Australian Government
Cotton Research and Development Corporation

FINAL REPORT 2013

Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

CRDC Project Number: **DAQ1202**

Project Title: Cross-industry preparedness for Cotton leaf curl disease

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CRDC Program: Farming systems & Human Capacity

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Part 3 – Final Report

(The points below are to be used as a guideline when completing your final report.)

Background

Cotton leaf curl disease (CLCuD) is caused by a complex of different begomoviruses and DNA-β satellite molecules. Both the viruses and the satellites are transmitted by the Silverleaf whitefly (SLW). CLCuD is responsible for serious economic losses to cotton production in several areas where it occurs. For example, CLCuD cost the Pakistan industry an estimated US$5 billion between 1992 and 1997. CLCuD represents a serious biosecurity risk to the Australian industry. Early detection of the disease if introduced into Australia is vital to minimise economic losses. This is, through containment to limit disease spread, eradication if feasible or early implementation of management packages if eradication is not feasible.

Begomoviruses are considered one of the major emerging viral threats to crop production worldwide (Mansoor et al. 2006; Varma and Malathi 2003). The number of begomoviruses causing CLCuD has risen from only a few species in 1992 to at least six species, with about 30 different genomic sequence variants within those six species, in 2008 (Fauquet et al. 2008). A similar emergence of different DNA-β satellites has also been observed. The continual succumbing of CLCuD-resistant cotton varieties to the disease in Pakistan and India indicates an ongoing emergence of new virus species and/or DNA-β satellites. Several of these viruses and DNA-beta satellites also infect and cause disease in a range of vegetables and ornamentals, including tomato, chilli, capsicum, cucurbits and hibiscus and as many of these plants species are susceptible to more than one begomvirus, there is opportunity for the emergence of new begomoviruses. This is because multiple virus infections within the one host plant can facilitate a genetic mixing between the different virus species, leading to emergence of new species with altered host ranges and pathogenicity. Given, new begomoviruses or DNA-β satellites, may arise in plant species other than cotton, the viruses of concern to the cotton industry are not just those currently associated with CLCuD, but should also include potential emergence of new species in other plant species. Hence detection and management of CLCuD will involve multiple commodities (eg vegetables and ornamentals) and not be limited to cotton.

Many begomovirus species, several of which potentially cause CLCuD, are present in countries to the immediate north of Australia, such as Indonesia. Establishment of begomoviruses in northern Australia could occur in native vegetation or weeds, including native and endemic *Gossypium* and *Hibiscus* species, or in vegetable crops, and once established represent a significant threat for spread to southern cropping areas. Incursions of these viruses could occur through importation of infected plant material, or in virus-laden whiteflies associated with the movement of people, plant material or in tropical cyclones.

Within the last 5 years, Australia had two incursions of whitefly-transmitted viruses, including the begomovirus, *Tomato yellow leaf curl virus* (TYLCV). The incursion of the two viruses in distinctly different Australian growing regions and their presence at multiple locations demonstrates the existence of multiple entry and spread pathways. *Abutilon mosaic virus* (AbMV), another begomovirus, has been present in Australia for decades. The ornamental industry sells infected Chinese lantern (*Abutilon* spp., family *Malvaceae*) plants as a variegated form due to the symptoms induced by the virus. The presence of this virus in a widely distributed ornamental species highlights a significant risk pathway for virus introduction and spread. Thus, a review of the regulations governing the importation of ornamental species are imported in Australia and what restrictions apply to these imports is paramount to thoroughly analyse the risk for introduction of biosecurity threats such as CLCuD.

Objectives

1. **To review current management practices of Silverleaf whitely (SLW) and the viruses it transmits and to identify research gaps.**

To complete this objective a literature review on control of SLW and the viruses it transmits was completed. The results from the literature review were used to develop a program and identify key note speakers for a three day workshop to inform industry, scientists and biosecurity agencies of current biosecurity threats and to discuss potential management strategies to be employed during an incursion of these exotic viruses. The three-day workshop entitled 'Epidemiology and management of whitefly-transmitted viruses' was delivered on the 15th to 17th of October 2012 at the Ecosciences Precinct, Dutton Park, Brisbane.

2. **Investigate surveillance strategies for the early detection of begomoviruses through sampling and testing their whitefly vector, the SLW**

Bowen, in northern QLD was chosen as the study site for investigation of surveillance strategies to detect begomoviruses, and studies commenced in mid 2012 using TYLCV as a model system. TYLCV is an endemic begomovirus, present in Bowen since 2011. A statistically-based strategy was developed, and then tested from mid 2012 until the completion of project DAQ1202 in June 2013. The data from this preliminary testing provides valuable information on the feasibility of using SLW as an early warning surveillance target for exotic viruses.

3. **Review the importation restriction on ornamental plant species.**

A review of plant species with current import permits and their import conditions was completed using the Australian Quarantine Inspection Service ICON database. The results were collated for all relevant permits issued prior to end of June 2013.

4. **Foster a cross-industry approach to management of whitefly-transmitted viruses.**

The 'Epidemiology and management of whitefly-transmitted viruses', workshop included participants from multiple industries including cotton, grains, vegetables and nurseries. This workshop provided many opportunities for fostering cross-industry approaches to the management of whitefly-transmitted viruses and national and international networks were established. Although there was little support for a formalised taskforce for ongoing development and implementation of management strategies, linkages between groups are in place and new work was developed.

Methods

1. **To review current management practices of Silverleaf whitely (SLW) and the viruses it transmits and to identify research gaps.**

A literature review on control of SLW and the viruses it transmits was completed. The literature review summaries published information on the management of *Bemisia tabaci* biotype B (Silverleaf whitefly, SLW) and the viruses it transmits. Relevant publications from 2001 to date were considered and where appropriate, publications earlier than 2001 included. The review mostly focussed on viruses affecting cotton but also included other cropping systems where SLW-transmitted viruses are considered a major constraint to production. The results from the literature review were used to develop a program and identify key note speakers for a three day workshop on the 'Epidemiology and management of whitefly-transmitted viruses'. The literature review and the workshop discussions helped identify research gaps.

A committee for delivering the workshop was established and consisted of Cherie Gambley (Project Leader), Murray Sharman (Project Researcher), Denis Persley (Project Researcher), John Thomas (Project Researcher), Linda Smith (DAFF, Plant Pathologist and Queensland Biosecurity contact for the Cotton Industry) and David Carey (DAFF, Senior Horticulturist for the Vegetable Industry). The list of topics for discussion during the workshop included overviews of whitefly management in key industries (e.g cotton, vegetables, grains and nursery), whitefly biology and genetics, resistance monitoring of insect populations, novel diagnostics for detection of whitefly species and the viruses they transmit, overviews of endemic whitefly-transmitted viruses and possibilities for biological control of whiteflies.

Supporting funds for the workshop were sought from Horticulture Australia Limited (HAL), the Grains Research Development Corporation (GRDC), key industry participants such as seed companies, chemical companies, biosecurity agencies (e.g Plant Health Australia), crop consultancy companies and other non-levy paying industries (e.g Melon Industry, Bundaberg and Bowen tomato growing associations).

Four key international virology experts were invited to attend including Dr Rob Briddon (NIBGE, Pakistan), Dr Bill Wintermantel (USDA,California), Dr Enrique Moriones (Instituto de Hortofruticultura Subtropical y Mediterránea, Spain) and Dr Scott Atkins (USDA Florida). All of these guests with the exception of Dr Briddon were able to attend the workshop. A further guest, Dr Abdel-Salam (Cairo University, Egypt) also attended and provided his experiences on whitefly transmitted viruses of malvaceous species and vegetable crops. The remainder of the program called upon Australian virologists and entomologists to present a range of different technical aspects of managing whitefly and the viruses they transmit.

2. **Investigate surveillance strategies for the early detection of begomoviruses through sampling and testing their whitefly vector, the SLW**

The surveillance strategy is proposed to be an alternative to visual or random sampling of crops for early detection of exotic begomoviruses. To determine the feasibility of the strategy, information on the prevalence of virus within SLW populations is needed. Dr Dan Pagendam (OCE Postdoctoral Research Fellow, CSIRO), an experienced biometrician, assisted in design of preliminary experiments to develop the surveillance strategy.

Initially, it was thought the prevalence of whiteflies carrying virus (i.e viruliferous whiteflies) within areas would be reasonably high and that samples of 100 SLW per study site would be sufficient. However, preliminary data indicated the prevalence of viruliferous whiteflies and the whitefly population itself was highly influenced by time of sampling. Instead, the sampling strategy was modified to be less influenced by ecological factors and used the following statistical formula: $P= (1-p)^n$, where p=the proportion of randomly tested whiteflies which carry virus and P=probability (Cannon & Roe, 1982). For a 95% confidence in detection of virus if present at a level of 1% or more, 300 samples were proposed to be tested. To evaluate the strategy, measuring the prevalence of viruliferous whiteflies in tomato crops affected by virus, in non-host crops and in other plant species such as weeds was planned.

Preliminary experiments commenced in mid 2012 using the endemic *Tomato yellow leaf curl virus* (TYLCV) as a model system. The experiments aimed at determining the numbers of individual insects within a given population of SLW capable of spreading TYLCV (i.e viruliferous), and determining the spatial limit of detection of the virus within the insect populations, that is, at what distance away from the primary disease source is the virus still detectable within its insect vector. To support these experiments, funds were sought from HAL through three separate VC proposals, all of which were unsuccessful. As a result not all of the proposed experiments (detailed in the May 2012 progress report) were completed.

The first proposed experiment, to test the prevalence of TYLCV in SLW individuals collected from virus-affected tomato crops was completed as a series of surveys in Bowen. The number of SLW to be collected from each crop was proposed to be 300; however, the actual number collected was determined by the number available during the survey and varied from as low as 18 to more than 1500. SLW were collected by vacuum using a modified leaf blower. It was also proposed to collect SLW from crops with 1, 10 and >50% of plants affected by TYLCV. However, outbreaks of *Potato leafroll virus* (PLRV) in the study area complicated visual estimations of TYLCV incidences. Instead, surveys were completed at multiple blocks and 300 plants sampled randomly to obtain the virus incidence. This was repeated several times during the growing season to ensure a range of different TYLCV incidences were compared. The SLW were processed individually or bulked in lots of ten and the random plant samples were bulked in lots of ten for molecular indexing. All samples were tested by PCR using TYLCV-specific primers.

3. Review the importation restriction on ornamental plant species.

Initially, species which were imported under four 'end use categories' were considered in the review of the AQIS ICON database (http://www.aqis.gov.au/icon32/asp/ex_querycontent.asp). These categories included (1) cut flowers and foliage, (2) nursery stock, (3) propagation and (4) processing. However, this was later refined to only include 'nursery stock' as the remaining end use categories were considered as a negligible risk for co-importation of exotic viruses such as the begomoviruses causing CLCuD.

The begomoviruses and DNA- β satellites which cause CLCuD infect a wide range of plant species including those in the *Cucurbitaceae*, *Malvaceae* and *Solanaceae*. The ICON database searches were restricted to species within these families and for the family *Malvaceae* further restricted to genera most closely related to *Gossypium*, *Hibiscus* and other genera containing species which are known hosts of begomoviruses (e.g *Abutilon* spp., *Sida* spp. etc).

4. Foster a cross-industry approach to management of whitefly-transmitted viruses.

The first step to fostering a cross-industry approach to the management of whitefly-transmitted viruses was to hold the 'Epidemiology and management of whitefly-transmitted viruses' workshop. The workshop committee included staff from different industries and funding support for the workshop was sought from the cotton, grains, vegetables and the nursery industries. The workshop program spanned topics of interest to all industries and served to demonstrate the linkages between the industries in relation to management options and key biosecurity threats.

Following on from the workshop was the formation of a taskforce. This, however, was not supported by the group. Instead, linkages between individuals were cultivated, both with local staff and with the international guests. New research areas were developed and progressed between some participants of the workshop.

Results

1. **To review current management practices of Silverleaf whitely (SLW) and the viruses it transmits and to identify research gaps.**

A review of current literature on control of SLW and the begomoviruses they transmit is completed and attached to this report (Attachment 1). An important finding from the literature review is the detection of CLCuD in China, reported in 2010 (Cai et al. 2010). A copy of the executive summary from the review is provided below:

SLW transmits a very large range of plant viruses to a very large number of host plant species. Recent reports state the insect was known to vector over 150 different plant viruses in the tropics and subtropics (Lapidot and Polston 2010; Navas-Castillo et al. 2011). Most of these virus species belong to the *Begomovirus* genus (Family *Geminiviridae*) with a handful of species belonging to the *Crinivirus* genus (Family *Closteroviridae*), *Ipomovirus* genus (Family *Potyviridae*), *Carlavirus* genus (Family *Betaflexividae*) and the *Torradovirus* genus (Family *Secoviridae*). The ICTV lists in excess of 190 individual species of begomoviruses (Brown et al. 2012), the majority of which, are confirmed to be SLW transmitted. Of concern to the Australian cotton industry are those viruses within the *Begomovirus* genus, and in particular, the group of monopartite viruses associated with the DNA-β satellite molecule.

Management of SLW-transmitted viruses was divided into the following subcategories: (i) insecticide usage, (ii) cultural practices, (iii) germplasm resistance, (iv) biocontrol control and (v) integrated pest management. To demonstrate what is currently used overseas for management, examples of area wide management of whitefly-transmitted viruses and control strategies used for CLCuD on the Indian subcontinent and in Africa are also provided. The overall conclusion from review of these subcategories and from overseas case studies is a need for an Integrated Pest Management – Insecticide Resistance Management (IPM-IRM) strategy. This strategy needs to be implemented at a regional level.

In addition to the literature review, a three-day workshop on the ‘Epidemiology and management of whitefly-transmitted viruses’, was held to identify further research gaps. The general feedback from delegates during and after the workshop was very positive. Many appreciated having the mix of entomology and virology to discuss the topic and improve knowledge in their reciprocal field. The discussion sessions provided valuable feedback for inclusion in the draft CLCuD contingency plan and helped to focus potential new areas of research. In particular, the discussion highlighted the need to protect previous investments in relation to minimising insecticide resistance within all pest populations which are present in cotton. Any actions required in an emergency response to an incursion of CLCuD will need to consider the long term effect on insecticide resistance and factor this into the cost-benefit analyses of the emergency response.

Importantly, several of the workshop discussion points for areas of proposed research are now incorporated into two new CRDC-funded projects and a regional based horticulture project co-funded by the Bowen and Gumlu Grower Association and other industry partners. The two CRDC projects are to further progress work on SLW and CLCuD preparedness. The two projects are linked and aimed to increase the knowledge of SLW biology including the role of endosymbionts, test Australian SLW populations for ability to transmit the CLCuD pathogen complex (work to be completed in the UK) and continued post and pre-border surveillance for CLCuD through sampling plants and SLW in high risk areas within Australia and within countries close by. The third project is aimed at investigating and implementing area wide management strategies for begomoviruses in the Bowen tomato growing region.

2. **Investigate surveillance strategies for the early detection of begomoviruses through sampling and testing their whitefly vector, the SLW**

Preliminary experiments to test a surveillance strategy based on collecting and indexing SLW for exotic begomoviruses, such as those causing CLCuD, commenced in July 2012. A second sampling was completed in early November 2012. Development and testing the strategy uses the endemic *Tomato yellow leaf curl virus* (TYLCV) and the Bowen tomato production district as a model system. The experiments are aimed at determining the numbers of individual insects within a given population of SLW capable of spreading TYLCV and determining the spatial limit of detection of the virus within the insect populations, that is, at what distance away from the primary disease source is the virus still detectable within its insect vector.

The first survey of the Bowen area was to map the tomato farms in the district and to ascertain where TYLCV had previously been detected, visit some known affected properties and identify survey sites for ongoing monitoring. At the commencement of this work, TYLCV was known to occur on several properties along Euri Creek Rd and around the river delta area but it was not known to be present on properties along Collinsville Rd (aka Bowen Development Rd), however, during surveys TYLCV was confirmed present on two properties along this road (Figure 1). The study sites comprises three to four tomato blocks along Euri Ck Rd, one to two blocks within the Delta area and two along Collinsville Rd and blocks are spread over four to six properties depending on the availability of sites. As growers utilise different land parcels each season it is not always possible to study the exact same location.

Figure 1. Map of survey sites in the Bowen district. The site with a pink marker is where the first detection of *Tomato yellow leaf curl virus* was in 2011. Blue and yellow markers indicate survey sites visited in November 2012 and June 2013, respectively. Virus was detected at all sites either in trapped Silverleaf whitefly, tomato samples or both. The exception is one site in the delta area, indicated with a yellow marker, where virus was not detected in either sample type.

Information on the feasibility of using SLW as a survey target for exotic virus detection and on the epidemiology of SLW-transmitted viruses was obtained from the preliminary surveys. Firstly, as a survey target the population of SLW needs to be sufficiently high to confidently detect virus if present. For example, TYLCV was not detected from SLW at three survey sites during the June 2013 studies but was detected in the random plant samples from those sites (Table 1). This is because very low numbers of SLW were trapped at these sites (i.e 18, to 106), much below the statistical target of 300 which predicts 95% confidence to detect virus at 1% incidence. By contrast, the virus was detected from the 300 random samples at very low levels (i.e 0.4 to 1.4%). Conversely, at almost all other sites the population of SLW was higher, more were trapped and the reliability of TYLCV detection was improved. There was a single site (Delta-1) during the June 2013 surveys from which TYLCV was not detected in either sample type. The data also shows detection of TYLCV from plants can be unreliable if insufficient samples are collected., For example, TYLCV was not detected in plant samplescollected during the November 2012 surveys at two sites (Euri Creek Rd sites 2 & 3, Table 1) but the virus was detected from 20-46% of the SLW collected from these sites. Again the number of plant samples (n=100) was only a third of that required for statistical confidence of virus detectionat low levels.

Table 1. Details of virus incidence in tomato crops and Silverleaf whitefly (SLW) populations in the Bowen growing district.

Survey site	Date	Plants tested	% TYLCV Tested[2]	SLW trapped	SLW population[1]	%SLW with TYLCV[3]
Euri Ck Rd – 1	Nov-12	100	1 (0-5.5)	>1500	High	30.0 (n=100)
Euri Ck Rd – 2	Nov-12	100	0 (0-3.6)	567	Mod	20.0 (n=100)
Euri Ck Rd – 3	Nov-12	100	0 (0-3.6)	70	Low	45.7
Collinsville rd – 1	Nov-12	300	1.8 (0.6-4.2)	63	Low	2.0
Collinsville rd – 2	Nov-12	300	0.4 (0-1.9)	261	Low	4.0 (n=100)
Delta – 1	Nov-12	48	50 (29-60)	1508	High	41.0 (n=100)
Delta - 2	Nov-12	300	0.4 (0-1.9)	754	Low	28.0 (n=100)
Euri Ck Rd – 1	Jun-13	300	1 (0.2-3)	200	Low	1.0
Euri Ck Rd – 2	Jun-13	300	2.6 (1-5.4)	534	Low	0.7 (n=300; 0.8-2.5%)[1]
Euri Ck Rd – 3	Jun-13	300	1.4 (0.4-3.6)	176	Low	0.6
Euri Ck Rd – 4	Jun-13	300	0 (0-1.2)	130	Low	1.5
Collinsville rd – 1	Jun-13	300	0.4 (0-1.9)	18	Very Low	0.0
Collinsville rd – 2	Jun-13	300	1.4 (0.4-3.6)	106	Very Low	0.0
Delta – 1	Jun-13	300	0.0	25	Very Low	0.0

[1]The SLW population size was rated by dividing the number of insects trapped per number of vacuum samples taken within the crop. Very low is <1, low 1-10, moderate 11-30, high 31-50 and very high >50 insects trapped per sample. Low and very low SLW populations were difficult to observe visually whereas for moderate and above, the infestation was obvious.

[2]The percent incidence of TYLCV infections or proportion of viruliferous SLW were estimated using a statistical system developed by M. Sharman, based on a formula from Moran et al. (1983) and Rohlf and Sokal, (1969). The figures in brackets indicate the upper and lower expected percent incidences based on a 95% confidence interval.

[3]All SLW trapped were tested unless otherwise stated as (n=).

Secondly, these surveys provided useful information on the spread of a SLW-transmitted virus under Australian growing conditions. TYLCV was introduced into the Bowen growing area in 2011 and although probably established in weed species in the region, it still takes several months for the levels of virus to become significant within tomato crops. Tomato crops are planted in mid to late February and final harvests are completed by mid to late November. Visual observations from inspections in July 2012 indicated low levels of virus in crops on most properties (results not shown). The exception to this was one block on Euri creek Rd which had an incidence of 38%. Data collected during June 2013 surveys also indicated low levels (<3% at any site) of virus within crops (Table 1). This is mirrored by the low proportion of viruliferous SLW detected (<2%).

Although the virus incidences detected in crops during the November 2012 surveys were also quite low, the proportion of viruliferous SLW detected was quite high. Five of the seven sites had 20% or more trapped SLW, laden with TYLCV (Table 1). This indicates a much higher level of inoculum present within the district late in the season, as expected. Surveys conducted during August and November 2013 under the new Area Wide Management Project will provide useful additional epidemiological data.

The correlation between virus incidence and the proportion of viruliferous SLW present within a crop is difficult to establish. This is due to a range of factors including migration of adult whitefly from old crops and weeds and the population density of the whitefly. For example, at Delta-1 survey site in November 2012 there was good correlation with 50% of plants infected with TYLCV and 41% viruliferous SLW. However, at the Delta-2 site during the same survey, the correlation was poor with 0.4 and 28% TYLCV incidence in crop and SLW, respectively. This is indicative of migration into the crop of already viruliferous SLW which was also supported by anecdotal evidence from the grower. Similarly, the results from the November 2012 Euri Creek surveys of sites 2 and 3 of 20 and 46% of SLW carrying TYLCV with no or very low virus infection of plants indicate virus acquisition of virus elsewhere then migration into this crop,

3. Review the importation restriction on ornamental plant species.

Initially, ornamental plant species with import permits applicable to four 'end use categories' were considered in the review of the AQIS ICON database (http://www.aqis.gov.au/icon32/asp/ex_querycontent.asp). These categories included (1) cut flowers and foliage, (2) nursery stock, (3) propagation and (4) processing. However, this was later refined to only include 'nursery stock' as the remaining end use categories were considered as a negligible risk for co-importation of exotic viruses such as those causing Cotton leaf curl disease 0028CLCuD). The full list of species and their import conditions is provided in Attachment 2.

The begomoviruses and DNA-β satellites which cause CLCuD infect a wide range of plant species including those in the *Cucurbitaceae*, *Malvaceae* and *Solanaceae*. The ICON database searches were restricted to species within these families and for the family *Malvaceae* further restricted to genera most closely related to *Gossypium*, *Hibiscus* and other genera containing species which are known hosts of begomoviruses (e.g *Abutilon* spp., *Sida* spp. etc).

There are no high risk import permits existing for *Gossypium* spp. There is, however, an import permit for unprocessed cotton with an end use category of 'all uses other than as animal foods, fertilisers or for growing purposes'. The permit conditions (C5170) for unprocessed cotton states

"These conditions apply to consignments of unprocessed cotton including raw or seed cotton, cotton lint, linters, cotton waste and cotton stuffing. All commercial consignments of unprocessed cotton require an Import Permit. There is a NIL tolerance for cotton trash (plant material and other contamination) in unprocessed cotton imported to Australia, all imported unprocessed cotton must be free from quarantine risk material or treated upon arrival."

Importation of unprocessed cotton under these conditions is considered a negligible risk for co-importation of exotic viruses.

The current conditions for importation of other high risk hosts of CLCuD such as *Hibiscus* spp. pose a potential risk for co-importation of exotic viruses and require further investigation. *Hibiscus* spp. are a known host of the CLCuD pathogen complex, however, the host status of all individual hibiscus species and commercial varieties is largely unknown. Given the host range of the CLCuD pathogen complex is quite wide both within and outside the *Malvaceae* family; it is highly likely that many *Hibiscus* spp. and varieties are also hosts. Thus, unless tested and proven otherwise, all *Hibiscus* spp. and varieties should be considered high risk imports.

Importation of *Hibiscus* spp. is allowed under condition C13672 which means live plants are released from post-entry quarantine following a minimum grow-out phase of three months in a Post Entry Quarantine (PEQ) facility, with two inspections for visual symptoms of disease. This represents a high risk scenario for co-importation of begomoviruses if any of the plants released from PEQ facilities are infected. The symptoms of begomovirus infections are not always obvious and in some cases entirely absent. It is possible that plants would not display symptoms during the three month growth phase and infections would remain undetected. Even with longer grow out phases infections may be missed, most certainly if the plant species is an asymptomatic host. There are other plant species with similar import permit conditions that would also be considered a similar risk as hibiscus. This includes species within the *Cucurbita* and *Luffa* genera.

In addition there are many genera which contain host species for a whole range of other begomoviruses. Co-importation of these viruses is also a risk as it increases the diversity of these viruses in Australia. This will ultimately facilitate genetic recombination between viruses creating new species as seen in other regions around the world.

This review was limited to documenting import conditions of plant species which represent a risk for co-importation of CLCuD. The existence of an import permit is not direct evidence of importation. The review does not provide information on which species are entering the country, in what numbers and to which PEQ facility. This further information is very valuable to evaluate risk of co-importation of exotic pathogens and whether risk mitigation such as molecular indexing of plants whilst in the PEQ facility is warranted.

4. Foster a cross-industry approach to management of whitefly-transmitted viruses.

There was good cross-industry support for the 'Epidemiology and management of whitefly-transmitted viruses' workshop, which was attended by 56 delegates spread across various sections of industry including representatives from chemical companies (Du Pont, Bayer CropScience and Monsanto), a vegetable seed company (Rijk Zwaan), biosecurity agencies (BQ, PHA and AQIS) and several crop consultants from both horticulture and broadacre production. The full list of delegates, including their contact details, is provided in the workshop proceedings (Attachment 3).

There was also good cross-industry financial support for the workshop. In addition to the funds provided by the CRDC, further funds for the workshop were obtained from Horticulture Australia Limited (HAL) in the form of conference support and from Rijk Zwaan Australia through direct financial sponsorship and attendance by three company representatives, including an a senior staff member from The Netherlands.

Following the workshop there was a tour to and around Bundaberg to further transfer knowledge from the international experts to our local researchers, extension officers, vegetable growers and private company representatives such as Du Pont, Bugs for Bugs, Hortus, Bayer and others. This was through a combination of short presentations at a grower/industry evening and field walks within the area. The tour was funded by local grower groups and private industry through direct sponsorship.

This tour very successfully embedded many aspects of the management strategies discussed during the workshop with local staff. It also enhanced our network with these international experts to allow further transfer of knowledge into the future. The grower/industry evening assisted to promote the idea of area wide management and cross-industry engagement with local growing regions as there was strong interest shown by many attendees.

A proposed outcome from the workshop was to form a taskforce to develop and implement potential management strategies for whitefly-transmitted viruses. An open call was provided to the delegates of the workshop; however, there was limited interest in this. Instead there was significant interest in attempting direct experimental work and in particular, attempting area wide management of some viruses affecting vegetable crops. This interest was independently expressed by chemical companies, entomologists, virologists, local grower groups and a biological control company. The results of many of these experiments will be of direct benefit to the cotton and grains industry, as will be improved knowledge on the logistics of implementing such a strategy. Preliminary outcomes from the work were presented at the Cotton Pathology Meeting (Fuscom) in July 2013 and will continue to be presented, if required, at cotton and grains industry forums into the future.

Through consultation with research staff and local consultants it is agreed the Bowen horticulture production area is an appropriate location to trial area wide management. During 2011, TYCLV spread by (SLW) was found infecting Bowen tomato crops. A second virus, *Potato leaf roll virus* (PLRV; *Polerovirus*) spread by the green peach aphid is also affecting the crop and complicates in-field diagnoses because of an overlapping symptom range with TYLCV. Incidences of both viruses fluctuate during the season and throughout the district from quite low to high. This, in combination with the geographical isolation of this tomato production area relative to others, makes Bowen an ideal location for evaluating area wide management strategies. The area wide management project commenced in July 2013 and is in collaboration with local researchers, consultants and the Bowen and Gumlu Growers Association (BGGA).

References:

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Outcomes

- **A literature review of published research on management strategies. A two-day workshop held to review & discuss this topic.**

The expected science outcome from these two outputs was the identification of research gaps in management of SLW & begomoviruses. Both the literature review and the workshop contributed to this outcome. In particular, the literature review assisted in the program design for the workshop and identified key researchers to approach for workshop presentations. During the workshop there was a discussion session and research gaps were documented from this discussion.

The proposed industry outcome from these two outputs was improved preparedness to CLCuD though knowledge of best management practices for its control. The literature review and workshop illustrated the importance of best management practices at a regional or area wide level to achieve the greatest success in controlling whitefly-transmitted viruses. This area wide management theme was repeatedly reported and discussed in recent literature and re-iterated by international and local researchers during the workshop. New learnings applicable to preparedness for CLCuD are documented in Version 2 of the CLCuD Contingency Plan (Attachment 4).

- **A non-crop based surveillance strategy using sampling and testing of insects for early detection of exotic diseases such as CLCuD.**

Improved diagnostics and surveillance for detection of begomoviruses was the expected science outcome for this output. Regular and large volume sampling of virus-infected tomato plants and viruliferous whiteflies has improved diagnostics through continual refinement of processes. Detection of begomoviruses from either plant tissue or whitefly samples is now a routine technique in the laboratory. The processing and analyses of the samples has also refined surveillance for these viruses with improved understanding on where and what to sample for maximal likelihood of virus detection.

The proposed outcome from this output was, improved preparedness to CLCuD though better surveillance strategies, with potential for virus detection prior to establishment in commercial crops. The proposed surveillance strategy was trialled under a range of different crop scenarios, with reliable detection of virus in almost all cases. As completion of all proposed experiments was not possible during this project, information gaps still remain in relation to virus detection prior to establishment in commercial crops. The information collated, however, provides very valuable insight in how quickly these viruses move into a commercial crop, establish and spread. In addition, important knowledge on the proportion of viruliferous whitefly present within affected crops was gained. This newly acquired epidemiology data improves preparedness to CLCuD as it is expected to behave similarly.

- **Recommendations on the potential ornamental plant species posing a risk for co-importation of exotic viruses and if the importation regulations are adequate for mitigating the risk.**

The expected science outcome for this output was improved knowledge on potential new exotic virus outbreaks. Documentation of the import conditions of plant species within three major families (*Cucurbitaceae, Malvaceae* and *Solanaceae*), of which contain species of major Australian commodities, provides an excellent resource for evaluating potential virus outbreaks that will cause significant economic losses. This list contains many known hosts of plant viruses. The review also highlights the need for improved importation regulations for high risk species (e.g *Hibiscus* spp.) if imported in high numbers.

The proposed outcome for industry for this output was improved preparedness to CLCuD though better knowledge on potential entry pathways for this disease. Importation of ornamental plant species was always considered by industry as a possible entry pathway for CLCuD. This risk is now confirmed through the review of import conditions of ornamental species. For example, the current import regulations applying to high risk CLCuD host plants such *Hibiscus* spp. are not sufficient to adequately mitigate hibiscus imports as an entry pathway because potential exists for latently-infected plants to be released as live propagation material. It is also important for co-importation of other exotic diseases of cotton such as Cotton blue disease and Cotton leaf crumple virus,

- **A taskforce for ongoing development & implementation of management approaches. The taskforce will be formed from representatives of all interested industries, including cotton & vegetables.**

The planned science outcome for this output was enhanced transfer of research and extension information to multiple industries via a far-reaching network of key contact people. This was achieved through the cross-industry workshop and post-workshop tour, during which there was ample opportunity for discussions and network establishment between local and international researchers. Although no formal taskforce was established, there was significant interest in attempting direct experimental work and in particular, attempting area wide management of some viruses affecting vegetable crops. This includes the Project Leader of DAQ1202 who has initiated and is leading a new project for area wide management of TYLCV in Bowen.

Improved preparedness for an incursion of CLCuD though cross-industry engagement was the outcome expected for industry from this output. The workshop program and discussion sessions served to educate and review management options for control of diseases such as CLCuD for all participating industries. This improved preparedness for incursions of whitefly-transmitted viruses for all those industries. Importantly, a major research area identified through the workshop was a regional management approach for diseases such as CLCuD. This will be attempted, as a direct result of the workshop, in a regional based horticulture project co-funded by the BGGA and other industry partners. This project is linked very closely with an ongoing ACIAR project in Indonesia investigating begomviruses of chilli and two newly funded CRDC projects aimed to increase the knowledge of the epidemiology of CLCuD and continued post and pre-border surveillance for the disease. The strong linkages between these projects are evidence of ongoing cross-industry engagement in this area with reciprocal transfer of information to participating industries planned.

Conclusion

A major conclusion for this project was a need for an area wide IPM approach to control whitefly-transmitted viruses, such as those that cause CLCuD. Review of published research and discussions at the 'Epidemiology and management of whitefly-transmitted viruses' workshop highlighted the benefits and limitations of area wide management. It also served to identify knowledge gaps in the epidemiology of whitefly-transmitted viruses and consequently potential gaps in management strategies. The workshop also promoted cross-industry awareness of these viruses, particularly through the acknowledgement of alternative hosts for CLCuD in horticulture and nursery industries.

As a direct result of the workshop, a regional based horticulture project co-funded by the Bowen and Gumlu Growers Association and other industry partners has commenced, and is aimed at regional control of the endemic *Tomato yellow leaf curl virus* within the Bowen growing district. This new project is linked very closely with an ongoing ACIAR project in Indonesia investigating begomviruses of chilli. Additionally the information from this CRDC project (DAQ1202) will be built upon in two newly funded CRDC projects aimed to increase the knowledge of the epidemiology of CLCuD and to continue post and pre-border surveillance for the disease. The strong linkages between these projects are evidence of ongoing cross-industry engagement in this area with reciprocal transfer of information to participating industries planned.

A second major conclusion from the project was confirmation that imports of ornamental plants pose a significant and largely unmitigated risk for co-importation of CLCuD. Documentation of the import conditions of plant species within three major families (*Cucurbitaceae, Malvaceae* and *Solanaceae*), of which contain species of major Australian commodities, provides an excellent resource for evaluating potential virus outbreaks that may cause significant economic losses. This list contains many known hosts of plant viruses. The review also highlights the need for improved importation regulations for high risk species (e.g *Hibiscus* spp.) if imported in high numbers. The current import regulations applying to *Hibiscus* spp. are not sufficient to adequately mitigate hibiscus imports as an entry pathway because potential exists for latently-infected plants to be released as live propagation material. Further evaluation of this pathway, including obtaining information on the volume of imports arriving and lobbying for access to test imported material for virus prior to release from quarantine, will be completed within a new CRDC project.

A non-crop based surveillance strategy using sampling and testing of insects for early detection of exotic diseases such as CLCuD was developed during this project. The major conclusion from this work is considered application of the whitefly surveillance strategy. The strategy complements a plant-based surveillance strategy that was developed in a previous project and a combination of both will provide time efficiencies for delimiting surveys during an incursion. It also has potential for monitoring non-crop based sites such as areas around post-entry quarantine facilities and ports of entry where high risk host plant material is unloaded. To be most effective, capture of reasonable numbers of whitefly is required and this is dependent on biological and environmental factors, thus this type of surveillance should be applied strategically.

Extension Opportunities

- Detail a plan for the activities or other steps that may be taken:

(a) to further develop or to exploit the project technology.
(b) for the future presentation and dissemination of the project outcomes.
(c) for future research.

Key findings from the project were incorporated in the CLCuD contingency plan (Version 2, Attachment 4). Research gaps in preparedness for CLCuD are to be addressed in two new CRDC funded projects and a regional based horticulture project in Bowen co-funded by the BGGA and other industry partners.

8. A. List the publications arising from the research project and/or a publication plan.
(NB: Where possible, please provide a copy of any publication/s)

Workshop proceedings (Attachment 3)

B. Have you developed any online resources and what is the website address?

No

Part 4 – Final Report Executive Summary

Cotton leaf curl disease (CLCuD) is caused by a complex of different begomoviruses and DNA-β satellite molecules. Both the viruses and the satellites are transmitted by the Silverleaf whitefly (SLW). CLCuD is responsible for serious economic losses to cotton production in several areas where it occurs and represents a serious biosecurity risk to the Australian industry. Early detection of the disease if introduced into Australia is vital to minimise economic losses.

The major industry outcome for this project was increased preparedness for CLCuD through cross-industry engagement, improved surveillance systems for its detection and knowledge on potential entry pathways for the disease. If an incursion of CLCuD occurred in Australia, the industry now has improved knowledge on how to delimit the extent of the incursion and what short and long-term management strategies have the greatest potential to contain and/or eradicate the disease.

The surveillance strategy using whitefly as the survey target, developed in this project, complements that developed in a previous project which used plants as the survey target. The combination of both strategies allows time effective delimiting surveys to be completed during an incursion. The whitefly surveys also provided valuable information on the proportion of individuals within insect populations which are capable of disease spread. This data assists in the prediction of detecting exotic viruses using whitefly as a survey target, and also on potential rates and distances of disease transfer. These studies were completed in Bowen, QLD using an endemic disease of tomato as a model system.

Improved knowledge on establishment and spread of begomoviruses within Australia was an added benefit from investigating surveillance strategies, particularly in the relationship between whitefly levels and virus incidence within crops. Although virus infection occurs when whitefly numbers are low, infections are also relatively low, and only when insect numbers begin to significantly build, does virus infections become economically important. However, the population level triggering economic impact from virus infection is still lower than that from whitefly colonisation and feeding.

Review of published research and discussions at the ‘Epidemiology and management of whitefly-transmitted viruses’ workshop has highlighted potential short and long term management strategies for whitefly-transmitted viruses. If CLCuD was introduced, short term strategies aimed at reducing inoculum levels through removal of plant hosts and reduction of whitefly populations in the affected areas is recommended. This should consider protection of the considerable investment in IPM by the industry to date, in particular, use of chemicals should be strategic and minimal. Eradication of the disease may be attempted if the incursion is very small and isolated.

If CLCuD became established, the recommended long-term strategy is an area wide IPM approach using a combination of cultural, chemical and genetic factors. Cultural practices to remove alternative plant hosts such as weed, native or other cultivated species, including volunteer cotton, will reduce the risk of transfer of CLCuD into cotton crops. Ideally, this would be completed pre-planting with ongoing control as required. This will also assist in minimising whitefly numbers which usually build on these other plants before moving into crops. Further control of whitefly populations through strategic chemical use and biological agents will prevent or minimise spread of the disease. Genetic resistance to CLCuD within Australian elite germplasm is not known nor expected. However, during initial phases of an incursion lines could be quickly screened for resistance or tolerance to CLCuD. Resistance to whitefly feeding should be evaluated prior to an incursion if not already known. Genetic resistance to CLCuD, whether natural or transgenic, is likely to be the most effective management strategy to limit economic impacts.

Due to its wide host range, introduction of CLCuD may not necessarily occur within cotton crops. Instead it could be on a vegetable or nursery premise, and as such, engagement for preparedness for this disease was sought with other industries. There was good support from both industries for the ‘Epidemiology and management of whitefly-transmitted viruses’ workshop and representatives from both industries expressed interest in further involvement in this area. Consequently, a project has commenced with the Bowen and Gumlu Growers Association to develop area wide management of an endemic whitefly-transmitted virus in their district. The threat of CLCuD introduction through import of ornamental species, highlighted via a review of current import permits, will be discussed with the nursery industry to further evaluate this risk and develop mitigation strategies for it.

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