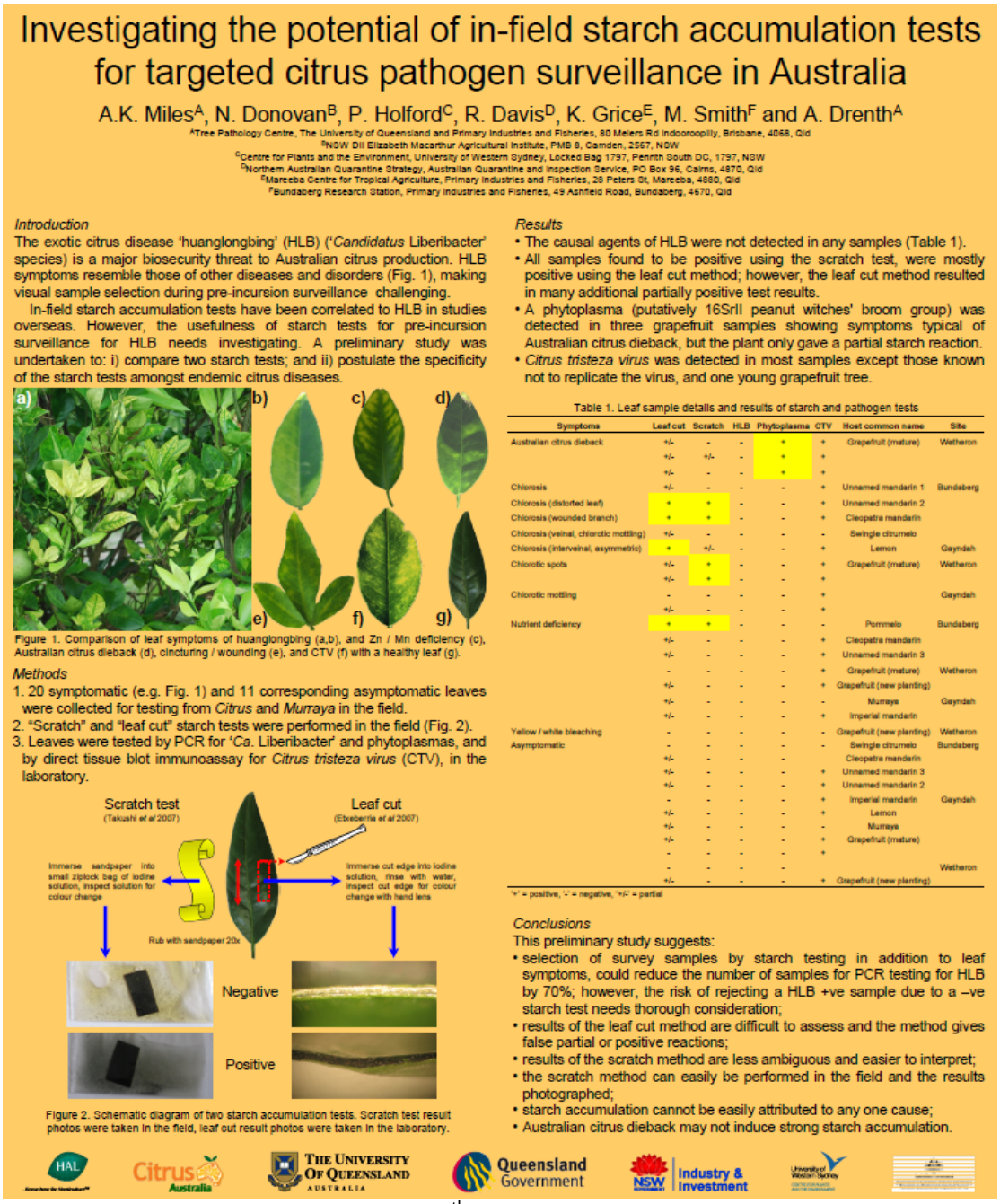


Figure 3.5.1 Poster presented at the 17th Australasian Plant Pathology Conference, Newcastle 2009.

HLB-ACP research by project collaborators

As previously noted in this report, HLB-ACP is not a direct research area of this project. However, it is important to acknowledge that HLB-ACP has been the focus of several CT07012 collaborators through projects primarily funded by the Australian Centre for International Agricultural Research (ACIAR). Much of the HLB-specific research undertaken is reflected through the journal publications listed in the 'Collaborator journal articles' section found later in this report.

3.6 Citrus canker

Citrus canker is a serious disease of *Citrus*. The most economically significant form of the disease is the Asiatic form caused by the bacterium *Xanthomonas citri* subsp. *citri* (Schubert *et al.*, 2001). Infection by the bacterium causes corky, raised pustules on leaves, fruit and stems of most *Citrus* sp. The presence of the disease in a production area causes direct impacts on production costs, as well significant export market access restrictions. Australia is currently free of the disease, but has undergone several eradication programs to eliminate outbreaks; the most recent being the eradication of citrus canker from Emerald, Qld. Citrus canker is not a specific research topic of project CT07012, however the importance of the disease requires the project team and collaborators to accumulate and share knowledge in this area. The following section outlines details of the CT07012 activities relating to citrus canker.

National Citrus Canker Eradication Program

In July 2004 citrus canker was detected in Emerald, Qld. As a result the National Citrus Canker Eradication Program was established to implement the eradication process. Intense surveillance was a key activity, with ~1800 diagnostic specimens collected and forwarded to the laboratory for analysis over the life of the NCCEP. Many of these diagnostic samples were triaged by Andrew Miles until the last diagnostic specimen was processed on the 4th December 2008 (Fig. 3.6.1). This specimen was the final negative result for the eradication program, before area freedom was declared in 2009.

Figure 3.6.1 Kathy Parmenter (back) and Andrew Miles (front) examining the final (negative) citrus canker surveillance specimen.

Citrus canker contingency plan gap analysis

A citrus canker contingency plan gap analysis meeting was conducted on the 12-13th June 2008 at the Indooroopilly Research Centre. Attendees included CT07012 project team member Andrew Miles, and representatives for several other agencies; Pat Barkley (CAL), Fiona Macbeth (OCPPO (Chair)), Bill Washington (DPI VIC), Cherie Gambley (QDPI&F), Grant Telford (NCCEP), Michael Benham (NCCEP), Mike Ashton (QDPI&F), Jo Slattery (PHA) and Sophie Peterson (PHA). A report on the meeting including recommended action items was provided to the Consultative Committee on Emergency Plant Pests. The fate of the recommendations is unclear, however, Grant Telford and Pat Barkley have prepared a book chapter for a soon to be published Citrus Biosecurity publication.

The distribution and spread of citrus canker in Emerald, Australia

In order to document the details of the outbreak of citrus canker in Emerald, Andrew Miles contributed to a journal publication by lead-author Cherie Gambley:

Gambley CF, Miles AK, Ramsden M, Doogan VJ, Thomas JE, Parmenter K, Whittle PJL (2009) The distribution and spread of citrus canker in Emerald, Australia. Australasian Plant Pathology 38, 547-557.

Abstract Citrus canker is a disease of *Citrus* and closely related species, caused by the bacterium *Xanthomonas citri* subsp. *citri*. This disease, previously exotic to Australia, was detected on a single farm [infested premise-1, (IP1). IP is the terminology used in official biosecurity protocols to describe a locality at which an exotic plant pest has been confirmed or is presumed to exist. IP are numbered sequentially as they are detected] in Emerald, Queensland in July 2004. During the following 10 months the disease was subsequently detected on two other farms (IP2 and IP3) within the same area and studies indicated the disease first occurred on IP1 and spread to IP2 and IP3. The oldest, naturally infected plant tissue observed on any of these farms indicated the disease was present on IP1 for several months before detection and established on IP2 and IP3 during the second quarter (i.e. autumn) 2004. Transect studies on some IP1 blocks showed disease incidences ranged between 52 and 100% (trees infected). This contrasted to very low disease incidence, less than 4% of trees within a block, on IP2 and IP3. The mechanisms proposed for disease spread within blocks include weather assisted dispersal of the bacterium (e.g. wind-driven rain) and movement of contaminated farm equipment, in particular by pivot irrigator towers via mechanical damage in combination with abundant water. Spread between blocks on IP2 was attributed to movement of contaminated farm equipment and/or people. Epidemiology results suggest: (i) successive surveillance rounds increase the likelihood of disease detection; (ii) surveillance sensitivity is affected by tree size; and (iii) individual destruction zones (for the purpose of eradication) could be determined using disease incidence and severity data rather than a predefined set area.

2010 phytosanitary survey of commercial citrus in Emerald, Qld

In December 2010, Andrew Miles and Dan Papacek (Bugs for Bugs) undertook a survey of commercial citrus in Emerald, Qld, for any symptoms or signs of citrus canker or other key exotic diseases such as HLB. This survey occurred approximately two years after the declaration of eradication of citrus canker from the area. Ten trees

across each of 18 irrigation bays in the area were inspected thoroughly. No symptoms or signs of citrus canker or any other exotic diseases were observed. This additional grower-initiated survey further supports the successful eradication of citrus canker from the Emerald region.

3.7 Citrus black spot (CBS)

CBS, caused by the fungus *Phyllosticta citricarpa* (synonym *Guignardia citricarpa*), is an important pathogen in terms of market access and biosecurity, as well as productivity. For example, gaining market access to the USA for fruit from regions where CBS is endemic requires methods for producing fruit free of the disease. The USA market for Murcott tangor from the Central Burnett production area alone has been estimated to be worth $67.5M pa. Therefore research aiming to overcome barriers to trade due to CBS has been a priority alongside research aiming to reduce negative impacts of CBS on productivity.

Completion of HAL CT00035 and Qld Government AMHI projects

Two CBS research projects were completed by Andrew Miles during project CT07012. These projects were HAL project CT03005 *Expanding citrus market access using a systems approach to control black spot* and the Queensland government funded Asian Markets for Horticulture Initiative (AMHI) project *Enhancing citrus black spot management to facilitate market access opportunities for Queensland citrus*. Both these projects were led by Pauline Wyatt (DPI&F) until Pauline relocated overseas. As a result, Andrew Miles took over the project leadership to complete the two projects. Final experiments and reporting were completed successfully, with the research findings forming the basis of an export submission to the USA for market access from CBS areas. The CT03005 final report is available from HAL, and the AMHI final report is available from DAFF Qld.

USDA Technical Working Group on *Guignardia citricarpa*

In March 2010 CBS symptoms were discovered near Immokalee, Florida, and subsequently confirmed as being caused by *Phyllosticta citricarpa* (Schubert *et al.*, 2012). In response the United States Department of Agriculture (USDA) established a Technical Working Group (TWG) on CBS, and invited Andrew Miles to participate along with other CBS researchers from around the world. The combined expertise within the TWG assisted the USDA to form the USDA plan for dealing with the outbreak. Given the latent nature of the disease, eradication was not attempted. Instead, the disease is being commercially controlled and its spread in Florida monitored.

USDA (2010) '*Guignardia citricarpa* (Citrus Black Spot, CBS) technical working group final report.' Animal and Plant Health Inspection Service, United States Department of Agriculture, Raleigh, USA. pp. 12.

Global *Phyllosticta citricarpa* population studies

Project CT07012 team members Andrew Miles and collaborator Nerida Donovan were invited in 2010 by Paul Fourie and PhD student Elma Carstens from Citrus Research International, South Africa, to contribute to a global population study of

Phyllosticta citricarpa. The study aimed to collect populations of the fungus from as many countries as possible, and then use molecular methods to determine the genetic diversity of the various populations. From the results of this study it will be possible to infer: i) the relative importance of pycnidiospores (clonal spores found within lesions) and ascospores (sexual spore produced from the leaf litter) in the different countries; ii) the likely evolutionary origin of *P. citricarpa*; and iii) the potential importance or otherwise of movement of individuals of *P. citricarpa* between the populations of *P. citricarpa* found around the world. In order to promote this material collaboration between South Africa and Australia, Andrew Miles and Nerida Donovan collected fruit with CBS symptoms from citrus orchards, then isolated *P. citricarpa* and forwarded the cultures under permit to South Africa for analysis. In total populations of ~40 isolates each were collected from Mundubbera, Gayndah and Gosford. At the time of writing the CT07012 final report the diversity study was not yet complete, but in June 2013 Elma Carstens visited Australia to work with Australian population genetics expert Celeste Linde (The Australian National University) on the data analysis.

Australian *Phyllosticta citricarpa* population studies

Phyllosticta spp. on cultivated *Citrus* in Australia

A. K. Miles[*1], Y.P. Tan[1], M.K. Tan[2], N.J. Donovan[2], A. Ghalayini[2], A. Drenth[3]

[1]Department of Agriculture, Fisheries and Forestry (Queensland), Ecosciences Precinct, 41 Boggo Road, Dutton Park, Qld 4102, Australia. [2]NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institute, Private Bag 4008, Narellan NSW 2567, Australia. [3]The University of Queensland, Centre for Plant Science, Ecosciences Precinct, 41 Boggo Road, Dutton Park, Qld 4102, Australia.

[*]Corresponding author; phone: +61 (07) 32554345, fax: +61 (07) 38444529, email: andrew.miles@daff.qld.gov.au

Miles AK, Tan YP, Tan MK, Donovan NJ, Ghalayini A, Drenth A (2013) *Phyllosticta* spp. on cultivated *Citrus* in Australia. Australasian Plant Pathology.

Key words: endophyte, taxonomy, black spot, disease

Abstract

The occurrence of pathogenic and endophytic species of *Phyllosticta* on cultivated *Citrus* in Australia was investigated by DNA sequence analysis of specimens held in plant pathology herbaria and culture collections. Sequences of the internal transcribed spacer region (ITS1, 5.8S, ITS2), and partial translation elongation factor 1-alpha (TEF) gene of 41 *Phyllosticta*-like isolates from *Citrus* were compared to those sequences from the type specimens of *Phyllosticta* recorded from around the world. Phylogenetic analysis resolved all the sequences of Australian accessions into two major clades. One clade corresponded to *P. citricarpa*, which causes citrus black spot (CBS) disease. The other clade contained *P. capitalensis*, which is a known endophyte of *Citrus* and many other plant species. No Australian isolates were identified as the

newly described pathogens of *Citrus P. citriasiana* or *P. citrichinaensis*, or the endophytes *Guignarida mangiferae*, *P. brazilianiae*, or *P. citribraziliensis*.

Domestic market access

In 2010 South Australia abruptly began the enforcement of changes to the South Australian domestic import requirements for *Citrus*, requiring *Citrus* to be inspected and found free of CBS. No risk assessment was provided to justify the change in ruling. The ruling may also be counter-productive to the market access negotiations about to commence with the USA for the export of *Citrus* from CBS regions in Australia. The sudden change resulted in Tahiti lime shipments being held in transit. Within a few days Andrew Miles, with assistance from Pat Barkley, Sandra Hardy, Nerida Donovan, Matt Weinert and Kathy Grice was able to provide the necessary information to have Tahiti limes listed as exempt from the new ruling. This was largely based on research conducted in South America which showed that Tahiti limes are "insensitive" to *P. citricarpa* and do not show symptoms of CBS (Baldassari *et al.*, 2008).

3.8 Project outputs

The Market Access and Biosecurity component of this project proposed to deliver a number of project outputs for industry. The proposed outputs and how these have been individually addressed is provided:

1. Adoption of a national approach to surveillance involving different organisations and stakeholders and the production of a joint surveillance report on a regular basis.

The adoption of a national approach to surveillance remains a challenge. The Review of surveillance for exotic citrus disease threats to Australia found this challenge stems from the wide range of agencies involved in surveillance, and also the limitations that exist in resourcing for a truly national approach to surveillance. What sections of the review therefore aimed to do was quantify the resources likely to be needed to achieve a spectrum of surveillance outcomes of interest to the industry, and then identify alternative and more cost effective approaches. The main conclusion from the review was that the most cost-effective approach to increasing surveillance is through training and awareness programs for industry people already deployed in the field; namely pest scouts and consultants. The Biosecurity training and awareness section of this report provides details of how CT07012 and its collaborators worked towards delivering this approach to surveillance. Wider delivery around Australia is needed, however.

Joint surveillance reporting during this project was delivered through the Technical field visits and diagnostics section of this report, and through the specific disease sections of this chapter of the report (huanglongbing, citrus canker, citrus scab). More specifically, the joint surveillance activities conducted during this project have included:

- Auscitrus budwood multiplication scheme inspection (Mildura/Dareton region);
- Investigating the potential of in-field starch accumulation tests for targeted citrus pathogen surveillance in Australia (Widebay and Burnett region);
- 2010 phytosanitary survey of commercial citrus in Emerald, Qld (Central Qld region);

- Citrus scab (see full report provided to HAL); and
- *Phyllosticta* spp. on cultivated *Citrus* in Australia (Qld, North Coast NSW, and Darwin).

2. Further training of growers and consultants in diagnosis of endemic and exotic diseases through workshops and the production of fact sheets.

The collaboration of project CT07012 with Ceri Pearce (Biosecurity Qld) to present Biosecurity training and awareness in Queensland was a major output of this project in this area. The Office of the Chief Plant Protection Officer (OCPPO) had expressed interest in supporting similar training exercises around Australia, with a willingness from the CT07012 team and collaborators to assist in the delivery of the training. The CT07012 project is not aware of any further developments in this area.

3. Development of a strong relationship between the industry Australia-wide and the plant pathologists.

Regular participation of the CT07012 project team and collaborators at industry events has unpinned the development of strong relationships between industry and the plant pathologists. For example, citrus pathology has been represented through poster and oral presentations at the 2009**,** 2010**,** 2011**,** and 2012 Citrus Australian National Conferences. Another important means for developing these relationships has been through the provision of technical field visits and diagnostics to individual growers. These visits give growers time to ask questions 'one on one' about citrus pathology that they may not be comfortable asking in a conference environment.

4. Development of strong and effective collaboration between Australian and overseas citrus pathologists.

The best examples of strong and effective collaborations between Australian and overseas citrus pathologists built during this project are those between Australia and Florida, and Australia and South Africa. Andrew Miles and Andre Drenth have worked with Megan Dewdney (University of Florida) to prepare a joint CBS research proposal that has been submitted to the Citrus Research and Development Foundation in the USA. Nerida Donovan and Andrew Miles have also worked with Paul Fourie and PhD student Elma Carstens (Citrus Research International) in South Africa on a global study of the genetic diversity of *Phyllosticta citricarpa*, the cause of CBS. The conferences and overseas study tours component of the project has also provided the opportunity to meet and network with research from many countries including the USA, Brazil, Japan, India, and China, just to list a few.

5. Other outputs

More generally, the aim of the pathology activities relating to market access and biosecurity has been to maintain, and where possible improve, the phytosanitary status of the Australian citrus industry. A large component of achieving this aim has been ensuring that market access and biosecurity decision making is held to a high level of scientific rigour. Major outputs for industry in this area have therefore been:

<u>Surveillance for exotic citrus diseases</u>

- The 'Review of surveillance for exotic citrus disease threats to Australia' made a number of key recommendations that if adopted, or continued to be adopted, will improve the biosecurity of Australian *Citrus*.

- Project CT07012 itself has contributed to actioning a number of the recommendations (see 'Delivery of action items during project CT07012'); in particular conducting a major biosecurity awareness and training exercise in Qld in collaboration with Biosecurity Queensland staff (see 'Biosecurity training and awareness') which provided training to 45 pest scouts and surveillance staff.
- It is the understanding of the CT07012 project team that Plant Health Australia has put forward a biosecurity proposal to HAL in 2012 which will further address a number of these recommendations.

Import risk analysis

- Scientific review of the draft risk analysis for the importation of fresh fruit from Japan (see 'Fresh fruit from Japan')
- Research advice regarding importation of fresh fruit from Korea (see 'Fresh fruit from Korea')

Throughout CT07012 there have been a number of project outputs that relate to *Citrus* pathogens of consequence to market access and biosecurity:

Huanglongbing (*Candidatus* Liberibacter spp.)

- Provision of HLB surveillance training to 45 pest scouts and surveillance staff.

Citrus canker (*Xanthomonas citri* subsp. *citri*)

- Post-eradication surveillance of *Citrus* in Emerald to further support the declaration of eradication.
- Provision of citrus canker surveillance training to 45 pest scouts and surveillance staff.

Citrus scab (*Elsinoë* spp.)

- Diagnosis of specimens collected during surveillance for exotic forms of citrus scab.
- Confirmation of past reports that the 'Lemon' and 'Tryon's' pathotypes of *E. fawcettii*, which mainly infect lemons, are the dominant pathotypes and species of this fungus in Australia. However, this work also identified novel genotypes of *E. fawcettii*, for which the pathotype is unknown.
- Provision of citrus scab surveillance training to 45 pest scouts and surveillance staff.

Citrus black spot (*Phyllosticta citricarpa*)

- Provision of CBS research findings to Biosecurity Australia in order to support a market access submission to the USA.
- Peer reviewed and published research confirming that the same fungus causes CBS in Australia and Florida, reducing the potential for market access restrictions.

3.9 Conclusions and future directions

During this project the issues having arisen under market access and biosecurity have been substantial and varied. These issues are dynamic and ongoing for the Australian

citrus industry, with limited certainty surrounding when matters needing pathology input would arise. It can be concluded with more certainty that access for the industry to expertise in this area will remain essential. Project CT07012 has contributed expertise for these issues, albeit for the finite life of the project.

Achieving a national approach to surveillance for exotic citrus pests and diseases is a logical aspirational goal for any horticultural industry in Australia. However, a conclusion that can be reached from the CT07012 activities is that this target for the citrus industry is unlikely to be met to achieve measurable increases in early detection without significant new financial investment from industry and/or government. The estimated cost of such a program was >$650k per round of surveillance (Miles, 2011a). Whilst this is a significant sum of money, for practical comparison, the Australian banana industry currently invests approximately $671,872 per annum in its Banana Bunchy Top virus eradication program, which involves six industry inspectors (not all full time) undertaking routine inspections of plantations in the northern NSW and southeast Qld region. If the citrus industry were to invest in a national surveillance program through existing funding streams such as the R&D levy through HAL, the program would utilise 50% or more of the existing annual R&D budget. Alternatively, this level of funding could be raised through an increase to the R&D levy of $0.50 per tonne, or as a standalone levy of $1.00 or more per tonne. In the absence of new investment, it has been concluded that training of existing industry personnel provides the most cost-effective approach to improving surveillance (Miles, 2011a). Biosecurity awareness training needs will presumably be met through a citrus biosecurity project which commenced in June 2013 (Mecham, 2013), however the specific details of how this will be provided are unknown to the CT07012 team.

It can also be concluded from CT07012 that availability of expertise is critical to market access and biosecurity. For example, the activity of this project requiring the highest level of pathology expertise was the response to the possible detection of the 'Sweet orange scab' pathotype of *E. australis*. What made this response particularly challenging was the inability to easily identify the fungus. Under these circumstances it is simply not possible to refer to existing literature to determine the implications of the finding. It also becomes very difficult for state and federal government agencies to determine their response when the potential impacts are unclear. However, this example does highlight the potential for complications should a similar situation arise for an extremely serious disease such as HLB; i.e. any deviation from the well defined *Ca.* Liberibacter spp. associated with HLB could cause very costly delays in biosecurity decision making. Similar levels of expertise are also required for the review of import risk analysis, such as that undertaken for *Citrus* fruit from Japan. The level of detail in this review was unlikely to be achieved without project CT07012, and similar arrangements are likely to be required to ensure detailed review of future import risk analyses. It is therefore critical that the expertise and infrastructure (such as quarantine containment facilities) is maintained/developed in Australia. However, a recent capability study of plant pathology and entomology expertise confirms that Australia's expertise is in decline, with a very strong shift towards the dominant age group being ≥55 y/o (Howie, 2012). A very cost-effective approach for industry to take the lead in developing expertise for itself, as well provide answers to relevant issues, would be through financial support for all levels of students; diploma to PhD. The South African citrus industry, for example, supports 55 students across a 58,000 ha industry (Harty, 2012). If the same rate of investment in education was adopted in Australia there would be approximately 25 students working on citrus in Australia.

The major threat to citrus production in Australia is the introduction of huanglongbing (HLB) disease. However, making measurable forward progress in protecting/preparing Australia from/for an incursion of the disease has proven notoriously difficult. For example, the HLB/ACP Taskforce, the HLB/ACP incursion management plan, and ACIAR research prioritising activities are defunct or remain unresolved as far as the project team are aware. The reasons for this are likely to include limited resourcing and availability of expertise, and reduced priority whilst Australia remains free of the disease. To maintain momentum the recently commissioned citrus biosecurity project will appoint a part-time biosecurity manager and include a HLB contingency plan as a project output (Mecham, 2013). Finalising of the incursion management plan for HLB will be a significant step forward, and make resources available to achieve progress in other areas such as awareness training and surveillance.

The CBS disease has been an impediment to expanding export market access for fruit from the areas where the disease occurs. This project has played an important part in providing research outcomes to Citrus Australia Limited and the Department of Agriculture, Fisheries and Forestry (DAFF) to keep market access submission to the USA a priority for bilateral discussions between DAFF and the USA. The most recent research outcome from project CT07012 was the collaborative paper *Phyllosticta* spp. on cultivated *Citrus* in Australia, which showed CBS in Australia and Florida to be caused by the same pathogen (Miles *et al.*, 2013). A significant development in this area has also been the recent pest risk analysis for CBS in fruit (USDA, 2010), and the proposed rule for the importation of *Citrus* fruit into the USA from Uruguay (USDA, 2013), which consider fresh fruit not to be an epidemiologically significant pathway for the movement of CBS. This outcome is expected to be positive for market access for Australian fruit from CBS areas.

The future direction for market access and biosecurity for the citrus industry will be determined largely by two recent developments. The first is the appointment of a full time Market Access Manager, David Daniels, to Citrus Australia Limited. The second is the commissioning of a citrus biosecurity project, led by Plant Health Australia, which will appoint a citrus biosecurity manager. The CT07012 project team is not aware of the specific plans to arise from these positions.

Huanglongbing (*Candidatus* Liberibacter spp.)

Recommendation: Finalise the incursion management plan, ensure industry personnel are trained to recognise the vector and disease and report suspicious samples, and ensure expertise exists for diagnosis and incursion management.
Action: A citrus biosecurity project led by Plant Health Australia has commenced in 2013.

Citrus canker (*Xanthomonas citri* subsp. *citri*)

Recommendation: Ensure industry personnel are trained to recognise the disease and report suspicious samples, and ensure expertise exists for diagnosis and incursion management.
Action: A citrus biosecurity project led by Plant Health Australia has commenced in 2013.

Citrus scab (*Elsinoë* spp.)

Recommendation: Complete the studies needed to fully characterise this disease in Australia.

Action: Due to unsuccessful attempts with the Plant Biosecurity Cooperative Research Centre, and Plant Health Australia, to fund a PhD scholarship to investigate this issue, no further action will be taken.

Citrus black spot (*Phyllosticta citricarpa*)

Recommendation: Focus on supporting market access opportunities by developing efficient and effective control strategies, with a change in emphasis from achieving 100% disease control in export fruit, to achieving very low levels of disease in the most profitable way possible.

Action: The CT07012 project team has applied for two new projects with specific activities in this area. The first is a Horticulture Australia Limited levy grant with a focus on new fungicides for CBS control. The second is a collaborative project with the University of Florida, through the Citrus Research and Development Foundation, which focuses on the disease cycle and potential for genetic resistance to CBS.

Chapter 4

Resource, Technical Support, Extension and Training

4.1 Introduction

The objectives of the activities outlined in this chapter are aimed **to build a strong field pathology skills base, recognise diseases and understand their epidemiology, improve collaboration (nationally and internationally), and build extension and adoption networks between the research providers and the industry on a nationally**. This objective was achieved through a number of key project activities including:

- facilitating citrus pathology workshops;
- undertaking technical field visits and diagnostics;
- participating in conferences and overseas study tours; and
- providing publications and reports for industry and other end users.

The citrus pathology workshops provided a unique opportunity to update all citrus pathology researchers in Australia on the range of research being undertaken, as well as plan and undergo collaborative activities. Prime examples of collaboration resulting from these workshops include the publication of the citrus diseases chapter in the CSIRO publication 'Diseases of Fruit Crops in Australia' and a joint field research exercise on the use of starch accumulation as a field test for symptoms of huanglongbing.

Undertaking technical field visits and diagnostics for growers was made possible through this project. These activities ranged from basic disease diagnosis, through to investigating more complex technical production issues. The latter were often tackled as joint efforts between various organisations involved with the project, with opportunities for travel between states for Andrew Miles and other project collaborators funded by the project.

The presentation of work undertaken in the project through participation in conferences and overseas study tours was made possible through CT07012 and associated projects. This included presenting at the Citrus Australia National Conferences, Australasian Plant Pathology Society Conferences, and international conferences such as the International Citrus Congress. This ensured that project activities were extended to industry end-users as well as to the wider plant pathology discipline.

Finally, publications and reports for industry and other end users produced at all levels, from presentations given directly to growers, through to peer-reviewed journal articles. This documentation of project activities not only gave growers access to new information, but also ensured the retention of "corporate knowledge" in the discipline for future access.

4.2 Citrus Pathology Workshops

Over the life of CT07012 four citrus pathology workshops were held to provide research updates and share expertise. The workshops typically included representatives from most state departments of agriculture, two universities and the industry peak body.

2008, Elizabeth Macarthur Agricultural Institute, NSW

The 2008 workshop held on the 18th - 19th March was the largest gathering of citrus pathologists in Australia for several years. The workshop was held at the Elizabeth Macarthur Agricultural Institute (EMAI) where project collaborators Nerida Donovan, Grant Chambers and others are based. The workshop agenda included updating current research activities, exotic citrus disease surveillance, and developing a plan for the production of a citrus pathology review paper. Positive aspects of the workshop were the fostering of professional relationships between the attendees, discussion of current research activities and opportunities for the group, and development of a firm plan for a pathology review paper; the latter of which became refocused on the preparation of a citrus chapter in the CSIRO book 'Disease of Fruit Crops in Australia.' Andrew Miles also spent additional time at EMAI with Nerida Donovan and Grant Chambers to collate citrus disease photos for the book chapter. Aspects of the workshop that were changed for the later workshops included a more specific agenda, all agenda items raised clearly prior to the workshop to ensure adequate discussion time, and the opportunity for pathology training exercises in the field. Attendees (Fig. 4.2.1) of the workshop were:

NSW DPI	DAFF (formerly AQIS)	UWS	Qld DAFF
Nerida Donovan	Richard Davis	Andrew Beattie	Andrew Miles
Deborah Hailstones	Mark Walker	Paul Holford	Ceri Pearce
Grant Chambers			
Tracy Berg			
CAL	**UQ**	**Vic DPI**	
Pat Barkley	André Drenth	Bob Emmett	

Figure 4.2.1 Attendees of the 2008 Citrus Pathology Workshop, from left to right: Nerida Donovan, Andre Drenth, Bob Emmett, Tracy Berg, Andrew Miles, Deborah Hailstones, Richard Davis, Pat Barkley, Mark Walker, Ceri Pearce, Grant Chambers and Paul Holford. Absent from photo: Andrew Beattie.

AGENDA Citrus Pathology Workshop March 18-19 2008

Elizabeth Macarthur Agricultural Institute, Camden, NSW 2750

Time	Speaker	Issue
Tuesday March 18		
10:30	Arrival-Coffee and Tea	
11:00	André Drenth	Welcome and Introduction
11:10	**Update of Activities**	
11:20	Nerida Donovan	Please provide a 1 page word document of activities related to citrus pathology you are involved in dot point format before the meeting. We intend to use this for the citrus capability document. Please no powerpoint presentations for this section.
11:30	Deborah Hailstones	
11:40	Richard Davis	
11:50	Grant Chambers	
12:00	Mark Walker	
12:10	Bob Emmett	
12:20	Andrew Beattie	
12:30	Andrew Miles	
12:40	Pat Barkley	
12:50	Paul Holford	
13:00	**Lunch**	
2:00	Grant Chambers	Travel report: Citrus diseases in Turkey
2:40	André Drenth	Coordination of surveillance activities in Australia
2:50	Ceri Pearce	Surveillance Biosecurity Queensland
3:20	**Afternoon tea**	
3:40	Richard Davis	Surveillance NAQS
4:00	Nerida Donovan	Area freedom surveillance
4:15	Andrew Miles	National database demonstration
4:30	André Drenth	Discussion of surveillance activities for quarantine and area freedom.
5:00	André Drenth	Actions and responsibilities. Issues to be resolved
5:30	**Finish**	
7:30	**Workshop Dinner**	The Crown Hotel
Wednesday March 19		
8:30	André Drenth	Citrus pathology capability document
9:15	Andrew Miles	Citrus pathology review paper
10:15	André Drenth	Discussion and assignment of tasks for review paper
10:30	**Morning Tea**	
11:00	Andrew Miles	Discussion of activities in the year ahead
11:45	André Drenth	Review of actions and responsibilities. Issues to be resolved
12:00	Nerida Donovan & Grant Chambers	Overview and background of EMAI and Auscitrus
12:30	**Lunch**	
1:00	Nerida Donovan & Grant Chambers	Tour of the EMAI & Auscitrus facilities
2:30	**Afternoon tea and finish**	

2009, Indooroopilly Research Centre, Qld

The Indooroopilly Research Centre, Brisbane, was the location for the second Citrus Pathology Workshop on the 30-31st March 2009. This site was the location of project leader André Drenth and team member Andrew Miles. The workshop agenda focused on research activity updates, travel reports, and biosecurity preparedness. The workshop also included an extended field trip component to the Central Burnett and Bundaberg Research Station. The purpose of the field trip was to investigate the feasibility of using in-field starch accumulation tests for pre-incursion surveillance for huanglongbing (HLB). This question had been raised at the HLB / Asian citrus psyllid (ACP) Incursion Management Plan Workshop, attended by several of the citrus pathology workshop attendees. The field trip exercise was presented as an abstract and poster at the 2009 Australasian Plant Pathology Conference (Fig. 3.5.1). Attendees (Fig. 4.2.2) of the workshop were:

Qld DAFF	NSW DPI	CAL	NT DPIF
Andrew Miles	Nerida Donovan	Pat Barkley	Lucy Tran-Nguyen
Ceri Pearce	Grant Chambers	Simon Powell	
Matt Weinert	Sandra Hardy		
Malcolm Smith			
Helen Hofman			
UWS	**DAFF (formerly AQIS)**	**UQ**	**Vic DPI**
Paul Holford	Richard Davis	André Drenth	Bob Emmett

Figure 4.2.2 Attendees at the 2009 Citrus Pathology Workshop, from left to right: Pat Barkley, Paul Holford, Matt Weinert, Bob Emmett, Sandra Hardy, Andrew Miles, Nerida Donovan, Richard Davis, Grant Chambers and Lucy Tran-Nguyen. Absent from photo: Ceri Pearce, Malcolm Smith, Helen Hofman, André Drenth, and Simon Powell.

AGENDA Citrus Pathology Program Meeting March 30-31 2009

Location Entomology Conference Room, DPI&F, 80 Meiers Road Indooroopilly 4068. Brisbane

Time	Speaker/convenor	Issue
Monday March 30		
10:30	Arrival- Coffee and Tea	
10:50	André Drenth	Welcome and Introduction
	Update of Activities	
11:00	Nerida Donovan	Please provide a 1 page word document of activities related to citrus pathology you are involved in dot point format before the meeting. Please no powerpoint presentations for this section.
11:15	Richard Davis	
11:30	Grant Chambers	
11:45	Ceri Pearce	
12:00	Bob Emmett	
12:15	Sandra Hardy	
12:30	Pat Barkley	
12:45	Andrew Miles	
13:00	**Lunch**	
2:00	Simon Powell	Industry perspective on citrus diseases – what are the concerns of Qld citrus growers?
2:15	Andrew Miles	Travel report: Citrus diseases in China, Japan and India
2:30	Sandra Hardy/ Nerida Donovan	Pathology components of ACIAR projects in Bhutan and Pakistan
2:45	Matthew Weinert	Citrus disease problems in North Queensland
3:00	Andrew Miles	Finalisation of surveillance report; discussion on a joint National Citrus Pathology surveillance project?
3:30	**Afternoon tea**	
3:45	Yu Pei Tan	PCR for *Elsinoë australis*
4:00	Pat Barkley	How can the Pathology group be involved in addressing some of the pre-incursion requirements for preparedness for HLB?
4:30	Sandra Hardy/Ceri Pearce	Information material for awareness and extension
5:00	André Drenth	Actions and Responsibilities. Issues to be resolved
5:30	Finish	Take participants to St Lucia Gardens
7:00	Workshop Dinner	
Tuesday March 31		
8:00		Pick up from St Lucia Gardens
8:30	Paul Holford	Overview of activities and Pathology components of ACIAR projects in Indonesia, Vietnam, China?
9:00	Andre Drenth	Citrus Pathology Capability Document
9:20	André Drenth	Citrus Pathology Review Paper Discussion and assignment of tasks for review paper
10:30	**Morning Tea**	
11:00	Andrew Miles	Discussion of activities in the year ahead
11:30	André Drenth	Discussion regarding new projects
12:00	André Drenth	Review of Actions and Responsibilities. Issues to be resolved
12:15	**Lunch**	
13:00	**Departure to field trip**	~380km (up to 6 hours by road)
15:00	Coffee break Gympie	
19:00	Arrive Bundaberg	

Wednesday April 1		
8:15	Depart for BRS	
8:45	Malcolm Smith & Helen Hofman	Discussions of citrus breeding/physiology activities and resources
9:30	**Early Morning Tea**	
10:00	Field tour (after decontamination)	• New screenhouse • Germplasm collection • Promising new varieties • Sampling and starch testing
12:00	**Lunch**	
12:30	Depart for Gayndah	~160km (2 hours)
14:30	Arrive Gayndah	Check into accommodation
15:30	Head to ACD site in Gayndah	Sampling and starch testing in Gayndah
Thursday April 2		
08:00	Continue sampling and starch testing	
PM	Return home	We need to be back at Brisbane airport between 4-5 to get everyone back home on time

2010, Dareton Agricultural Research and Advisory Station, NSW

The Dareton Agricultural Research and Advisory Station, Dareton NSW, was the location for the third Citrus Pathology Workshop on the 29 November-1st December 2010. The workshop agenda focused on citrus industry updates, research activity updates, travel reports, and biosecurity preparedness. The workshop also included a tour of the Dareton field station, Auscitrus, and surrounding orchards to learn about issues such as blight, sudden death, and phytophthora. A full report on the workshop was circulated to all attendees and others. The full report has been provided to HAL and is available upon request. Attendees (Fig. 4.2.3) of the workshop were:

NSW DPI	Qld DAFF	SARDI	CAL	Vic DPI
Deborah Hailstones	Andrew Miles	Peter Taverner	Pat Barkley	Bob Emmett
Grant Chambers	Malcolm Smith	Nancy Cunningham	Andrew Harty	
Tahir Khurshid	Yu Pei Tan			
Graeme Sanderson				

UWS	UQ	CSIRO	MVCB
Paul Holford	André Drenth	Steve Sykes	Mary Cannard

Figure 4.2.3 Attendees of the 2010 Citrus Pathology Workshop, from left to right: Malcolm Smith, Peter Taverner, Yu Pei Tan, Paul Holford, Deborah Hailstones, Andre Drenth, Nancy Cunningham, Pat Barkley, Steve Sykes, Grant Chambers, Andrew Miles and Bob Emmett. Absent from photo: Andrew Harty, Mary Cannard, Tahir Khurshid, Graeme Sanderson.

AGENDA Citrus Pathology Program Meeting November 29-30 2010

Location: Dareton Research Station, Silver city Highway NSW 2717

Time	Speaker/convenor	Issue
Monday November 29		
8:00		Depart from Motel
8:30	André Drenth	Welcome and Introduction
9:00	Update of Activities	Roundtable discussion where everyone has 10 minutes to give an update of activities
10.30	Morning tea	
11:00	Andrew Miles and Yu Pei Tan	Update of citrus scab developments
11:30	Nerida Donovan	Trip Report Florida and new incursions in the USA
12:00	Andrew Harty	Citrus industry, the big picture Discussion about industry and research perspectives
13:00	Lunch	
14:00	Peter Taverner and Nancy Cunningham	Overview of post harvest pathology Followed by discussion
15:15	Mary Cannard	Discussion of the activities of the Murray Valley Citrus Board
15:30	Tahir Khurshid	Citrus research at Dareton
15:45	Graeme Sanderson	Dareton Station Tour and citrus trials
17:00		Travel back to Motel
19:00		Workshop dinner:
Tuesday November 30		
8:00		Depart from Motel
8:30	Paul Holford	Update of ACIAR project activities
9:00	Andrew Miles	Discussion Surveillance Report
9:20	André Drenth	Citrus Pathology Review Paper Discussion and assignment of tasks for review paper
9:40	Malcolm Smith	Breeding for disease resistance
10:30	Morning Tea	
11:00	André Drenth	Discussion regarding new projects
11:30	André Drenth	Review of Actions and Responsibilities. Issues to be resolved Including distribution of milestone reports, project updates and workshop reports.
12:00	Lunch	
13:00	Nerida and Tim Herrmann	Visit Auscitrus facilities
15:00	Steven Sykes	Visit CSIRO breeding in Merbein
16:00	Travel to airport	Paul and Deb leave at 5 pm
Wed Dec 1	Mary Cannard Murray Valley Citrus Board	Leave Mildura at 8:00am Arrive Mansell's Farm 9:00am – Blight on Citrus Leave Mansells Farm 10:00am Arrive Nick Praght's Farm 10:15 – Possible ***Phytophthora*** Leave Nick Praght's Farm 11.15 Arrive Redcliffs for lunch 12:00 Leave Redcliffs 12:45 Arrive Sevenfields 1:00pm Leave Sevenfields 2:00pm Arrive DPI Victoria 2:30pm Leave DPI Victoria 3:30pm for Airport

2012, Ecosciences Precinct, Qld

The 2012 workshop was help at the Ecosciences Precinct, Brisbane, on the 6th-7th September 2012. This site was the new location of project leader André Drenth and team member Andrew Miles. Like previous workshops, the agenda focused on citrus industry updates, research activity updates, travel reports, and biosecurity preparedness. However a significant inclusion to the agenda was time dedicated to the planning of future research proposals. A summary of the project planning activities was published in Australian Citrus News (Davis, 2012). A full report on the workshop was circulated to all attendees and others. The full report has been provided to HAL and is available upon request. Attendees (Fig. 4.2.4) of the workshop were:

Qld DAFF	NSW DPI	UWS	HAL
Andrew Miles	Nerida Donovan	Paul Holford	Brad Wells
Malcolm Smith	Shane Hetherington	Andrew Beattie	Ben Callaghan
Yu Pei Tan		Namgay Om	
Ceri Pearce			

SARDI	UQ	Vic DPI	NT DoR
Peter Taverner	André Drenth	Bob Emmett	Lucy Tran-Nguyen

CAL	DAFF (formerly AQIS)
Andrew Harty	Richard Davis

Figure 4.2.4 Attendees of the 2012 Citrus Pathology Workshop, from left to right: Andrew Beattie, Peter Taverner, Andrew Miles, Yu Pei Tan, Shane Hetherington, Nerida Donovan, Brad Wells, Lucy Tran-Nguyen, Paul Holford, Ceri Pearce, Andrew Harty, Namgay Om, Richard Davis, Malcolm Smith, Bob Emmett and Andre Drenth. Absent from photo: Ben Callaghan.

AGENDA Citrus Pathology Workshop September 6th & 7th 2012

Foyer meeting room GA-604, EcoSciences Precinct, Boggo Rd, Brisbane

Day 1 Citrus Pathology Research Forum		
Time	**Presenter/s**	**Issue**
08:30	***Arrival and coffee***	
09:00	Andre Drenth	Welcome and introduction
09:05	Participant introductions	Names, organisation and area of expertise
09:15	Andrew Harty	Australian citrus industry update, major challenges & successes – setting the scene for whom and why we are undertaking research.
Specific research topics (allocated times to include 5 minutes for questions)		
09:30	Nerida Donovan	Orange stem pitting strains of CTV in Australia
09:50	Paul Holford	Powdery mildew of citrus and taxonomy of *Murraya*
10:10	Andrew Miles	Novel *Elsinoë* occurring in Australia
10:25	Yu Pei Tan	The modern plant pathology herbarium: new technologies and conventions
10:40	***Morning tea***	
10:55	Andrew Beattie	Heat therapy for HLB and potential new vectors
11:15	Peter Taverner	Recent advances in postharvest pathology and disinfestation.
11:35	Malcolm Smith	Integrating disease resistance breeding into a commercial scion breeding program.
11:55	Andrew Harty & Andrew Miles	Strategic Agrochemical Review Process for citrus and fungicide evaluation for brown spot and black spot.
12:15	Lucy Tran-Nguyen and Richard Davis	*Cryptosporiopsis citri* in northern Australia
12:30	***Lunch***	
Biosecurity surveillance and extension (allocated times to include 5 minutes for questions)		
01:00	Andrew Beattie	International biosecurity developments of interest to Australia and incursion management plans.
01:30	Richard Davis	HLB field survey tactics and recent developments in the Pacific
01:45	Ceri Pearce	Engaging industry personnel in biosecurity surveillance: Biosecurity Queensland's exotic citrus pest training.
02:00	Chair: Ceri Pearce	Updates on each state's surveillance activities and discussion re future activities:
02:30	Chair: Ceri Pearce	Open discussion on training, awareness programs, and industry resources materials.
03:00	David Spence	**ESP site tour**
04:00	End of days formal proceedings	We would invite the participants to use this time before dinner to meet up with any other ESP-based researchers/staff not directly involved with citrus or pathology.

06:30	***Workshop dinner***	(Morrison Hotel, 640 Stanley Street)

Day 2 Citrus pathology research planning forum		
8:30	***Arrival and coffee***	
8.45	Andrew Harty	Citrus Industry Strategic Plan : Pathology and Biosecurity strategies & outputs
9.00	Andrew Harty	Market access issues faced by Australian citrus industry; overview of South African pathology research (including discussion and questions)
09:45	Andre Drenth & Andrew Miles	Recap of CT07012, outcomes and achievements against milestones
10:45	***Morning tea***	
11:00	Chair: Brad Wells	HAL pathology proposals for 2012 call: Andrew Miles Nerida Donovan Others
12:00	Chair: Nerida Donovan	Pathology proposals for CRC PB, ACIAR and other funding sources
12:30	***Lunch***	
01:00	Shane Hetherington	National Horticulture Research Framework
01:05	Chair: Shane Hetherington	Future research and extension priorities for citrus pathology.
02:30	Chair: Andre Drenth	PhD's, higher education and career paths in citrus pathology
02:50	Andrew Harty	Concluding summation
03:00	**Workshop concluded**	
03:26	**Train to airport**	

4.3 Technical field visits and diagnostics

During this project a number of technical production issues arose requiring various levels of input from the CT07012 team and collaborators to assist growers. These issues ranged from basic diagnosis of disease symptoms in samples sent to the laboratory, through to more complex issues requiring more thorough investigation including field visits. The following section provides details of the more complex issues and investigations undertaken to determine their cause and best management strategies.

IrM2 Murcott Disorder in Mundubbera

In June 2008, Grant Chambers (NSW DPI), Pat Barkley (ACG) and Andrew Miles (DPI&F) visited the Central Burnett with John Owen-Turner (consultant) and Simon Powell (QCG IDO) to investigate a bark scaling disorder (Fig. 4.3.1 – top). The problem was evident in a significant proportion of trees (~25%) in a block of IrM2 Murcott mandarins on Troyer citrange rootstock with a Sunburst mandarin interstock. Unique to the disorder was the often narrow band of bark cracking progressing up one side of the trunk (Fig. 4.3.1 – bottom left). Symptoms were somewhat similar to those caused by *Citrus psorosis virus*. Field inspection and follow up biological indexing and molecular testing did not support *Citrus psorosis virus* being the problem. Uprooting and dissection of an affected tree found compartmentalised sectors of dead wood (Fig. 4.3.1 – bottom right) and fungal mycelium similar to that formed by basidiomycetes growing under the decaying bark (Fig. 4.3.2 – top). It was noted in the field that every 4th tree in a row had been removed to reduce the planting density (Fig. 4.3.2 – bottom left). Trees were topped low to the ground and the stumps painted with 1 part Grazon herbicide (triclopyr and picloram) to 5 parts water to prevent regrowth (Fig. 4.3.2 – bottom right). An alternative cause of the disorder is phytotoxicity resulting from the possible movement into adjacent trees of the herbicide from the treated stumps, either through soil diffusion or grafted roots. The narrow band of bark cracking up the trunk may then be the result of localised phytotoxic effects to the vascular tissue corresponding with the root that came into contact with any herbicide. However, signs of phytoxicity were not observed in the foliage. The role of the fungus observed on the decaying roots is unclear, but it is most likely present as a saprophyte feeding on the already dead roots or has been able to parasitise roots that may have been compromised by the herbicide. It is also possible, but less likely, that the decaying stumps had increased the biomass of decay fungi sufficiently to become pathogenic to the adjacent trees. Garry Fullelove (DPI&F) has also visited the site and offered the same possibilities as above, with the additional possibilities of significant root death occurring during the topworking process or frost damage occurring soon after topworking.

In August 2008, Andrew Miles and Andre Drenth (UQ) returned to this site to make quantitative observations of the distribution and severity of the condition (Fig. 4.3.3). The aim was to be able to demonstrate any significant association between symptomatic trees and the proximity to the cut stumps, and to be able to detect any spread of the condition in the future. A lack of spread would indicate the disorder was more likely physiological than pathological. A significant association of symptomatic trees with a close proximity to cut stumps would support the possibility of the disorder being directly related to the cut stumps (i.e. herbicide damage or fungal damage). Using the data collected in August, a significant (<0.001) association between symptomatic trees and close proximity to cut stumps was demonstrated by a

two sample binomial test. No further evidence for spread was subsequently observed. It was concluded that the symptoms were that of herbicide phytotoxicity.

Figure 4.3.1 Symptoms of an unidentified disorder occurring in a block of IrM2 Murcott mandarins in Mundubbera.

Figure 4.3.2 Fungal mycelium possibly of a Basidiomycete on (top - left) and under (top - right) the bark and removal of every fourth tree, leaving stumps in the ground (bottom).

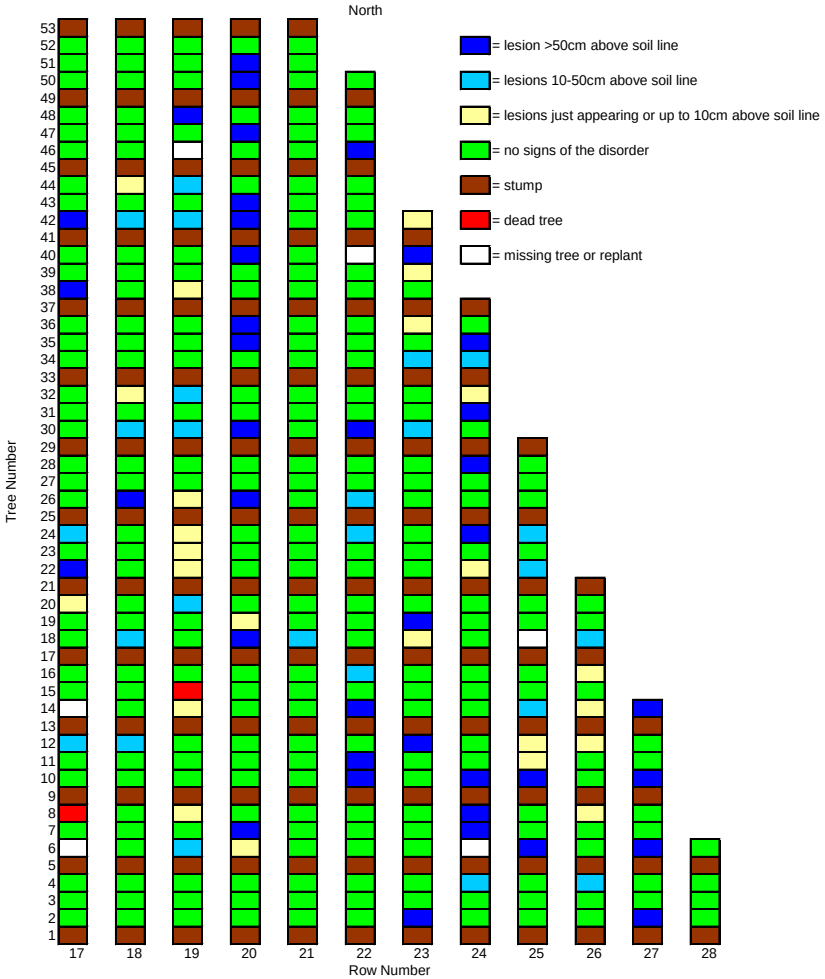


Figure 4.3.3 Distribution and severity (height of symptoms above soil line) of IrM2 Murcotts showing symptoms of an unknown disorder.

Possible psorosis in Murcott tangors at Mundubbera

In June 2008, Grant Chambers (NSW DPI), Pat Barkley (ACG) and Andrew Miles (DPI&F) visited the Central Burnett with John Owen-Turner (consultant) and Simon Powell (QCG IDO) to investigate symptoms typical of *Citrus psorosis virus* observed in Murcott mandarins in Mundubbera. Bark scaling and chlorotic flecking in leaves were observed on a number of trees (Fig. 4.3.4). Samples were sent under permit to Grant Chambers at NSW DPI for testing by RT-PCR and biological indexing. RT-PCR tests were negative for *Citrus psorosis virus*, *Citrus exocortis virus* and group I and III *Citrus* viroids, but positive for *Citrus* viroid II. It was not determined which group II viroid was present – though this viroid is unlikely to be the definitive cause of the bark scaling observed. Biological indexing was inconclusive even after two attempts by Grant Chambers. The causes of the symptoms remain unconfirmed.

Figure 4.3.4 Psorosis-like symptoms in Murcott tangor. Causal agent undetermined.

Hail-exacerbated EBS outbreak

In 2008 a block of Murcott near Mundubbera that was damaged by a severe hail storm was visited by the pathology team and Dan Papacek (Bugs For Bugs). Since the storm damage, a severe epidemic of Emperor brown spot (EBS) (*Alternaria alternata*) was causing significant shoot infection and leaf abscission (Fig. 4.3.5 – left). Significant translocation of the host specific toxin produced by *A. alternata* was evident in young shoots (Fig. 4.3.5 - right). The likely factors contributing to the epidemic were the uncharacteristic growth flushes in response to the hail damage, and the use of overhead irrigation. As leaf wetness duration is a key factor in *A. alternata* infection (Canihos *et al.*, 1999), it was recommended that changing to under-tree irrigation would significantly reduce the impact of EBS in the block. In addition to registered fungicide applications, any horticultural practices such as nitrogen application and/or pruning to synchronise growth flushes should also make fungicide applications easier to target, as well as improve their efficacy.

Figure 4.3.5 Shoot infection and leaf abscission caused by Alternaria alternata (left) and veinal necrosis and leaf chlorosis (right) caused by the translocation of the host specific toxin produced by the fungus.

Suspected blight outbreak

A potential citrus blight outbreak in a block of Navelina trees near Mundubbera was investigated by Dan Papacek (Bugs for Bugs) and Andrew Miles in October 2009. Above ground symptoms were typical of blight (Fig. 4.3.6 top left), and tended to occur in patches down the rows (Fig. 4.3.6 top right) However, stem water infiltration tests (Fig. 4.3.6 bottom left) indicated that blight was not likely to be the cause of the decline in these trees (Chapman and Hutton, 1988). Affected trees showed reduced root mass and signs of phytophthora root rot. Soil structure was notably poor, most probably contributing to the cause of the decline. The soil profile of affected and healthy trees was investigated using an auger (Fig. 4.3.6 bottom right). It was evident from investigating the soil profile that the topsoil depth was highly variable, with poor performing trees found to be in very shallow top soil over a heavy clay pan. Healthy trees were in deeper, more friable top soil. The shallow clay pan was likely to be leading to waterlogging and conditions favourable for *Phytophthora nicotianae* to cause root rot and decline symptoms in the trees. Management strategies for phytopthora root rot were recommended.

Figure 4.3.6 Blight-like symptoms (top left) occurring in Navelina trees in patches moving down the rows (top right). Performing water infiltration tests (bottom left) and soil profile examinations (bottom right).

North-western NSW

On the 8th-10th March 2011 Dan Papacek (Bugs for Bugs) arranged for Andrew Miles to travel with him to the North-western NSW centres of Moree, Gunnedah and Narromine. Andrew gave a presentation to local growers on *Phytophthora*, a significant problem in some orchards. The growers from the general area were together for a meeting with the owners of Grove Juice.

The first orchard visited consisted of mostly Hamlin on Trifoliata rootstock. The trees were planted by a previous owner, and then abandoned after planting. The current owners had subsequently put a lot of effort into improving tree health. Despite being ~ 5 years old, the trees were generally quite small, only lightly cropping and in some cases declining (Fig. 4.3.7 left). The soil had a very high clay content, and was not ideal for *Citrus*. There was substantial evidence of past *Phytophthora* damage that had been treated with phosphorous acid. The owners indicated the trees were responding well, though feeder root damage wasn't hard to find on roots.

Figure 4.3.7 Example of a tree in a state of decline (left) and of a 'j' rooted tree in the field (right).

The second orchard visited consisted of mostly Hamlins and Salustiana. This site had a much lighter soil, and the younger trees looked like they would outperform the older trees in the previous orchard. Tree quality with regards to size, j-roots (Fig. 4.3.7 right) and vigour was very inconsistent. No pathology problems appeared to be limiting performance. A nursery stock problem was evident.

In Moree, Dan Papacek noted that the close proximity of cotton production (i.e. a roadway away) might be disrupting beneficial insect populations.

The third orchard consisted of Hamlin and Salustiana. It was a very healthy and productive orchard. A few wet spots were observed due to minor irrigation issues, but only a small number of trees were affected. The orchard had recently received a few pallets of rootstock seedlings (bags and flats), however many of the seedlings were very root bound, and arguably not worth planting. To make the most of the nursery material, Andrew Miles provided literature on nursery hygiene and the use of high health status propagating material.

The fourth orchard was younger and had been flooded, but surprisingly the flooded portion seemed to be performing better, because the inundation had suppressed fleabane weed. The two biggest issues for this orchard were the fleabane

(nearly as tall as the *Citrus* in some cases) and locust damage on the young trees. There was some concern about *Phytophthora*, but poor looking trees tended to be associated with poor irrigation. Apparently the drip lines laid in cool weather subsequently "walked" when they expanded in hot weather, shifting the drippers too far away from some trees. There was also some inconsistency in tree quality. Lucerne in the inter-rows was growing well as a potential mulch source.

The final destination was a nursery that had received ~5000 trees from another nursery. The same nursery problems seen elsewhere were evident in these trees, i.e. inconsistent size, j-roots, severe root binding, and evidence of interstocks and *Phytophthora nicotianae* (although root rot was not the main issue in these trees in Andrew Miles' opinion). It was suggested that the new nurseryman should sort the trees according to tree quality and plant rows of consistent trees instead of mixing the trees together.

Overall, the biggest challenge to the growers we visited in the area appeared to be the planting material which was all obtained from the same nursery. Other than *Phytophthora*, no other significant pathology issues were identified in the region.

The CT07012 project team thank Dan Papacek and Bugs for Bugs for making the trip possible. We also thank the local growers for their hospitality.

Lemon blossom mould

In June 2011 the occurrence of a mould on lemon blossoms (Fig. 4.3.8) in an orchard near Bundaberg was investigated by Andrew Miles and Malcolm Smith. It was possible that the mould was *Botrytis cinerea*, which can lead to loss of flowers and downgrading of fruit. However, in this case the fungus was identified as a *Cladosporium* sp. Advice was sought from New Zealand plant pathologist Bob Fullerton, who has prior experience with *B. cinerea* in *Citrus* (Fullerton *et al.*, 1999). The experience in New Zealand is that *Cladosporium* can colonise senescent flower parts and give a grey mould-like appearance in the field. However, yield loss does not commonly result from *Cladosporium* infection in this manner. As such, it was recommended that the orchardist tag affected blossoms and see if fruit set was being reduced and a fungicide application was required

Figure 4.3.8 *Cladosporium* sp. colonising senescent lemon blossom parts.

Late season Murcott fruit drop

In the 2011-12 production season, a block of Murcott at Gayndah experienced an unusual fruit abscission pattern. Fruit of all sizes and stages of colour break had been abscising steadily since March/April 2012 until harvest. The drop was particularly bad in a few rows (closest to the river) of a single block. The drop was apparent in other blocks, but not as severe. According to the orchard manager, there is a history of this kind of drop in the block.

There was concern that the drop was caused by a pathogen. This was largely based on the presence of discoloration around the calyx end of some of the abscised fruit on the ground. Larger fruit samples showed that the discoloration was present in about half the fallen fruit, however it was not known for how long different fruit had been abscised. Therefore a more structured sampling was performed to determine the role of a pathogen.

Methods

Mal Wallis (Citricare) sampled freshly abscised fruit by shaking tree limbs and collecting ~30 fallen fruit. He also sampled ~30 firmly adhered fruit by clipping. Andrew Miles then inspected the fruit at the lab and found that about half the fallen fruit had stem ends healthy in appearance, and the other half discoloured stem ends (Fig. 4.3.9). Buttons were removed from 10 clipped fruit and the stem ends found to be healthy in appearance.

Figure 4.3.9 Sample of abscised fruit showing various sizes and stages of colour break, and both healthy and discoloured calyx ends.

Fungal isolations were made from the stem ends of abscised fruit with healthy and discoloured stem ends, and also from the stem ends of clipped fruit. The remaining fruit were incubated in a fruit ripening room to look for the development of stem end

and/or core rots. Stem ends healthy in appearance were generally free of fungi, discoloured stems had more activity but no fungus was consistently isolated. Fungi isolated included *Aspergillus, Colletotrichum*, and some sterile mycelium. The fruit incubating in the ripening room remained largely unchanged from their condition when sampled i.e. healthy stem ends still looked healthy, discoloured stem ends showed very limited progressing of the discoloration.

After two weeks incubated fruit were removed from the fruit ripening room and inspected for signs of disease. In some cases abscised fruit with discoloured calyx ends showed signs of progression of the discoloration. However, when fruit were cut there were no signs of pathogen progression into the fruit. Fruit from each batch above were cut in half to check for rots, but not significant signs of decay were evident in any batch of fruit (Fig. 4.3.10).

Figure 4.3.10 Cut fruit samples following incubation. Top left = abscised fruit w/ healthy calyx ends. Top right = abscised fruit w/discoloured calyx ends. Bottom left = clipped fruit with buttons removed. Bottom right = clipped fruit.

Conclusions:

Without consistently isolating any fungal pathogen, and with no significant disease progress in the incubating fruit, it is unlikely the drop is the result of a pathogen. With a physiological cause looking more likely, the recommendation was to consider alternative lines of investigation as discussed by Andrew Harty and Andrew Miles:

- Basic soil and leaf analysis comparison between rows with and without the drop.
- Comparison of final tree yields between rows with and without the drop to confirm the extent of any ultimate yield loss.

In January 2013 Andrew Miles followed up with the orchardists regarding the final yield differences between the rows where the abscission was more or less evident. Final numbers of harvested bins did not clearly indicate a pattern of final fruit loss due to the abscission. In order from closest to the river (where the drop was most severe), the number of bins harvested from each row were 29, 33, 32, 31, 20 and 34.

4.4 Grower Meetings, Conferences and overseas study tours

16th Australasian Plant Pathology Conference, Adelaide, 2007

Andrew Miles attended and presented an oral presentation at the 16th Australasian Plant Pathology Conference, Adelaide, 2007. Andrew gave the presentation on behalf of first author Cherie Gambley (DPI&F Qld). The presentation slides are available from Andrew Miles:

Gambley C, Benham M, Miles AK, Smith LS, Whittle PJL (2007) Evaluation of potential citrus canker inoculum reservoirs in Emerald, Queensland. In '16th Biennial Australasian Plant Pathology Society Conference - Back to Basics: Managing Plant Diseases'. Adelaide, Australia, 24th-27th September.

Mexico and United States, May 2008

The Australian Citrus Growers Inc and the North American Plant Protection Organisation (NAPPO) funded Andrew Miles to attend the "Workshop on Huanglongbing (*Candidatus* Liberibacter spp.) and the Asian Citrus Psyllid (*Diaphorina citri*)", Hermosillo, Mexico (May 7-9, 2008) and a pre-workshop visit to Riverside, California, USA. Andrew presented a paper on the behalf of Pat Barkley and Andrew Beattie titled "Contingency plans for HLB and its vectors in Australia". A comprehensive trip report was forwarded to ACG Inc and the citrus pathology group. The report is available on request from Andrew Miles:

Miles AK (2008) 'Report on the Workshop on Huanglongbing (*Candidatus* Liberibacter spp.) and the Asian Citrus Psyllid (*Diaphorina citri*), Hermosillo, Sonora State, Mexico, May 7-9, and pre-workshop visit to Riverside, California, USA.' Tree Pathology Centre (University of Queensland and Department of Primary Industries and Fisheries)

China, Japan, India, October 2008

The Australian Centre for International Agricultural Research (ACIAR) funded Malcolm Smith and Andrew Miles to travel to China, Japan and India in 2008 to attend the 11th International Citrus Congress and 8th International Congress of Citrus Nurserymen in China, the National Institute of Fruit Tree Science in Japan, and assess the status of citrus field trials in India. This study tour provided the opportunity to learn about the latest advances in huanglongbing disease and citriculture generally. Malcolm Smith and Andrew Miles also presented aspects of their research at these international fora. The full trip report as provided to ACIAR and the pathology group is available from Malcolm Smith or Andrew Miles:

Smith MW, Miles AK (2009) Status of field trials established in NE India under "Improving subtropical citrus production in Sikkim and Australia CS1/2002/030" and related international HLB and citrus research activity in China and Japan. A report prepared for the Australian Centre for International Agricultural Research.

Citrus Research, Development and BBQ Afternoon, Gayndah, 2009

In March 2009, Andrew Miles assisted Helen Hofman (DEEDI) and Simon Powell (Citrus Industry Development Officer) to hold a Citrus Research, Development and BBQ Afternoon in Gayndah for the local growers. This meeting was organised as a research communication event like this had not been held for several years. The program for the afternoon and IDO newsletter report about the event are below.

Queensland Government
Department of Primary Industries and Fisheries

Inviting all citrus growers…

Citrus Research, Development and BBQ Afternoon

Thursday 5th March, 1pm to 5pm

at Bevan and Judy Young's packing shed at
Greenhaven Orchard
16309 Burnett Highway, Gayndah

On the agenda….

1:00	**Welcome and Introduction**	(Simon Powell)
1:15	**Granulation ('dryness') in Imperial mandarins (a)**	Results of first year of field trials of management practices (Helen Hofman)
1:45	**Granulation ('dryness') in Imperial mandarins (b)**	Results of investigations into possible technologies for non-invasive assessment in the packing line (Kerry Walsh/Phul Subedi)
2:15	**Area Wide Management - fruit flies as a case study**	(Ed Hamacek/Gu Hainan)
2:45	**Break**	
3:15	**Citrus Black Spot**	(Andrew Miles)
3:45	**Developments in local breeding programs**	(Malcolm Smith)
4:15	**China Exports - Trade developments and market research**	(Bruce McGrath)
4:45	**Close and BBQ**	(and opportunity to raise any future/immerging issues not yet discussed)

Please RSVP to Helen Hofman by 26th February
(ph. 4155 6244, 0408 732801, helen.hofman@dpi.qld.gov.au)

DPI Project reporting forum a huge success.

I am not sure whether it was the lure of a BBQ and a few drinks, or the chance to kick the tyres on Bevan Young's new packing line, but the DPI reporting forum was one of the best grower attended workshop I have ever facilitated.

Researchers at the DPI are the first to admit that it has been too long since we have held such a workshop and are keen to make this an annual event. It was great as an observer to see first hand the results of citrus projects conducted in Queensland with the calibre of presentations representing the skill and hard work of the DPI researches.

Much thanks must go to Bevan and Judy for letting us use their packing shed, and to all Bevan's workers who helped us set up the BBQ and "morgue" fridge. Thanks must also go to all the researchers who presented on the day, particularly Helen Hoffman, and Andrew Miles who helped organised the event, and Brigid Mclelland who did a great job on the BBQ.

Lastly I would like to thanks all those growers and industry representative who attended the day. Without your presence the forum is a pointless exercise.

Powel, S (2009) DPI Project reporting forum a huge success. In 'Queensland Citrus Grower IDO Report', Citrus Australia Limited, 10th March 2009.

CITT Group Meeting, Mareeba, 2009

In September 2009 Andrew Miles travelled with Queensland Citrus Industry Development Officer, Simon Powell, to Mareeba to present pathology information at a CITT Group Meeting. Andrew Miles gave presentations on citrus black spot (CBS) and phytophthora diseases. Locally these two diseases were the most important pathology issues. Later Andrew Miles forwarded a PDF version of the presentations including a summary of the various questions and answers asked at the meeting.

Miles AK (2009) Phytophthora diseases of citrus. In 'CITT Groups'. (Mareeba, Queensland. 17th September).

Miles AK (2009) Citrus black spot. In 'CITT Groups'. (Mareeba, Queensland. 17th September).

Citrus Australia Limited National Conference, Mildura, 2009

Andrew Miles attended the 2009 CAL National Conference and presented a poster outlining the key contributions to industry of citrus pathology (Fig. 4.4.1). In addition, Andrew Miles assisted Nerida Donovan and Grant Chambers to undertake inspections of the Auscitrus Budwood Multiplication Scheme and other field visit, as outlined in section 1.3 of this report.

Figure 4.4.1 Poster presented by Andrew Miles and Nerida Donovan at the 2009 Citrus Australia National Conference.

17th Australasian Plant Pathology Conference, Newcastle, 2009

Australian citrus pathology researchers were represented at the 17th APP conference through four research abstracts. Nerida Donovan circulated these abstracts to the pathology group after the conference.

Chohan SN, Aftab O, Qamar R, Mannan S, Ibrahim M, Ahmed I, Shah MKN, Holford P, Beattie GAC (2009) Management and distribution of huanglongbing in Pakistan. In 'APPS 2009 Plant Health Management: An Integrated Approach'. Newcastle, SNW, Australia p. 176. (Australasian Plant Pathology Society).

Donovan NJ, Barkley P, Hardy S (2009) Pathotypes of *Elsinoë fawcettii* on citrus in Australia. In '17th Australasian Plant Pathology Conference, Plant Health Management'. Newcastle, NSW p. 146.

Mannan S, Chohan SN, Ibrahim M, Aftab O, Qamar R, Shah MKN, Ahmad I, Holford P, Beattie GAC (2009) Phytoplasma diseases in citrus orchards of Pakistan. In 'APPS 2009 Plant Health Management: An Integrated Approach'. Newcastle, NSW, Australia p. 80. (Australasian Plant Pathology Society).

Miles AK, Donovan N, Holford P, Davis R, Grice K, Smith M, Drenth A (2009) Investigating the potential of in-field starch accumulation tests for targeted citrus pathogen surveillance in Australia. In '17th Australasian Plant Pathology Conference, Plant Health Management'. Newcastle, NSW p. 180. (see section 3.5.1 for poster image)

Citrus Australia Limited National Conference, Hervey Bay, 2010

Andrew Miles attended and presented a field talk at the 2010 CAL national conference. The technical presentations program, with images of the presented posters, were circulated to the pathology group after the conference for those unable to attend.

Miles AK (2010) Expanding citrus market access using a systems approach to control black spot. In 'Citrus Australia Limited National Conference, 31st October - 1st November'. (Hervey Bay, Queensland).

Citrus Australia Limited National Conference, Barossa Valley, 2011

Andrew Miles attended and presented two posters at the 2011 CAL national conference. The technical presentations program, with images of the presented posters, were circulated to the pathology group after the conference for those unable to attend.

Miles AK, Smith MW, Drenth A (2011) Fighting brown spot of mandarins. In 'Citrus Australia National Conference'. Wolf Blass Visitor Centre, Barossa Valley, South Australia. (Fig. 4.4.2)

Pearce CA, Miles AK (2011) Engaging industry personnel in biosecurity surveillance: Biosecurity Queensland's exotic citrus pest training. In 'Citrus Australia National Conference'. Wolf Blass Visitor Centre, Barossa Valley, South Australia. (Fig. 4.4.3)

Fighting brown spot in mandarins

Andrew Miles[A], Malcolm Smith[B] and Andre Drenth[C]

[A]Department of Employment, Economic Development and Innovation, 41 Boggo Road, Dutton Park, Qld 4102
[B]Department of Employment, Economic Development and Innovation, 49 Ashfield Road, Bundaberg, Qld 4670
[C]The University of Queensland, Centre for Plant Science, 41 Boggo Road, Dutton Park, Qld 4102

The fungus *Alternaria alternata* causes the destructive disease 'brown spot'. Symptoms include sunken brown lesions on fruit and leaves, defoliation and death of young shoots in susceptible varieties (e.g. Murcott, Daisy). The fungus thrives during warm, wet weather.

Economic impact of brown spot in Queensland?

- 709113[†] susceptible trees × $5 / tree in estimated fruit losses = ~$3.5M annually
- 1624 hectares[†] of susceptible trees × $300 / hectare / fungicide application × 3 applications = ~$1.5M annually
- **The estimated annual cost of brown spot to Qld is ~$5 million**

(note: worst reported fruit loss is $35 / tree, and often more than 3 fungicide applications)

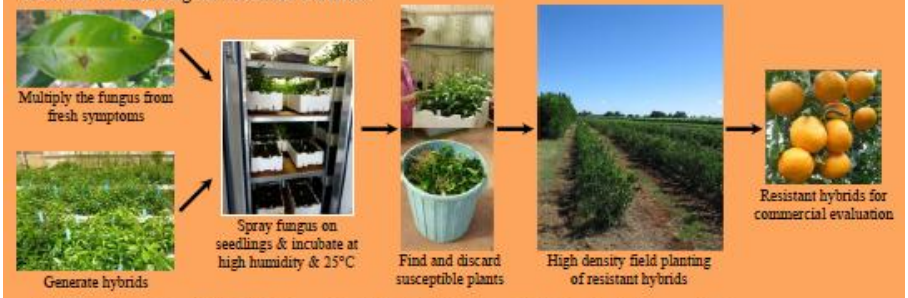
What is being done about brown spot in the short-term?

- Emergency use permits for two additional fungicides; azoxystrobin (Amistar®) and iprodione (e.g. Rovral®)
- The permitted use patterns for these fungicides provide growers with up to 13 weeks of additional protection[‡]
- National registration of Amistar® is expected as early as March 2012
- Registration of iprodione requires additional residue data to be provided to the APVMA

What is the best solution to brown spot in the long-term?

- Selection of resistant hybrids – resistance to brown spot is under simple genetic control
- Resistant hybrids have the potential to eliminate the brown spot problem altogether

How are we selecting for resistant varieties?



- So far we have tested 6411 hybrids and identified 4001 resistant hybrids for further evaluation
- Several thousand more hybrids have been generated and are ready for testing this summer

Resistance offers the only true 'clean and green' solution to the brown spot problem.

References:
[†]Argus S (2008) 'CT07055 National Citrus Plantings Database Management 2008 Citrus Report.' Horticulture Australia Limited, Sydney.
[‡]Miles AK (2011) Tips for the emergency use of iprodione and Amistar® *Citrus Australia Website*.

Figure 4.4.2 Poster presented by Andrew Miles at the 2011 Citrus Australia National Conference.

Figure 4.4.3 Poster presented by Andrew Miles at the 2011 Citrus Australia National Conference.

18th Australasian Plant Pathology Conference, Darwin, 2011

Andrew Miles attended and presented a poster at the 18th Australasian Plant Pathology Conference, Darwin, 2011. Images of all the citrus research papers were made available to the pathology group after the conference for those unable to attend.

Pearce CA, Miles AK (2011) Engaging industry personnel in biosecurity surveillance: Biosecurity Queensland's exotic citrus pest training. In 'ACPP APPS Darwin 2011, New Frontiers in Plant Pathology for Asia and Oceania'. Darwin Convention Centre, Darwin, NT p. 139. (Fig. 4.4.3)

Queensland citrus study tour with Megan Dewdney, University of Florida, 2011

University of Florida citrus pathologist, Dr Megan Dewdney, visited Queensland from the 19th-21st October 2011. Project CT07012 funded Dr Dewdney's Queensland travel component. Dr Dewdney and Andrew Miles visited: the Bundaberg Research Station to see and discuss *Alternaria* resistance breeding and citrus scab inoculation techniques with Malcolm Smith; the Bugs for Bugs insectary with Dan Papacek; orchards with Mal Wallis (Citricare) to see hand pruning operations, brown spot epidemics, and fungicide application methods; and orchards with Brian Gallagher (Citrus Monitoring Services) to see the use of compost tea for possible disease control. Dr Dewdney gave a seminar at the Golden Orange, Gayndah, for growers covering an update of HLB in Florida, copper residue modelling, and fungicide resistance.

Citrus Australia Limited National Conference, Leeton, 2012

Andrew Miles attended and presented a poster at the 2012 CAL national conference. The technical presentations program, with images of the presented posters, were circulated to the pathology group after the conference for those unable to attend.

Miles AK, Papacek DF, Harty A, Drenth A (2012) New fungicides to protect citrus. In 'Citrus Australia National Conference'. 21st-24th October, Leeton Soldiers Club, Leeton, NSW. (Citrus Australia Limited). (Fig. 4.4.4)

Citrus Australia Limited Regional Forum, Gayndah, 2013

Andrew Miles attended and gave an oral presentation at the Citrus Australia Regional Forum in Gayndah. The presentation provided an update on the progress of various fungicide research results. Slides of the presentation are available from Andrew Miles.

Figure 4.4.4 Poster presented by Andrew Miles at the 2012 Citrus Australia National Conference.

4.5 Publications and reports for industry and other end users produced during this project

Book Chapter – Diseases of Fruit Crops in Australia

During project CT07012 a citrus diseases chapter was prepared for the CSIRO publication 'Diseases of Fruit Crops in Australia'. The new book was a replacement to a previous Qld DPI publication from 1993, 'Diseases of Fruit Crops'. The updated chapter was prepared jointly by authors from Qld DPI&F, NSW DPI, DPI VIC, and Australian Citrus Growers Inc. The chapter was updated to contain more nationally significant content than the 1993 publication, including new content such as the five most important exotic citrus diseases for the Australian industry. Since publication in 2010 the book has sold ~750 hard copies plus additional e-book sales. A complimentary copy of the book was provided to each author, key collaborators and Citrus Australia Limited.

Miles AK, Donovan N, Gambley C, Emmett RW, Barkley P (2009) Citrus. In 'Diseases of Fruit Crops in Australia'. (Eds AW Cooke, D Persley and S House) pp. 91-118. (CSIRO Publishing: Collingwood, Victoria).

Journal articles

Gambley CF, Miles AK, Ramsden M, Doogan VJ, Thomas JE, Parmenter K, Whittle PJL (2009) The distribution and spread of citrus canker in Emerald, Australia. *Australasian Plant Pathology* **38**, 547-557.

Miles AK, Tan YP, Tan MK, Donovan NJ, Ghalayini A, Drenth A (2013) *Phyllosticta* spp. on cultivated *Citrus* in Australia. *Australasian Plant Pathology* **42**, 461-467.

Miles AK, Newman TK, Gultzow DL, Parfitt SC, Drenth A, Smith MW (Submitted) Commercial-scale alternaria brown spot resistance screening as the first step in breeding new mandarins for Australia. *Acta Horticulturae*.

Journal articles in preparation

Miles AK, Wright C, Kopittke R, Wyatt P, Eelkema M, Drenth A (In preparation) Improving quantitative pest risk analysis using observed data to refine pest distribution models: a case study of citrus black spot. *Risk Analysis*.

Collaborator journal articles (not CT07012-funded)

Liu ZM, Meats A, Beattie GAC (2008) Seasonal dynamics, dispersion, sequential sampling plans and treatment thresholds for the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera : Gracillariidae), in a mature lemon block in coastal New South Wales, Australia. *Australian Journal of Entomology* **47**, 243-250.

Davis R, Tsatsia H (2009) A survey for plant diseases caused by viruses and virus-like pathogens in the Solomon Islands. *Australasian Plant Pathology* **38**, 193-201.

Xue YG, Beattie GAC, Meats A, Spooner-Hart R, Herron GA (2009) Impact of nC24 agricultural mineral oil deposits on the searching efficiency and predation rate of the predatory mite *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae). *Australian Journal of Entomology* **48**, 258-264.

Xue YG, Beattie GAC, Meats A, Spooner-Hart R, Herron GA (2009) Relative toxicity of nC24 agricultural mineral oil to *Tetranychus urticae* Koch (Acari: Tetranychidae) and *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) and its possible relationship to egg ultrastructure. *Australian Journal of Entomology* **48**, 251-257.

Lloyd AC, Hamacek EL, Kopittke RA, Peek T, Wyatt PM, Neale CJ, Eelkema M, Gu H (2010) Area-wide management of fruit flies (Diptera: Tephritidae) in the Central Burnett district of Queensland, Australia. *Crop Protection* **29**, 462-469.

Zaka SM, Zeng X-N, Holford P, Beattie GAC (2010) Repellent effect of guava leaf volatiles on settlement of adults of citrus psylla, *Diaphorina citri* Kuwayama, on citrus. *Insect Science* **17**, 39-45.

Forster PI, Smith MW (2010) *Citrus wakonai* P.I.Forst. & M.W.Sm. (Rutaceae), a new species from Goodenough Island, Papua New Guinea. *Austrobaileya* **8**, 133-138.

Holford P, Donovan NJ, Thinlay T, Kabanoff E, Wildman O, Hardy S, Beattie GAC, Om N, Dorjee D, Wangdi P (2010) First report of *Oidium citri* in Bhutan. *Australasian Plant Disease Notes* **5**, 55-57.

Lopes SA, Frare GF, Camargo LEA, Wulff NA, Teixeira DC, Bassanezi RB, Beattie GAC, Ayres AJ (2010) Liberibacters associated with orange jasmine in Brazil: incidence in urban areas and relatedness to citrus liberibacters. *Plant Pathology* **59**, 1044-1053.

Liang WG, Meats A, Beattie GAC, Spooner-Hart R, Jiang L (2010) Conservation of natural enemy fauna in citrus canopies by horticultural mineral oil: Comparison with effects of carbaryl and methidathion treatments for control of armored scales. *Insect Science* **17**, 414-426.

Miyata S-i, Kato H, Davis R, Smith M, Weinert M, Iwanami T (2011) Asian-common strains of 'Candidatus Liberibacter asiaticus' are distributed in Northeast India, Papua New Guinea and Timor-Leste. *Journal of General Plant Pathology* **77**, 43-47.

Donovan N, Beattie G, Chambers G, Holford P, Englezou A, Hardy S, Dorjee D, Wangdi P, Thinlay T, Om N (2011) First report of '*Candidatus* Liberibacter asiaticus' in *Diaphorina communis*. *Australasian Plant Disease Notes* **7**, 1-4.

Taverner PD, Sutton C, Cunningham NM, Dyson C, Lucas N, Myers SW (2011) Efficacy of several insecticides alone and with horticultural mineral oils on light brown apple moth (Lepidoptera: Tortricidae) eggs. *Journal of Economic Entomology* **104**, 220-224.

Masaoka Y, Pustika A, Subandiyah S, Okada A, Hanundin E, Purwanto B, Okuda M, Okada Y, Saito A, Holford P, Beattie GAC, Iwanami T, (2011) Lower concentrations of microelements in leaves of *Citrus* infected with '*Candidatus* Liberibacter asiaticus'. *Japan Agricultural Research Quarterly* **45**, 569-275.

Taverner PD, Sutton C, Cunningham NM, Myers SW (2012) The potential of mineral oils alone and with reduced rates of insecticides for the control of light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae), on nursery plants. *Crop Protection* **42**, 83-87.

Katoh H, Davis R, Smith MW, Weinert M, Iwanami T (2012) Differentiation of Indian, East Timorese, Papuan and Floridian '*Candidatus* Liberibacter asiaticus' isolates on the basis of simple sequence repeat and single nucleotide polymorphism profiles at 25 loci. *Annals of Applied Biology* **160**, 291-297.

Rathé AA, Pilkington LJ, Gurr GM, Hoddle MS, Daugherty MP, Constable FE, Luck JE, Powell KS, Fletcher MJ, Edwards OR (2012) Incursion preparedness: anticipating the arrival of an economically important plant pathogen *Xylella fastidiosa* Wells (Proteobacteria: Xanthomonadaceae) and the insect vector *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) in Australia. *Australian Journal of Entomology* **51**, 209-220.

Cen YJ, Yang CL, Holford P, Beattie GAC, Spooner-Hart RN, Liang GW, Deng XL (2012) Feeding behaviour of the Asiatic citrus psyllid, *Diaphorina citri*, on healthy and huanglongbing-infected citrus. *Entomologia Experimentalis et Applciata* **143**, 13-22.

Smith MW, Gultzow DL, Newman TK (2013) First fruiting intergeneric hybrids between *Citrus* and *Citropsis*. *Journal of the American Society for Horticultural Science* **138**, 57-63.

Potts JM, Cox MJ, Barkley P, Christian R, Telford G, Burgman MA (2013) Model-based search strategies for plant diseases: a case study using citrus canker (*Xanthomonas citri*). *Diversity and Distributions* **19**, 590-602.

Vanaclocha P, Papacek D, Monzo C, Verdu MJ, Urbaneja A (2013) Intra-guild interactions between the parasitoid *Aphytis lingnanensis* and the predator *Chilocorus circumdatus*: Implications for the biological control of armoured scales. *Biological Control* **65**, 169-175.

Hall DG, Gottwald TR, Stover E, Beattie GAC (2013) Evaluation of management programs for protecting young citrus plantings from huanglongbing. *HortScience* **48**, 330-337.

Yang YP, Beattie GAC, Spooner-Hart RN, Huang MD, Barchia I, Holford P (2013) Influences of leaf age and type, non-host volatiles, and mineral oil deposits on the incidence, distribution, and form of stylet tracks of *Diaphorina citri*. *Entomologia Experimentalis et Applciata* **147**, 33-49.

Industry and other publications

Miles, A.K. and Hardy, S. (2008) Anthracnose – a potential problem this season. *Coastal Fruit Growers' Newsletter* **68**, 8.

Tree Pathology Centre (2008) 'Submission on: BA Policy Memorandum 2008/23. Draft import risk analysis report for fresh Unshu mandarin fruit from Japan (July 2008).' Plant Biosecurity, Biosecurity Australia, Canberra, Australia.

Tree Pathology Centre (2009) 'Biosecurity Australia advice 2009/07 Provisional final import risk analysis report for fresh unshu mandarin fruit from Japan.' Import Risk Analysis Appeals Panel, Department of Agriculture, Fisheries and Forestry, Canberra, Australia.

Miles AK (2010) New mandy varieties show signs of brown spot resistance. *Australian Citrus News* **87**, 17.

Drenth A, Miles AK (2010) Workshop report. In 'Third Citrus Pathology Workshop'. (Dareton Research Station and Mildura, Victoria. 29th-30th November).

Miles AK (2011) Tips for the emergency use of iprodione and Amistar. *Citrus Australia Website*.

Miles, A. K. and Harty, A. (2011) Fungicides in Queensland. *Australian Citrus News* **87**, 20-21.

Smith MW, Miles AK, Fullelove G (2011) Managing citrus orchards affected by wet weather. *DEEDI factsheet*.

Miles AK (2011) Review of surveillance for exotic citrus disease threats to Australia. The University of Queensland and AgriScience Queensland - a part of the Department of Employment, Economic Development and Innovation Brisbane, Australia.

Davis F (2012) Pathology experts plan priority projects. *Australian Citrus News* **89**, 9.

Smith MW, Miles AK, Fullelove G (2013) Managing citrus orchards affected by wet weather. *DAFF factsheet* (updated from 2011)

General media articles

Powell S (2009) DPI Project reporting forum a huge success. In 'Queensland Citrus Grower IDO Report', Citrus Australia Limited, 10th March 2009.

Lamond S (2009) Screen house to give citrus growers clean plants. In 'ABC Rural Report'. (ABC: Radio).

Lamond S (2009) Citrus industry prepares for threat of exotic disease. In 'ABC Rural Report'. (ABC: Radio).

Fisher K (2012) Seeking approval for a trusty defence against brown spot - iprodione. *Australian Citrus News* **89**, 36-37.

Mecham L (2013) R&D focus on better fungal disease control. *Australian Citrus News* **89**, 29.

Related project final reports and travel reports completed during this project

Miles AK (2008) 'Report on the Workshop on Huanglongbing (*Candidatus* Liberibacter spp.) and the Asian Citrus Psyllid (*Diaphorina citri*), Hermosillo, Sonora State, Mexico, May 7-9, and pre-workshop visit to Riverside, California, USA.' Tree Pathology Centre (University of Queensland and Department of Primary Industries and Fisheries)

Wyatt P, Miles AK, et al. (2008) 'Enhancing citrus black spot management to facilitate market access opportunities for Queensland citrus.' Department of Primary Industries and Fisheries, Asian Markets for Horticulture Initiative, Brisbane, Queensland.

Smith MW, Miles AK (2009) Status of field trials established in NE India under "Improving subtropical citrus production in Sikkim and Australia CS1/2002/030" and related international HLB and citrus research activity in China and Japan. A report prepared for the Australian Centre for International Agricultural Research.

Miles AK, Wyatt P, Kopittke R, Wright C, Eelkema M, Drenth A (2010) 'CT03005 Expanding citrus market access using a systems approach to control black spot.' Horticulture Australia Limited, Sydney.

Posters and abstracts

Miles AK, Gambley C, Telford G (2008) Verifying the eradication of citrus canker (*Xanthomonas citri* subsp. *citri*) from Emerald, Australia. In '11th International Citrus Congress'. Wuhan, China, 26th-30th October.

Miles AK, Donovan N, Holford P, Davis R, Grice K, Smith M, Drenth A (2009) Investigating the potential of in-field starch accumulation tests for targeted citrus

pathogen surveillance in Australia. In '17th Australasian Plant Pathology Conference, Plant Health Management'. Newcastle, NSW p. 180.

Miles AK, Smith MW, Drenth A (2011) Fighting brown spot of mandarins. In 'Citrus Australia National Conference'. Wolf Blass Visitor Centre, Barossa Valley, South Australia.

Pearce CA, Miles AK (2011) Engaging industry personnel in biosecurity surveillance: Biosecurity Queensland's exotic citrus pest training. In 'ACPP APPS Darwin 2011, New Frontiers in Plant Pathology for Asia and Oceania'. Darwin Convention Centre, Darwin, NT p. 139.

Pearce CA, Miles AK (2011) Engaging industry personnel in biosecurity surveillance: Biosecurity Queensland's exotic citrus pest training. In 'Citrus Australia National Conference'. Wolf Blass Visitor Centre, Barossa Valley, South Australia. (poster & oral)

Miles AK, Papacek DF, Harty A, Drenth A (2012) New fungicides to protect citrus. In 'Citrus Australia National Conference'. 21st-24th October, Leeton Soldiers Club, Leeton, NSW. (poster & oral)

Miles AK, Newman TK, Gultzow DL, Parfitt SC, Drenth A, Smith MW (2012) S15P12 Commercial-scale Alternaria Brown Spot resistance screening as the first step in breeding new mandarins for Australia. In 'XII International Citrus Congress'. Valencia, Spain p. 263.

Oral presentations

Gambley C, Benham M, Miles AK, Smith LS, Whittle PJL (2007) Evaluation of potential citrus canker inoculum reservoirs in Emerald, Queensland. In '16th Biennial Australasian Plant Pathology Society Conference - Back to Basics: Managing Plant Diseases'. Adelaide, Australia, 24th-27th September.

Miles AK, Donovan N (2007) Key exotic disease threats to the Australian citrus industry. In 'Australasian Plant Pathology Society Seminar Series'. (Indooroopilly Research Centre, Brisbane. 30th October).

Barkley P, Beattie GAC (2008) Contingency planning for HLB (huanglongbing) and its vectors in Australia. In 'Taller Internacional sobre el Huanglongbing y el Psilido Asiatico de los Citricos, 7 al 9 de mayo de 2008'. Hermosillo, Sonora, Mexico. (Oral presentation given by Andrew Miles on behalf of the authors).

Miles AK, Wyatt P, Kopittke R, Eelkema M, Missenden B, Hamacek E, Shivas M, Drenth A (2008) Integrated disease management of citrus black spot (*Guignardia citricarpa* Kiely) in Queensland, Australia. In '11th International Citrus Congress'. Wuhan, China. (Eds X Deng, J Xu, S Lin and R Guan) pp. 1070-1076. (China Agriculture Press).

Miles AK (2009) Integrated Disease Management of Citrus Black Spot (*Guignardia citricarpa* Kiely) in Queensland, Australia. In 'DPI&F R&D Day'. Gayndah.

Miles AK (2009) Phytophthora diseases of citrus. In 'CITT Groups'. (Mareeba, Queensland. 17th September).

Miles AK (2009) Citrus black spot. In 'CITT Groups'. (Mareeba, Queensland. 17th September).

Miles AK, Donovan N (2009) Citrus pathology - key contributions to industry. In 'Citrus Australia Limited National Conference, 8-11th November'. (Mildura, Victoria).

Miles AK, Smith MW, Drenth A (2011) Breeding for disease resistance in citrus: the uncommon common sense solution. In 'Australasian Plant Pathology Society Seminar Series'. (23rd June, Maroochy Research Station, Nambour, Queensland).

Miles AK (2010) Citrus canker and citrus scab. In 'Exotic Citrus Diseases Awareness Training Workshop. June 7th'. (Mareeba, Queensland).

Miles AK (2010) Citrus canker and citrus scab. In 'Exotic Citrus Diseases Awareness Training Workshop. June 9th'. (Mundubbera, Queensland).

Miles AK (2010) Citrus canker and citrus scab. In 'Exotic Citrus Diseases Awareness Training Workshop. June 11th'. (Brisbane, Queensland).

Miles AK (2010) Expanding citrus market access using a systems approach to control black spot. In 'Citrus Australia Limited National Conference, 31st October - 1st November'. (Hervey Bay, Queensland).

Miles AK, Tan YP (2010) Updates on citrus scab (*Elsinoë* spp.). In '3rd Citrus Pathology Workshop. 29th-30th November'. (Dareton Research Station, Dareton, New South Wales).

Miles AK, Donovan N (2011) Key exotic disease threats to the Australian citrus industry. In 'Staff Seminar'. (Berrimah Research Farm, North Territory. 16th September).

Miles AK, Wyatt P (2012) Citrus black spot & market access. In 'Citrus Postharvest Disinfestation for Market Access Planning Workshop'. (Holiday Inn, Melbourne Airport. 25th July).

Miles AK, Papacek DF, Harty A, Drenth A (2012) New fungicides to protect citrus. In 'Citrus Australia National Conference'. 21st-24th October, Leeton Soldiers Club, Leeton, NSW. (Citrus Australia Limited).

Miles AK (2013) Fungicide evaluation trials. In 'Citrus Australia Regional Forum'. 12th March, Gayndah, Queensland.

4.6 Project outputs

The Resource, Technical Support, Extension, Training issue of this project proposed to deliver a number of project outputs for industry. The proposed outputs and how these have been individually addressed is provided:

1. Research workshops with researchers and industry leaders to discuss and review ongoing research and plan new activities.

Project CT07012 delivered a total of four citrus pathology workshops. Each workshop included representatives of all research agencies actively involved in citrus pathology research, as well as at least one representative from the industry peak body. At the commencement of CT07012 a total of five workshops were intended, however this fifth workshop (scheduled for 2011) was removed from the project milestones in order to re-prioritise resources for undertaking work on citrus scab, iprodione registration, and the Queensland study tour with Megan Dewdney as per a milestone variation processed in December 2012. To ensure that in the absence of a workshop in 2011 the pathology group remained informed of the CT07012 project activities, Andrew Miles circulated a comprehensive written update of 2011 activities.

2. At least one written extension article per year in a relevant publication.

A comprehensive list of all the written and oral material produced during this project is provided in Section 4.5, and exceeds the project requirements for this output. Key examples from each year of the project are:

Miles AK, Hardy S (2008) Anthracnose - a potential problem this season. *Coastal Fruitgrowers' Newsletter* **68**, 8.

Miles AK, Donovan N, Gambley C, Emmett RW, Barkley P (2009) Citrus. In 'Diseases of Fruit Crops in Australia'. (Eds AW Cooke, D Persley and S House) pp. 91-118. (CSIRO Publishing: Collingwood, Victoria).

Miles AK, Smith MW (2010) New mandy varieties show signs of brown spot resistance. *Australian Citrus News* **87**, 17.

Miles AK (2011) Tips for the emergency use of iprodione and Amistar. *Citrus Australia Website*.

Miles AK, Papacek DF, Harty A, Drenth A (2012) New fungicides to protect citrus. In 'Citrus Australia National Conference'. 21st-24th October, Leeton Soldiers Club, Leeton, NSW. (Citrus Australia Limited).

Smith MW, Miles AK, Fullelove G (2013) Managing citrus orchards affected by wet weather. *DAFF factsheet* (updated from 2011)

3. Updating of citrus pathology scientific information as a team effort.

The best example of the updating of citrus pathology scientific information as a team effort through project CT07012 is the publication of the citrus chapter in the CSIRO publication 'Diseases of Fruit Crops in Australia'. The chapter was authored by citrus

researchers from Qld DPI&F, NSW DPI, DPI VIC, and Australian Citrus Growers Inc.

4. Attendance and strong representation at grower forums.

Throughout project CT07012, Andrew Miles represented project CT07012 at the following grower forums:

2009 - Citrus Research, Development and BBQ Afternoon, Gayndah, and the Citrus Australia National Conference, Mildura.

2010 – Citrus Australia National Conference, Hervey Bay.

2011 – Citrus Australia National Conference, Barossa Valley, and Queensland citrus study tour with Megan Dewdney.

2012 – Citrus Australia National Conference, Leeton.

2013 – Citrus Australia Regional Forum, Gayndah.

5. Attendance and/or presentation at one or more plant pathology forums nationally.

Andrew Miles has represented project CT07012 and citrus pathology research at the 16th, 17th, and 18th Australasian Plant Pathology Society Conferences held in 2007, 2009 and 2011, respectively.

6. Australian representation of one or more pathologists, at one or more international conferences, workshops, and study tours of benefit to the industry.

The details of Australian citrus pathology representation at international fora are provided in section 4.4 Conferences and overseas study tours. A prominent example was the attendance of the 11th International Citrus Congress and 8th International Congress of Citrus Nurserymen held in China in 2008. Andrew Miles was able to attend and present research findings at these events through the Australian Centre for International Agricultural Research (ACIAR) project CS1/2002/030 and project CT07012.

7. Grower field days to facilitate technology transfer during the project.

During project CT07012, Andrew Miles presented research findings at five field days/grower technical sessions. These field days/technical sessions were the DPI&F R&D Day, Mareeba CITT Group meeting, Citrus Australian National Conference technical sessions in 2010 and 2012, and the Citrus Australia Regional Forum in 2013.

8. Provision of technical pathology support to the Australian citrus industry as required.

Technical pathology support has been provided to the Australian citrus industry through project CT07012 across several areas of importance to industry. Section 4.3 Technical field visits and diagnostics describes examples of pathology support at the orchard level. At the germplasm level, the collaboration between pathology and breeding has made significant progress towards the development of new varieties resistant to brown spot. Project CT07012 has also provided technical pathology

support to industry in ensuring that import risk analyses are undertaken to a high level of scientific rigour.

4.7 Conclusions and future directions

Project CT07012 'Citrus Pathology Resource Scientist' has provided the citrus industry with access to a network of citrus pathology expertise that were formally interlinked through the citrus pathology workshops. Issues ranging from orchard problems through to assessment of import risk analysis were primarily handled by Andrew Miles as the plant pathologist resourced by the project, with input and advice provided in-kind by other project collaborators. This arrangement was generally beneficial to all involved, as issues were resolved for industry, whilst collaborators not directly resourced by the project did not hold ultimate responsibility for resolving the issues. Whilst generally this approach delivered results for industry, it remains a compromise in the absence of industry support to a wider range of expertise in Australia.

The various issues having required technical field visits and diagnostics demonstrate the scope of problems that can arise for citrus producers. Therefore, access for the citrus industry to Resource, Technical Support, Extension and Training needs to be maintained at all times. However, the expertise needed to provide services in this area continues to decline. Project CT07012 primarily retained the expertise of just one plant pathologist, Andrew Miles. The project financed the majority (70%) of the salary cost, with the Department of Agriculture, Fisheries and Forestry, Queensland, providing the remainder. Despite the ability of this project to retain one full time plant pathologist during the term of the project, expertise has continued to decline, including reductions in expertise within industry and government. The decline in expertise will reduce the industry's ability to access information in the future. In the short term, maintaining expertise for the citrus industry is heavily relying on already stretched industry levy funding. Therefore at the final citrus pathology workshop a range of project proposals were drafted to continue to support citrus pathology activities through industry levies. Four project areas were drafted: biosecurity (initially Ceri Pearce, DAFF, and later Plant Health Australia); graft transmissible diseases (Nerida Donovan, NSW DPI); postharvest integrity (Peter Taverner, SARDI) and; fungal pathogens (Andrew Miles, DAFF). Full proposals were submitted to HAL in November 2012. Together these projects would greatly improve industry's access to Resource, Technical Support, Extension and Training.

Project CT07012 has shown a strong commitment to building a national and international profile for citrus pathology in Australia through publication of research in peer-reviewed journals, presentation at national and international conferences, and participating in study tours with international collaborators. Many specific examples of this are presented throughout this chapter of the report. A major benefit of building this profile is the increased opportunity for international collaboration and investment in research areas that are priorities for the industry. This opportunity materialised into an invitation from Megan Dewdney, University of Florida, to Andrew Miles and Andre Drenth to prepare a collaborative CBS project proposal for submission to the Citrus Research and Development Foundation (CRDF) in Florida. A proposal was prepared and submitted to the CRDF in November 2012. A complementary voluntary contribution proposal was also prepared and submitted to HAL in November 2012. If these proposals are successful the research undertaken is likely to benefit both Florida

and Australia in terms of managing CBS, but also increases external investment in citrus pathology research.

The new project proposals submitted to HAL, if granted, will be critical to maintaining grower access to Resource, Technical Support, Extension and Training. All the proposals include formal technology transfer components as required by HAL, but resourcing of the Australian expertise through these grants will also provide a significant amount of informal technology transfer and access to expertise. Therefore, without the resourcing of expertise through HAL, or their respective service providers, this formal and informal access to Resource, Technical Support, Extension and Training will be at risk.

Acknowledgements

We thank Horticulture Australia Limited and the citrus growers of Australia for the financial support of this project. We also thank the team at Citrus Australia Limited for their support of the project. Particular thanks go to B.J. & J.E. Trott & Sons, Spencer Ranch, Abbotsleigh Citrus and Glengrove Citrus for hosting field trials, and the many other citrus orchards that have participated in project activities. The project team thanks all the project collaborators for their input to this project. Particular thanks go to Dan Papacek and the team at Bugs for Bugs for their significant in-kind contributions to several activities of the project. We also thank Malcolm Wallis (Citricare) and Brian Gallagher (Citrus Monitoring Services). We thank Malcolm Smith and the team at the Bundaberg Research Station, and Helen Hofman and the team at the Maroochy Horticultural Research Station, Department of Agriculture, Fisheries and Forestry (DAFF). We are grateful for the assistance of Peter Dal Santo (Agaware Consulting) and Dale Griffin (Crop Protection Research) for input into matters of fungicide access and registration. Thanks to Carole Wright (DAFF) for providing biometry services to the project. We thank the Horticultural Pathology Team (DAFF) and the Tree Pathology Team (The University of Queensland) for their many and varied contributions to the project. We also thank Bob Emmett, Richard Davis, Malcolm Smith and Paul Holford for comments on the draft final report.

Bibliography

Agostini, J. P., Mackenzie, S. J. & Adaskaveg, J. E. (2006). Effect of fungicides and storage conditions on postharvest development of citrus black spot and survival of *Guignardia citricarpa* in fruit tissues. *Plant Disease* 90(11): 1419-1424.

Aubert, B. (1990).Integrated activities for the control of huanglungbing-greening and its vector *Diaphorina citri* Kuwayama in Asia. In *Proceedings of the 4th International Asia Pacific Conference on Citrus Rehabilitation*, 133-144 (Eds B. Aubert, S. Tontyaporn and D. Buangsuwon). Chiang Mai, Thailand, 4-10th Feb: UNDP-FAO.

Avenot, H. F. & Michailides, T. J. (2007). Resistance to boscalid fungicide in *Alternaria alternata* isolates from pistachio in California. *Plant Disease* 91(10): 1345-1350.

Baayen, R. P., Bonants, P. J. M. & Carroll, G. C. (2003). Diagnostic protocols for regulated pests: *Guignardia citricarpa*. *EPPO Bulletin* 33: 271-280.

Baldassari, R. B., Reis, R. F. & de Goes, A. (2006). Susceptibility of fruits of the 'Valencia' and 'Natal' sweet orange varieties to *Guignardia citricarpa* and the influence of the coexistence of healthy and symptomatic fruits. *Fitopatologia Brasileira* 31(4): 337-341.

Baldassari, R. B., Wickert, E. & de Goes, A. (2008). Pathogenicity, colony morphology and diversity of isolates of *Guignardia citricarpa* and *G-mangiferae* isolated from *Citrus* spp. *European Journal of Plant Pathology* 120(2): 103-110.

Barkley, P. (1991). Outbreak of orange stem pitting in Queensland. *Australian Citrus News* 67(January): 7-10.

Barkley, P. & Forsyth, J. (1987). Funding for virus testing of trees in NSW citrus budwood multiplication scheme. *Australian Citrus News* 63(November): 12-15.

Barkley, P., Weppler, R., Eyles, G., Hardy, S., Donovan, N. & Pearce, C. (2010).Huanglongbing and citrus canker study tour to USA, 19th January to 6th February. 85: Horticulture Australia Limited and Citrus Australia Limited.

Bhuiyan, S., Croft, B., James, R. & Cox, M. (2012). Laboratory and field evaluation of fungicides for the management of sugarcane smut caused by *Sporisorium scitamineum* in seedcane. *Australasian Plant Pathology* 41(6): 591-599.

Broadbent, P., Brlansky, R. H. & Indsto, J. (1996). Biological characterization of Australian isolates of Citrus iristeza virus and separation of subisolates by single aphid transmissions. *Plant Disease* 80(3): 329-333.

Brodrick, H. T. & Rabie, C. J. (1970). Light and temperature effects on symptom development and sporolation of *Guignardia citricarpa* Kiely, on *Citrus sinenisis* (Linn) Osbeck. *Phytophylactica* 2(3): 157-164.

Campbell, C. L. & Madden, L. V. (1990).Temporal analysis of epidemics. I. Description and comparison of disease progress curves. In *Introduction to plant disease epidemiology*, 161-202 (Eds C. L. Campbell and L. V. Madden). New York, USA: Wiley.

Canihos, Y., Peever, T. L. & Timmer, L. W. (1999). Temperature, leaf wetness, and isolate effects on infection of Minneola tangelo leaves by *Alternaria* sp. *Plant Disease* 83: 429-433.

Chapman, J. C. & Hutton, D. G. (1988). Citrus blight in Australia. *Australian Citrus News* 64: 15-16.

CRI (2012).CRI Group Annual Report 2011/2012. Citrus Research International.

Dalkilic, Z., Timmer, L. W. & Gmitter, F. G. (2005). Linkage of an Alternaria disease resistance gene in mandarin hybrids with RAPD fragments. *Journal of the American Society for Horticultural Science* 130(2): 191-195.

Daus, A., Horev, B., Dvir, O., Ish-Shalom, S. & Lichter, A. (2011). The efficacy of ultrasonic fumigation for disinfestation of storage facilities against postharvest pathogens. *Postharvest Biology and Technology* 62(3): 310-313.

Davis, F. (2012). Pathology experts plan priority projects. *Australian Citrus News* 89(Oct/Noc): 9.

Dubois, J. W., Byrne, A. & Clark, J. E. (2000). Canadian bluestain fungi: Variation in tolerance to sapstain control biocides. *Forest Products Journal* 50(1): 60-66.

Fernandez-Ortuno, D., Chen, F. & Schnabel, G. (2012). Resistance to pyraclostrobin and boscalid in *Botrytis cinerea* isolates from strawberry fields in the Carolinas. *Plant Disease* 96(8): 1198-1203.

Fullerton, R. A., Harris, F. M. & Hallett, I. C. (1999). Rind distortion of lemon caused by *Botrytis cinerea* Pers. *New Zealand Journal of Crop and Horticultural Science* 27: 205-214.

Garnier, M. & Bove, J. M. (2000).Huanglongbing (Greening). In *Compendium of Citrus Diseases Second Edition*, 46-48 (Eds L. W. Timmer, S. M. Garnsey and J. H. Graham). St. Paul, Minnesota: APS Press.

Garnier, M., Jagoueix-Eveillard, S., Cronje, P. R., Le Roux, H. F. & Bove, J. M. (2000). Genomic characterization of a liberibacter present in an ornamental rutaceous tree, Calodendrum capense, in the Western Cape Province of South Africa. Proposal of 'Candidatus Liberibacter africanus subsp. capensis'. *International Journal of Systematic and Evolutionary Microbiology* 50(6): 2119-2125.

Glienke, C., Pereira, O. L., Stringari, D., Fabris, J., Kava-Cordeiro, V., Galli-Terasawa, L., Cunnington, J., Shivas, R. G., Groenewald, J. Z. & Crous, P. W. (2011). Endophytic and pathogenic *Phyllosticta* species, with reference to those associated with Citrus Black Spot. *Persoonia* 26: 47-56.

Gramaje, D., Aroca, A., Raposo, R., Garcia-Jimenez, J. & Armengol, J. (2009). Evaluation of fungicides to control Petri disease pathogens in the grapevine propagation process. *Crop Protection* 28(12): 1091-1097.

Harty, A. (2010).Response to OHMA: USA citrus market access submission, 15th October. Mildura, Victoria: Citrus Australia Limited.

Harty, A. (2012).7th Citrus Research Symposium - Citrus Research International, South Africa 19-22 August 2012. Mildura, Australia: Citrus Australia Limited.

Hewitt, H. G. (1998). *Fungicides in crop protection.* Wallingford, UK: Cab International.

Hincapie, M., Wang, N.-Y., Peres, N. A. & Dewdney, M. M. (*Accepted*). Baseline sensitivity of *Guignardia citricarpa*, the causal agent of citrus black spot, to azoxystrobin and pyraclostrobin. *Plant Disease* 97.

Hodges, A. W. & Spreen, T. H. (2012).Economic impacts of citrus greening (HLB) in Florida, 2006/07–2010/11. EDIS document FE903 Gainesville, FL Food and Resource Economics Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, .

Howie, B. (2012).Plant Pathology and Entomology Capability Study 2012. 73 Cabarita, NSW: C-Qual Agritelligence Pty Ltd.

Hutton, D. (1989). The Appearance of Iprodione Resistance in Alternaria Alternata, the Cause of Brown Spot of Murcott Tangor. *Australasian Plant Pathology* 18(2): 32-32.

Juergensen, L., Busnarda, J., Caux, P.-Y. & Kent, R. A. (2000). Fate, behavior, and aquatic toxicity of the fungicide DDAC in the Canadian environment. *Environmental Toxicology* 15(3): 174-200.

Jwu-guh, T. & Tsai-young, C. (1989).Ecology of citrus melanose caused by *Diaporthe citri* (Faw.) Wold and its control in Taiwan. In *Symposium on the Researches and Development of Citrus in Taiwan*, 191-199 Taiwan: Special Publication of TARI No. 27.

Kiely, T. B. (1948). Preliminary studies on *Guignardia citricarpa*, n.sp.: the ascigerous stage of *Phoma citricarpa* McAlp. and its relation to black spot of Citrus. *Proceeding of the Linnean Society of New South Wales* 73(5-6): 249-292.

Kohmoto, K., Akimitsu, K. & Otani, H. (1991). Correlation of resistance and susceptibility of citrus to *Alternaria alternata* with sensitivity to host-specific toxins. *Phytopathology* 81(7): 719-722.

Kohmoto, K., Scheffer, R. P. & Whiteside, J. O. (1979). Host-selective toxins from *Alternaria citri*. *Phytopathology* 69(6): 667-671.

Korf, H. J. G. (1998).Survival of *Phyllosticta citricarpa*, anamorph of the citrus black spot pathogen. In *Microbiology and Plant Pathology*, Vol. Master's Dissertation, 75 Pretoria, South Africa: University of Pretoria.

Kotze, J. M. (1981). Epidemiology and control of citrus black spot in South Africa. *Plant Disease* 65(12): 945-955.

Luo, Y., Ma, Z., Reyes, H. C., Morgan, D. P. & Michailides, T. J. (2007). Using real-time PCR to survey frequency of azoxystrobin-resistant allele G143A in Alternaria populations from almond and pistachio orchards in California. *Pesticide Biochemistry and Physiology* 88(3): 328-336.

Mayers, P. E. & Hutton, D. G. (1986).Full registration for iprodione for control of Alternaria brown spot in Murcott and Emperor mandarins. Nambour, Queensland: Department of Primary Industries, Queensland.

McOnie, K. C. (1964). The latent occurence in citrus and other hosts of a *Guignardia* easily confused with *G. citricarpa*, the citrus black spot pathogen. *Phytopathology* 54(1): 40-43.

Mecham, L. (2013). Biosecured - protecting the industry's production base. *Australian Citrus News* 89(June/July): 24-28.

Miles, A. K. (2011a).Review of surveillance for exotic citrus disease threats to Australia 46 Brisbane, Australia: The University of Queensland and AgriScience Queensland - a part of the Department of Employment, Economic Development and Innovation

Miles, A. K. (2011b). Tips for the emergency use of iprodione and Amistar® *Citrus Australia Website*.

Miles, A. K., Smith, M. W. & Drenth, A. (2011).Fighting brown spot of mandarins. In *Citrus Australia National Conference*Wolf Blass Visitor Centre, Barossa Valley, South Australia.

Miles, A. K., Tan, Y. P., Tan, M. K., Donovan, N. J., Ghalayini, A. & Drenth, A. (2013). *Phyllosticta* spp. on cultivated Citrus in Australia. *Australasian Plant Pathology* 42(4): 461-467.

Miles, A. K., Willingham, S. L. & Cooke, A. W. (2004). Field evaluation of strobilurins and a plant activator for the control of citrus black spot. *Australasian Plant Pathology* 33(3): 371-378.

Miles, A. K., Willingham, S. L. & Cooke, A. W. (2005). Field evaluation of a plant activator, captan, chlorothalonil, copper hydroxide, iprodione, mancozeb and strobilurins for the control of citrus brown spot of mandarin. *Australasian Plant Pathology* 34: 63-71.

Miles, A. K., Wyatt, P., Kopittke, R., Eelkema, M., Missenden, B., Hamacek, E., Shivas, M. & Drenth, A. (2008).Integrated disease management of citrus black spot (*Guignardia citricarpa* Kiely) in Queensland, Australia. In *11th International Citrus Congress*, Vol. 2, 1070-1076 (Eds X. Deng, J. Xu, S. Lin and R. Guan). Wuhan, China: China Agriculture Press.

Owen-Turner, J. (1990). Suspected severe pitting strain of tristeza virus discovered in Washington navels. *Queensland Citrus Bulletin* Autumn: 2-15.

Pegg, K. G. (1966). Studies of a strain of *Alternaria citri* Pierce, the causal organism of brown spot of Emperor mandarin. *Queensland Journal of Agricultural and Animal Sciences* 23: 15-28.

Poland, J. A., Balint-Kurti, P. J., Wisser, R. J., Pratt, R. C. & Nelson, R. J. (2009). Shades of gray: the world of quantitative disease resistance. *Trends in Plant Science* 14(1): 21-29.

Possiede, Y. M., Gabardo, J., Kava-Cordeiro, V., Galli-Terasawa, L. V., Azevedo, J. L. & Glienke, C. (2009). Fungicide resistance and genetic variability in plant pathogenic strains of *Guignardia citricarpa*. *Brazilian Journal of Microbiology* 40(2): 308-313.

Reis, R. F., de Almeida, T. F., Stuchi, E. S. & de Goes, A. (2007). Susceptibility of citrus species to *Alternaria alternata*, the causal agent of the alternaria brown spot. *Scientia Horticulturae* 113(4): 336-342.

Rohel, E. A., Cavelier, N. & Hollomon, D. W. (2001). Microscopic analysis of the effect of azoxystrobin treatments on *Mycosphaerella graminicola* infection using green fluorescent protein (GFP)-expressing transformants. *Pest Management Science* 57(11): 1017-1022.

Rosenzweig, N., Atallah, Z. K., Olaya, G. & Stevenson, W. R. (2008). Evaluation of QoI fungicide application strategies for managing fungicide resistance and potato early blight epidemics in Wisconsin. *Plant Disease* 92(4): 561-568.

Schubert, T. S., Dewdney, M. M., Peres, N. A., Palm, M. E., Jeyaprakash, A., Sutton, B., Mondal, S. N., Wang, N.-Y. & Rascoe, J. (2012). First report of citrus black spot caused by *Guignardia citricarpa* on sweet orange [*Citrus sinensis* (L.) Osbeck] in North America. *Plant Disease* 96(8): 1225.

Schubert, T. S., Rizvi, S. A., XiaoAn, S., Gottwald, T. R., Graham, J. H. & Dixon, W. N. (2001). Meeting the challenge of eradicating citrus canker in Florida-again. *Plant Disease* 85(4): 340-356.

Schutte, G. C. (2008). Reduction of contact fungicide tank rates by addition of a patented DDAC (Sporekill(r)) for the control of *Guignardia citricarpa* and *Alternaria alternata* on citrus in South Africa. *Journal of Plant Pathology* 90(3): 601-602.

Schutte, G. C., Beeton, K. V. & Kotze, J. M. (1997). Rind stippling on valencia oranges by copper fungicides used for control of citrus black spot in South Africa. *Plant Disease* 81(8): 851-854.

Schutte, G. C., Mansfield, R. I., Smith, H. & Beeton, K. V. (2003). Application of azoxystrobin for control of benomyl-resistant *Guignardia citricarpa* on Valencia oranges in South Africa. *Plant Disease* 87(7): 784-788.

Sette, F., Hawtin, J. & Argus, S. (2011).National Citrus Plantings Database CT10035. 89 Sydney, Australia: Horticulture Australia Limited.

Smith, D. (2002).Culturing, preservation and maintenance of fungi. In *Plant Pathologist's Pocketbook, 3rd Edition*, 390-391 (Eds J. M. Waller, J. M. Lenne and S. J. Waller). Wallingford, UK: CABI Publishing.

Smith, D. & Papacek, D. F. (1991). Studies of the predatory mite *Amblyseius victoriensis* (Acarina: Phytoseiidae) in citrus orchards in south-east Queensland: control of *Tegolophus australis* and *Phyllocoptruta oleivora* (Acarina: Eriophyidae), effect of pesticides, alternative host plants and augmentative release. *Experimental and Applied Acarology* 12(3-4): 195-217.

Solel, Z., Oren, Y. & Kimchi, M. (1997). Control of Alternaria brown spot of Minneola tangelo with fungicides. *Crop Protection* 16(7): 659-664.

Stevenson, K. L., Langston, D. B. & Sanders, F. (2008). Baseline sensitivity and evidence of resistance to boscalid in *Didymella bryoniae*. *Phytopathology* 98(6S): S151.

Swart, S. H., Wingfield, M. J., Swart, W. J. & Schutte, G. C. (1998). Chemical control of alternaria brown spot on Minneola tangelo in South Africa. *Annals of Applied Biology* 133(1): 17-30.

Timmer, L. W. (2000).Scab Diseases. In *Compendium of Citrus Diseases*, 31-32 (Eds L. W. Timmer, S. M. Garnsey and J. H. Graham). St Paul, MN: Phytopathological Society Press, Inc.

Timmer, L. W., Darhower, H., Zitko, S. E., Peever, T. L., Ibanez, A. & Bushong, P. M. (2000). Environmental factors affecting the severity of alternaria brown spot of citrus and their potential use in timing fungicide applications. *Plant Disease* 84: 638-643.

Timmer, L. W., Priest, M., Broadbent, P. & Tan, M. K. (1996). Morphological and pathological characterization of species of *Elsinoe* causing scab diseases of citrus. *Phytopathology* 86(10): 1032-1038.

Timmer, L. W., Solel, Z., Gottwald, T. R., Ibanez, A. & Zitko, S. E. (1998a). Environmental factors affecting production, release, and field populations of conidia of *Alternaria alternata*, the cause of brown spot of citrus. *Phytopathology* 88: 1218-1223.

Timmer, L. W. & Zitko, S. E. (1997). Evaluation of fungicides for control of alternaria brown spot and citrus scab. *Proceedings of the Florida State Horticultural Society* 110: 71-76.

Timmer, L. W., Zitko, S. E. & Albrigo, L. G. (1998b). Split applications of copper fungicides improve control of melanose on grapefruit in Florida. *Plant Disease* 82(9): 983-986.

Tsay, J.-G. & Chuang, T.-Y. (1986). Ecological study of citrus melanose caused by *Diaporthe citri*. *Chinese gardening* 32(2): 109-116.

Tsuge, T., Harimoto, Y., Akimitsu, K., Ohtani, K., Kodama, M., Akagi, Y., Egusa, M., Yamamoto, M. & Otani, H. (2013). Host-selective toxins produced by the plant pathogenic fungus *Alternaria alternata*. *FEMS Microbiology Reviews* 37(1): 44-66.

USDA (2010).Risk assessment of *Citrus* spp. fruit as a pathway for the introduction of *Guignardia citricarpa* Kiely, the organism that causes Citrus Black Spot disease Raleigh, North Carolina: Animal and Plant Health Inspection Service, United States Department of Agriculture.

USDA (2013). Importation of Fresh Citrus Fruit From Uruguay, Including Citrus Hybrids and *Fortunella* spp., Into the Continental United States, 7 CFR Part 319 [Docket No. APHIS–2011–0060] RIN 0579–AD59. *Federal Register* 78(25): 8435-8441.

Vicent, A., Armengol, J. & Garcia-Jimenez, J. (2007). Rain Fastness and Persistence of Fungicides for Control of Alternaria Brown Spot of Citrus. *Plant Disease* 91(4): 393-399.

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

Whiteside, J. O. (1990). Prospects for improved control of citrus scab with fungicide spray treatments. *Proceedings of the Florida State Horticultural Society* 103: 4-7.

Wyatt, P., Miles, A. K., Eelkema, M., Missenden, B., Shivas, M., Kopittke, R., Hamacek, E., van Brunschot, S., Peek, T. & Lloyd, A. C. (2008).Enhancing

citrus black spot management to facilitate market access opportunities for Queensland citrus. In *Asian Markets for Horticulture Initiative*, 58 Brisbane, Queensland: Department of Primary Industries and Fisheries, Asian Markets for Horticulture Initiative.

Xiao, Y. & Kreber, B. (2005). Effect of IPBC/DDAC on spore germination and hyphal growth of the sapstaining fungus *Ophiostoma piceae*. *Holzforschung* 53(3): 237-243.