If you are participating in the presentations this year, please provide a written report and a copy of your final report presentation by 31 October. If not, please provide a written report by 30 September.

Part 1 - Summary Details

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

CRDC Project Number: **DAQ0001**

Project Title: Developing the capacity to manage cotton viral diseases.

Project Commencement Date: 01/07/08 **Project Completion Date:** 01/07/2011

CRDC Program: 3 Crop Protection

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Part 3 – Final Report Guide (due 31 October 2011)

Background

1. Outline the background to the project.

Worldwide, virus diseases, especially Cotton leaf curl disease (CLCuD) cause serious economic losses to cotton production. For example, CLCuD cost the Pakistan industry an estimated US$5 billion between 1992 and 1997. However, Australian research on virus-like diseases of cotton has been largely limited to cotton bunchy top disease (CBTD). CLCuD is not yet known to be present but represents a serious biosecurity risk to the Australian industry. A contingency plan was needed to assist in the prevention of an incursion or to minimise the impact if an incursion of this disease was to occur.

CLCuD is caused by a complex of different begomoviruses and DNA-β satellite molecules. Okra, hibiscus and papaya are alternative hosts of the CLCuD complex. However, the survival of the disease between cotton seasons is not fully understood and other alternative weed and crop hosts may also harbour the disease. The CLCuD complex is transmitted by the silverleaf whitefly, which is widespread and abundant in many Australian cotton production areas. In countries to the immediate north of Australia, there is a range of plant species infected with various begomoviruses and DNA-β satellites, many of which may have the potential to cause CLCuD. Therefore, establishment of CLCuD, in native and endemic *Gossypium* and *Hibiscus* species present in northern Australia is a risk. This, in addition to the ubiquitous distribution of silverleaf whitefly, means CLCuD has the potential to establish and spread throughout all of the cotton growing regions within Australia. The recent incursion of *Tomato yellow leaf curl virus* into Qld demonstrates that pathways into Australia exist for these begomoviruses.

CBTD has previously been a significant issue for the industry. An extended period of dry conditions which are unfavourable for establishment and spread of this disease is the likely explanation for the relative low significance of the disease in recent years. However, under wetter and milder conditions the disease may cause problems again. The distribution of CBTD in different growing regions is not clearly known. Mapping the disease distribution during annual surveys will assist in identifying areas of concern when environmental conditions are conducive for disease. Surveys will also assist in detection of any current outbreaks.

Tobacco streak virus (TSV) was first detected at low levels in cotton in central Qld in 2007 as part of CRDC project 03DAQ005. A subsequent project (DAQ0002) has investigated various aspects of TSV in cotton and the continued surveillance activities for TSV in this project (DAQ0001) will support those of project DAQ0002. Continued monitoring of cotton production areas for several seasons will allow a thorough evaluation on the likely risk of TSV to production.

Objectives

2. Project objectives and the extent to which these have been achieved.

    a. obtain new information on the epidemiology of CLCuD to assist in the drafting of a contingency plan for this disease. Collaborate with researchers at NIBGE, in Pakistan to identify potential alternative hosts of CLCuD, in particular evaluating weed species common to both Pakistan and Australia

And

b. determine if Australian native *Gossypium* species are susceptible to CLCuD. Collaborative research with NIBGE to determine if new plantings of a range of Australian native *Gossypium* species, established under high CLCuD are hosts of the disease

A draft of the CLCuD contingency plan was prepared and is available for distribution to the Cotton Industry Biosecurity Committee for comment. The plan incorporates information obtained from Pakistan, India and Egypt through discussions with leading scientists in this area. Further information obtained from literature searches of published research results was also included. Furthermore the contingency plan incorporates surveillance strategies for CLCuD which were developed within this project using TSV and CBTD as endemic model systems.

The proposed collaborative research with NIBGE to test Australian native species and evaluate weed species as alternative hosts for CLCuD was not undertaken because travel to this country was not possible due to safety concerns. Although networks with virologists in India and Egypt were established there was insufficient time remaining to complete the required formalised arrangements with those virologists to progress the work. However, despite these complications at least four Australian native *Gossypium* species were evaluated, valuable information on the host range of CLCuD in Egypt and a list of potential CLCuD-host weed species in India was obtained.

c. participate in annual disease surveys currently being coordinated by the Australian Cotton Growers Research Association (ACGRA) to monitor commercial production areas for endemic diseases and viruses such as cotton bunchy top disease and *Tobacco streak virus*, in addition the exotic diseases CLCuD and cotton blue disease.

Monitoring of commercial cotton production areas for endemic viruses and CLCuD was via participation in annual disease surveys where possible and through additional separate specific virus surveys. Surveillance officers in QLD and NSW in addition to key extension staff were trained in the identification of CLCuD and high quality photographs supplied to them as an ongoing reference.

d. collect disease absence data to a WTO phytosanitary standard during surveys of weeds, native gossypium and commercial cotton.

CLCuD absence data was collected for commercial Australian cotton crops and native *Gossypium* and related species. Specific surveys of weed species were not conducted but any plants present within or nearby other survey areas were inspected.

Methods

3. Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.

The methodology for CLCuD surveillance was incorporated into the draft contingency plan for this disease. Samples collected during surveillance of commercial cotton or native gossypiums during this project were tested using the assays as described in the National Diagnostic Standard for CLCuD (Appendix 1).

The recommended strategy for detection of CLCuD is based on using confidence intervals for detection of disease at given incidences as described by (Cannon and Roe 1982) and used in the eradication of citrus canker from the Emerald growing area (2004-2009). This model suggests if disease is present at an incidence of at least 1%, inspection of 300 individuals will provide a 95% confidence in detecting that disease. This assumes

a 100% success in detection of the disease-affected individuals and that individuals are selected randomly for inspection. In practice, a 100% success in disease detection is unlikely, thus the number of individuals inspected is increased to accommodate this. For example, assuming detection success is only 50%, inspection of 600 individuals is required to maintain the same 95% confidence interval. It is also impractical to inspect randomly selected plants. Therefore, the strategy was modified to provide a practical approach to survey cotton crops for CLCuD incorporating both the expected surveillance sensitivity and the expected higher incidence of disease on crop edges.

In summary, the strategy uses multiple subareas distributed around the block to maximise the likelihood of disease detection. The strategy involves survey of approximately 3000 plants per block located in subareas each of at least 300 plants. There are 1 to 3 subareas per block edge plus one subarea per corner of the block. The survey should be conducted along rows and either limited to the first three rows from the block edge where the rows run parallel or for a distance no greater than approximately 20 m into the crop, where the rows are not parallel to the block edge. Optimal plant age for inspection is between 15 cm height (approximately four to five leaves) and pre-defoliation treatment or on-set of natural senescence. The average inspection time for a subarea is estimated to be between 10-15 min.

The survey area can be flexible and should be determined dynamically. For example, where there are multiple blocks of cotton cultivation, separated by a short distance of fallow ground (e.g road &/or irrigation ditch) the blocks can be combined to give a single survey area, for which only the outer edges would require inspection. However, if volunteer cotton and/or weed hosts are present between the blocks it may be prudent to inspect them as separate survey areas, rather than combining into one.

This strategy was successfully applied for the detection of Cotton bunchy top disease (CBTD) in cotton in Australia. It was also successfully trialled in India for detection of CLCuD in an area where it was known to occur but was not widely distributed.

Survey of surrounding areas for volunteer and ratoon cotton plants is essential and all detected plants should be subsequently inspected. Collection of random samples of these plants may be required as they are often naturally abnormal in growth and this could mask symptoms of CLCuD.

Selected samples of plants suspected to be infected with TSV were collected during surveys and given to Mr Murray Sharman for laboratory testing as part of his CRDC project DAQ0002 "Tobacco streak virus (TSV) in cotton". Selected samples of plants suspected to be infected with CBTD were collected during surveys and tested for polerovirus by RT-PCR assays. The assays used were developed by Mr Murray Sharman in preparation for his new project DAQ1201. Further evaluation of the detected poleroviruses was done using assays developed by Mr Murray Sharman to test for the two virus strains described in his DAQ0002 "*Tobacco streak virus* (TSV) in cotton" final report. The first strain, sequenced by Mr Murray Sharman is from Mondure/Emerald (strain MS) and the other, sequenced by Dr Mark Ellis is from Narrabri (strain ME). The assays developed for detection of the MS and ME strains are only partially optimised and validated, thus results generated using these assays are considered preliminary and may require confirmation.

Results

4. Detail and discuss the results for each objective including the statistical analysis of results.

Objectives 1 & 2:

- obtain new information on the epidemiology of CLCuD to assist in the drafting of a contingency plan for this disease. Collaborate with researchers at NIBGE, in Pakistan to identify potential alternative hosts of CLCuD, in particular evaluating weed species common to both Pakistan and Australia
- determine if Australian native *Gossypium* species are susceptible to CLCuD. Collaborative research with NIBGE to determine if new plantings of a range of Australian native *Gossypium* species, established under high CLCuD are hosts of the disease

As explained above the proposed collaborative research with NIBGE to test Australian native species was not undertaken because travel to this country was not possible due to safety concerns. However, during a visit to Pakistan in 2007, samples were collected from four Australian native *Gossypium* species grown in a Multan germplasm collection under high CLCuD pressure from surrounding affected crops and other plants within the collection. Only single plants of all four species were available for assessment in the collection and only one species, *G. sturtinanum*, displayed typical symptoms of CLCuD (Figure 1a). In this project, the four species were evaluated for susceptibility to CLCuD by indexing the samples for the presence of begomoviruses and DNA-β satellite molecules. Begomovirus was detected by PCR in the samples from the *G. sturtinanum* and *G. robinsonii* plants. Samples from the two remaining species *G. bickensii* and *G. nelsonii* tested negative for begomovirus by symptoms and PCR indexing. Additionally, the *G. sturtinanum* tested positive for the DNA-β satellite molecule by PCR whereas the *G. robinsonii* sample did not.

The DNA fragments amplified by PCR from the two virus isolates were sequenced to confirm their identity. The sequence from *G. robinsonii* was 99% identical to that obtained from *G. sturtinanum* and 90-92% identical to previously published sequences of Begomoviruses associated with Cotton leaf curl disease. To be certain of the identity of these viruses, the complete DNA-A genome will need to be sequenced and analysed. The absence of the DNA-β satellite molecule from the *G. robinsonii* sample may explain why typical CLCuD symptoms were not apparent in this plant as compared to the *G. sturtinanum* plant (Figure 1). The negative results for *G. bickensii* and *G. nelsonii* only indicates these species were not infected with the CLCuD agents at the time of sampling. The species are still considered potentially susceptible until a more thorough evaluation is conducted using multiple pathogen species.

Samples of capsicum and melon collected during a visit to Egypt in 2010 on a DAFF scholarship have tested positive for *Cotton leaf curl Geriza virus*. These plant species were not previously known to be susceptible to this virus. Although, the plants showed leaf distortions, the symptoms were not typical of CLCuD (Figure 2). DNA-β satellite molecules were detected from these samples but were atypical and require further evaluation.

A summary of plant species from which a CLCuD-associated begomovirus was detected is provided in Table 1. The preliminary identification of the viruses in these plants is based on sequencing a fragment of the DNA-A viral genome. For certainty the entire DNA-A

genome requires sequencing. Of these 13 samples 12 tested positive for the DNA-β satellite molecule.

Table 1. Summary of plant samples collected and tested positive for Begomoviruses associated with CLCuD.

Host	Symptoms CLCuD	Location	Country
G. robinsonii	mild	Multan	Pakistan
G. sturtinanum	mild	Multan	Pakistan
G. hirsutum	moderate	Multan	Pakistan
G. hirsutum Australian hybrid	moderate	Faisalabad	Pakistan
G. hirsutum Pakistan hybrid	severe	Faisalabad	Pakistan
Hibiscus spp.	mild	Faisalabad	Pakistan
Digeria spp.	mild	Faisalabad	Pakistan
G. hirsutum Indian hybrid	severe	New Delhi	India
G. hirsutum Indian hybrid	severe	New Delhi	India
G. barbadense	severe	New Delhi	India
Hibiscus spp.	moderate	New Delhi	India
Capsicum	mild/atypical	Cairo	Egypt
Melon	atypical	Cairo	Egypt

Discussions with key researchers such as Dr Monga at the Central Institute for Cotton Research (CICR) in India during 2010 identified a range of weed species known to occur in CLCuD-affected areas of India which are also present in Australia. These species include: *Phalaris minor*, *Phalaris paradoxa*, nutgrass, parthenium, argemone Mexicana (yellow flowered form of the one we have), *Tribulus terrestris* (CLCuD host), wild carrot, wild radish, wild mustard, flaxleaf fleabane, Mexican poppy, wild sunflower, white eye Mexican clover, *Amaranthus viridus*, fat hen, European bindweed (round leaf form), paddymelon, *Sida* spp. and a gooseberry species.

The CICR research staff completed some studies on the weed host range of CLCuD in India and will be submitting their results for publication soon. In addition to this, other researchers presented a poster of their alternative host range studies of CLCuD in India, at the International Plant Virus Epidemiology and Ecology Workshop held at Cornell University, Ithaca NY in June 2010. However, these results are also awaiting publication. Once these results become publically available they will be incorporated into the CLCuD contingency plan.

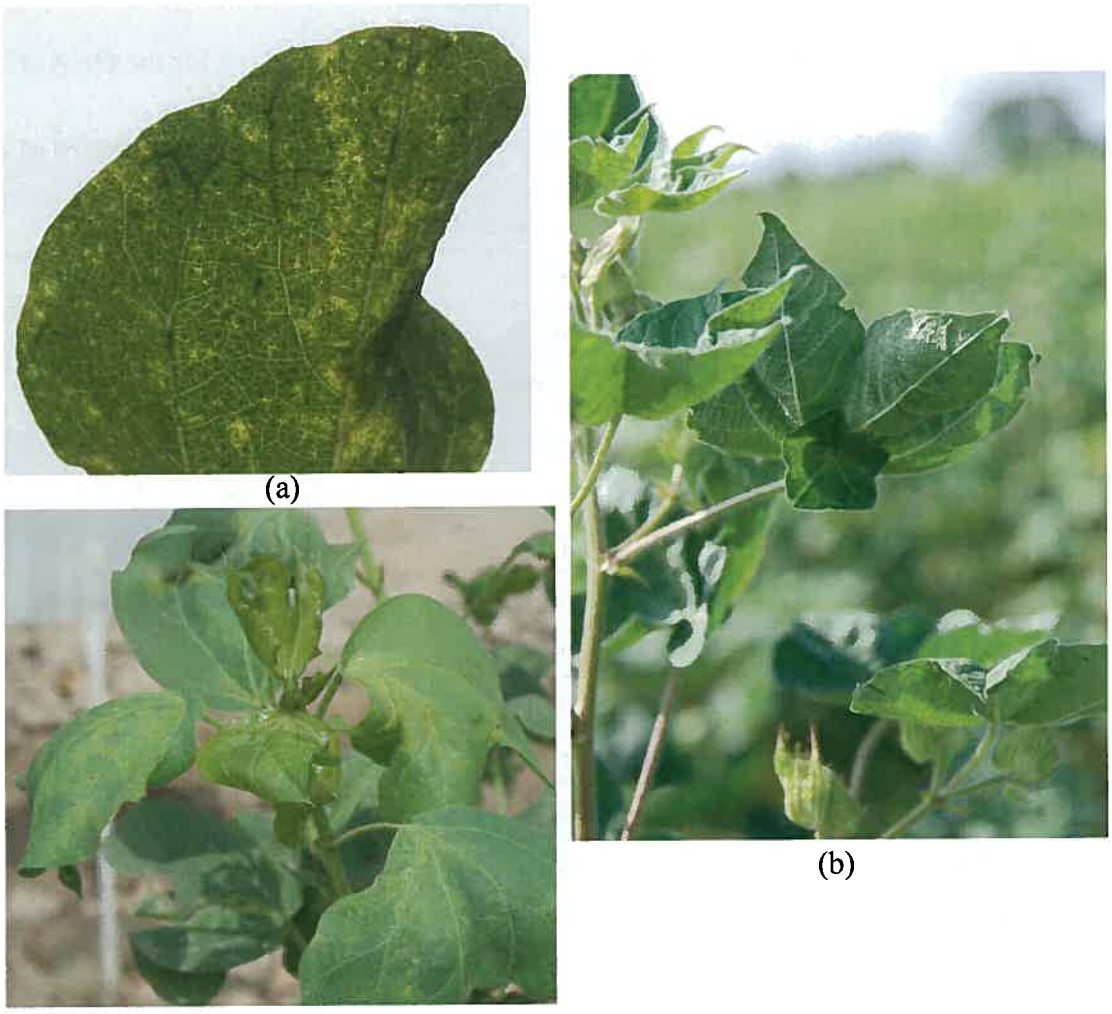

Figure 1. Symptoms of begomovirus infection on (a) *Gossypium sturtinanum*, (b) *Gossypium hirsutum* and (c) *Gossypium robinsonii*.

Figure 2. Symptoms of *Cotton leaf curl Gezira virus* infection on (a) capsicum and (b) melon. The symptoms are possible synergistic affects with other viruses such as potyviruses.

Objective 3:

- participate in annual disease surveys currently being coordinated by the Australian Cotton Growers Research Association (ACGRA) to monitor commercial production areas for endemic diseases and viruses such as cotton bunchy top disease and Tobacco streak virus, in addition the exotic diseases CLCuD and cotton blue disease.

No CLCuD or Cotton blue disease was detected during any of the surveys. No plants were observed with symptoms resembling either disease thus it was not necessary to collect samples.

Surveys for TSV were conducted initially but discontinued. This was because the virus induces subtle symptoms which can be confused with a multitude of other syndromes, especially in mature cotton making completion of surveys in a timely manner impractical. The virus was however detected from a range of properties in the Emerald area and on a ratoon cotton plant from Moura.

CBTD by contrast was relatively easy to distinguish from other syndromes, although there is some overlap in symptomology with herbicide damage. Table 2 lists the properties surveyed, the number of plants inspected, the number of properties CBTD was detected on and its prevalence. The prevalence listed in the table is a percentage of the plants inspected and not indicative of the overall prevalence within the crop. In most cases and based on general observations, the overall prevalence was negligible. The exception to this is individual properties in the Jimbour, Mondure and Emerald growing districts where during the 2011 season the prevalence of CBTD was unusually high. The disease was also regularly detected on ratoon cotton plants in many growing districts.

Table 2. Survey results for Cotton bunchy top disease surveys 2008-2011.

Year	Month	Regions	Properties Surveyed	Plants Inspected[1]	Properties with CBTD	Prevalence of CBTD in survey area
2008	November	Emerald, Theodore & Moura	12	23800	3	<1%
2009	March	Warra, Salby, MacAlistor	5	2750	2	<1%
2009	December	MacAlistor, Dalby, Bongeen, Warra, Emerald & Acturus Downs	12	22110	2	<1%
2010	December	Emerald	3	4500	2	2.6-5%
2011	March/April	Gwydir, McIntyre, Boggabilla, North Star, Namoi & Lower Namoi	10	8200	8	<1 - 5%
2011	March/April	Mondure, Jimbour	2	1450	2	50 - 78%

[1]The numbers of plants surveyed on each block varied depending on the time available to do the survey, the health status of the crop and the distance between survey subareas.

A range of samples with and without typical CBTD symptoms were collected from crops and ratoon cotton during annual disease surveys. The results of diagnostic testing of these samples are listed in Table 3. In all cases where samples were collected from plants with typical CBTD symptoms the samples tested positive for polerovirus. Virus detection was less reliable from crop plants where plants were stunted but didn't have the typical dark green blotches on their leaves. In these cases, only 6 samples from a total of 30 tested were positive for polerovirus. By contrast, only one sample was collected from a ratoon plant with typical CBTD symptoms, and it tested positive for polerovirus. A further 36 samples were collected from other ratoon plants which had abnormal growth. Of these 36

display damage from a range of other causes thus detection of typical CBTD symptoms is often complicated. The high proportion of positive results from the ratoon plants is dominated by samples collected on two properties, one of which (7.28) had a high prevalence of CBTD in the crop (Table 3). The other property (2.8) had a CBTD prevalence of less than 1% on the block near to the ratoon plants.

The 46 polerovirus positive samples were further evaluated for the two distinct virus variants detected in cotton and described in Mr Murray Sharman's final report 'DAQ0002'. As seen in Table 3, there is a wide distribution of strain MS but strain ME is quite limited and was only detected in 14 samples, all as dual infections with strain MS. Of interest are the eight samples which were positive for polerovirus but negative for both the known variants. These results indicate the presence of a possible third polerovirus species or strain in cotton. All samples tested negative for a further distinct polerovirus species which was detected in Phasey bean (Sharman, unpublished).

Table 3. Polerovirus indexing results for samples collected during surveys. The property identification reference, region and sample reference is listed with observed symptoms and host status as either a crop or ratoon plant. The virus status was determined by RT-PCR.

Property_ID	Region	Sample	Strain MS[1]	Strain ME[1]	Symptoms	Crop/Ratoon
2.1	Lower Namoi	NCBT013	+	-	T	C
2.10	McIntyre	GCBT015	+	+	T	C
2.10	McIntyre	GCBT017.1	-	-	A	R
2.10	McIntyre	GCBT017.2	+	+	A	R
2.10	McIntyre	GCBT017.7	+	+	A	R
2.3	Namoi	NCBT002	+	-	T	C
2.3	Namoi	NCBT004	+	-	T	C
2.3	Namoi	NCBT005	+	-	T	C
2.3	Namoi	NCBT006	+	-	T	C
2.3	Namoi	NCBT007	+	-	T	C
2.3	Namoi	NCBT008	+	-	T	C
2.3	Namoi	NCBT009	+	-	T	C
2.3	Namoi	NCBT010	+	-	T	C
2.3	Namoi	NCBT011	+	-	T	C
2.3	Namoi	NCBT012	+	-	A	R
2.3	Namoi	NCBT014	+	-	T	C
2.3	Namoi	NCBT015	+	-	T	C
2.7	North Star	GCBT005	-	-	S, NB	C
2.7	North Star	GCBT007	-	-	S, NB	C
2.8	Boggabilla	GCBT008.1	-	-	A	R
2.8	Boggabilla	GCBT008.3	+	-	A	R
2.8	Boggabilla	GCBT008.4	+	-	A	R
2.8	Boggabilla	GCBT008.5	+	-	A	R
2.8	Boggabilla	GCBT008.6	+	-	A	R
2.8	Boggabilla	GCBT008.7	+	-	A	R
2.8	Boggabilla	GCBT008.8	+	-	A	R
2.8	Boggabilla	GCBT009	+	-	T	C
2.8	Boggabilla	GCBT010	+	-	T	C
2.8	Boggabilla	GCBT012.1	+	+	A	R

2.9	McIntyre	GCBT013.1	-	-	S, NB	C
2.9	McIntyre	GCBT013.13	-	-	S, NB	C
2.9	McIntyre	GCBT013.15	+	-	S, NB	C
2.9	McIntyre	GCBT013.16	-	-	S, NB	C
2.9	McIntyre	GCBT014	+	+	T	C
7.27	Mondure	FrD2747	+	+	T	C
7.27	Mondure	MCBT001	-	-	T	C
7.27	Mondure	MCBT002	+	-	T	C
7.28	Jimbour	FrD2749	+	-	T	C
7.28	Jimbour	MCBT003	+	+	A	R
7.28	Jimbour	MCBT004	+	+	A	R
7.28	Jimbour	MCBT005	+	+	A	R
7.28	Jimbour	MCBT006	+	+	A	R
7.28	Jimbour	MCBT007	+	+	A	R
7.5[3]	Emerald	Em004	+	+	A	R
7.7[3]	Emerald	Em002	+	+	A	R
7.8[3]	Moura	Theo001	+	+	T	R

[1]Result of RT-PCR tested is indicated as a + for positive and - for negative. The assays were developed by Mr Murray Sharman in preparation for project DAQ1201. Variant MS refers to the virus sequenced by Mr Murray Sharman from the Emerald and Mondure growing areas and variant ME refers to the virus sequenced by Dr Mark Ellis from Narrabri.
[2]Symptoms are described as: NB = no blotches, A = atypical growth, T = typical CBTD, S = stunted only
[3]Samples collected in 2008, all remaining samples collected in 2011.

In conjunction with preparatory work by Mr Murray Sharman for project DAQ1201, a detailed study of CBTD at two properties where outbreaks were observed during the 2011 growing season was completed. At the first property, near Jimbour, there was a gradient of disease extending away from nearby infected ratoon plants. Near to the inoculum source the crop plants showed severe CBTD symptoms and the prevalence was 181/300 (60.3%) compared to only 129/300 (43.0%) at the opposite side, about 200 m away. The degree of severity also decreased, with the most distal affected plants only having very mild symptoms and limited to only one or two branches per plant. By contrast, the affected block on the second property, near Mondure was more uniformly affected and no obvious inoculum source was identified. In this block, the disease incidence was taken at several points and ranged from 31/50 to 50/50. Interestingly, the adjacent block had a CBTD incidence of <1% which is typical of most disease detections.

Objective 4:

- collect disease absence data to a WTO phytosanitary standard during surveys of weeds, native Gossypium and commercial cotton.

The surveillance methodology used in this project was developed in accordance with WTO phytosanitary standards, in particular those described in the 'International standards for phytosanitary measures ISPM No. 6 Guidelines for surveillance (1997)'. The methodology was developed for early detection of a specific pest, CLCuD. The scope of ISPM No. 6 is limited to a description of the components of survey and monitoring systems used for the purpose of pest detection and the supply of information for use in pest risk analyses, the establishment of pest free areas and, where appropriate, the preparation of pest lists. It does not provide detailed methodology on statistical rates for survey and/or sampling.

The surveillance methodology developed in this project for early detection of CLCuD is also applicable for delimiting surveillance during an incursion and for the establishment of pest

free area status. As such it was incorporated into the draft CLCuD contingency plan (Appendix 2). The methodology was used to monitor Australian cotton at various times for several seasons. The frequent detection of CBTD as described above is evidence of the effectiveness of the methodology to detect viral diseases in cotton at a very low prevalence.

Furthermore the methodology was successfully trialled in India for the detection of CLCuD. The disease was detected on a single block of the three blocks of cotton breeding lines at the Indian Agricultural Research Institute (IARI) in New Delhi. In this block there was a definite bias of disease at one end where the prevalence was quite high and not restricted to the edges. By contrast, at the other end of the plot the prevalence was much lower and more restricted to the edges. During inspection of this end of the block, only 3 plants of the 300 plants on the edge row inspected, had CLCuD symptoms. This confirms detection of the disease at a prevalence of at least 1%.

The annual early disease surveys are not ideal for collection of absence data for CLCuD because very close inspections of the plants is needed and this limits the number of plants that can be inspected in a timely manner. During the annual disease surveys it is routine to closely inspect 100 random plants per inspector per block for a range of disease and agronomic problems. Collection of absence data for CLCuD from these inspections is valid and recommended to continue. However, the optimal time for detection of CLCuD is by inspection of mid-late season plants as the plants are mostly very healthy, green and easy to inspect by visual scanning and this increases the potential number of plants and blocks inspected per day. The minimum size recommended for inspection by visual scanning is considered 15 cm with at least 4-5 leaves and the latest time is prior to defoliation and/or plant senescence. This is because survey of very small plants by visual scanning is not possible and many if not all plants would need individual leaves inverted to ensure they are free of symptoms. Older crops may have multiple symptoms, mostly associated with damage or senescence that will complicate inspections and therefore may not be suitable for survey.

Further confidence in determining the appropriate plant age for detection of CLCuD was gained from the recent DAFF funded trip by the Project Leader to India where the disease is endemic. Visiting commercial crops and breeding programs in the north-western Indian growing regions where CLCuD was present allowed inspection of plants of various ages including very young seedlings to those closer to harvest but not yet senescing. These inspections highlighted a need for plants to be within the age bracket stated previously for efficient and reliable CLCuD detection.

No specific surveys of weed species were conducted; however, general observations during surveys of commercial cotton and native gossypium plants did not detect any weed plants suspected of infection by begomovirus.

A survey of native and endemic *Malvaceae* species in northern Western Australia was completed. The area chosen to survey was based on species distributions detailed on the Flora Database of Western Australia (http://florabase.calm.wa.gov.au) and using GPS coordinates of previously located plants kindly provided by Rowena Eastwick, Northern Territory Government. The survey was conducted mostly along roadsides and tracks from Kununurra to Broome and included the Mitchell Plateau area. Figure 3 shows the locations of plant detections. About 570 individual plants were inspected; most (222) were *Gossypium australe* (native cotton) and the remaining were *G. rotundifolium*, *Hibiscus sabdariffa* (Rosella, edible), *H. meraukensis* (Merauke hibiscus), *H. panduriformis* (Yellow hibiscus), *G. costulatum*, *H. aphelus*, *H. austrinus* var. *austrinus*, *H. setulosus*, *G. pilosum*, *G. exiguum*, *Decaschistia occidentalis*, *Abutilon andrewsianum*, *A. leucopetalum* (Desert Chinese lantern), and *H. tiliaceus*. Ornamental hibiscus plants were also observed in the townships of

Darwin, Kununurra, Derby and Broome and at most homestead stations visited. No virus-like symptoms were observed on any of the plants but a total of 33 random samples were collected and tested negative for latent begomovirus infections. No whiteflies were detected during the survey.

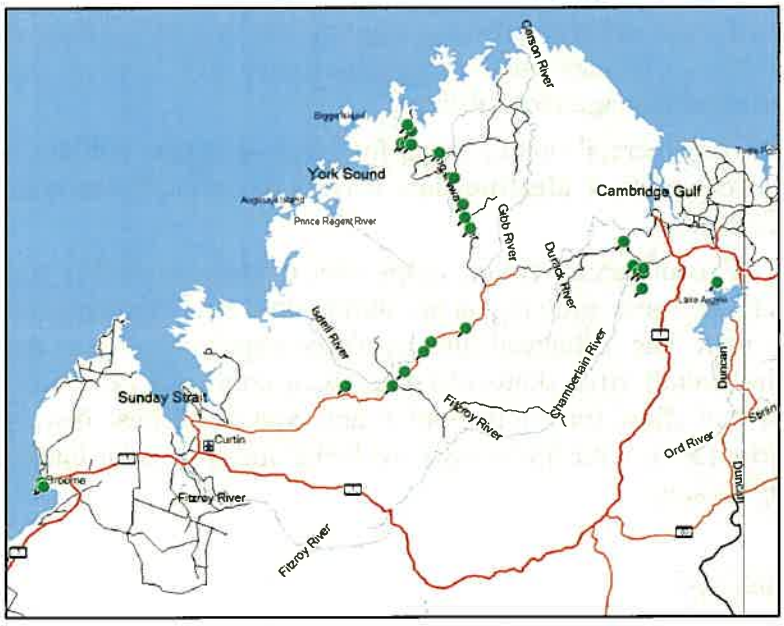

Figure 3. The locations of native and ornamental gossypium or related species surveyed for Cotton leaf curl disease in northern Western Australia are shown by green pins.

The distribution of these native and endemic plants varied from individuals to stands of 100-300 plants. Where large stands of plants were detected, 20-30 plants were randomly selected and inspected. It was not typical to find more than one *Gossypium* or *Hibiscus* species growing in close proximity, more often just single species were detected. The results of this survey also show that there exists a corridor of potential CLCuD host plants across northern Western Australia.

Outcomes

5. Describe how the project's outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.

   a. Clarifications of what weed species are present in and around cotton crops and in Australia and countries with CLCuD. Evaluation of which weed species show CLCuD-like symptoms and thus are a potential reservoir of the disease.

The knowledge of the potential susceptibility of weeds species in Australia was increased through discussions with CLCuD experts in India. The list of alternative hosts for CLCuD was expanded and the information included in the draft contingency plan. This will aid in the management of CLCuD if it was introduced into Australia and in targeted surveys for early warning of disease introduction.

   b. Data on the susceptibility of a range of Australian native *Gossypium* species.

Two Australian native *Gossypium* species were confirmed susceptible to CLCuD pathogens in Pakistan and two alternative crop plants (capsicum and melon) were detected as alternative hosts of the CLCuD pathogens in Egypt. The subsequently expanded list of alternative hosts

CLCuD if it was introduced into Australia and in targeted surveys for early warning of disease introduction.

c. Surveys of alternative & commercial hosts of CLCuD. A number of workshops held to train industry workers in recognition of virus symptoms.

CLCuD was not detected using methods in accordance with WTO phytosanitary standards. This was completed for several growing regions but not all. Demonstration of disease absence will assist in overseas trade negotiations. Industry workers now have increased ability to recognise and manage virus diseases.

d. Surveys of commercial cotton crops for virus diseases. Training other surveillance staff in the detection & identification of virus diseases. Production of technical notes on viral diseases

The virus status of commercial cotton crops was evaluated. CBTD was identified as an emerging problem in some growing areas during the 2011 growing season. Transfer of knowledge and skill has enhanced the virology capacity of the Australian industry. Knowledge of the overall virus status of commercial crops and detection of emerging viral disease problems will allow early intervention and control of these diseases. Knowledge on CBTD was contributed to industry to assist with risk mitigation for badly affected areas for the 2012 growing season.

6. Please describe any:-

a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);

None

b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and

None

c) required changes to the Intellectual Property register.

None

Conclusion

7. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?

The major outcome of the project was a draft contingency plan for CLCuD. This plan will allows the industry opportunity for input on what aspects are included in an emergency response for CLCuD. The plan contains details on how to detect, contain and eradicate or manage the disease if introduced to Australia. Furthermore, the industry was provided with training on the symptoms of the disease and how to find it which will assist in early reporting and subsequently improve the chance of containment and eradication or management of the disease if introduced.

The identification of melon and capsicum as alternative hosts of CLCuD highlighted the importance of cross-industry preparedness to this disease. Detection of two native *Gossypium* species infected with CLCuD-associated begomoviruses in Pakistan indicates a need for periodic monitoring of Australian native gossypium populations.

Surveys of commercial cotton and native gossypium provided confidence that CLCuD is not present in Australia. It also allowed a thorough evaluation of endemic viral diseases and confirmation that endemic strain of TSV is not causing economic damage to Australian cotton. CBTD was regularly detected at low levels but during the 2011 season was causing economic damage on a number of properties. This recent outbreak allowed opportunity to evaluate the disease under highly conducive conditions and thereby enhance knowledge on its management. Multiple samples were collected to confirm ratoon cotton as an important disease reservoir.

Extension Opportunities

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

(a) to further develop or to exploit the project technology.

None

(b) for the future presentation and dissemination of the project outcomes.

Continued involvement with the Cotton Industry Biosecurity Committee will occur to finalise the CLCuD contingency plan beyond a draft version. A summary of the CLCuD work was presented at a Biosecurity Awareness and Training day held at the Cotton Australia, Sydney on the 15th of September 2011.

During the project, outcomes were presented at several seminars and extension opportunities as listed below:

Allen S, Scheikowski L, Gambley CF, Sharman M, Maas S (2009-10) Cotton Pest Management Guide. In 'Integrated Disease Management'. (Ed. CCE Team) pp. 118-119.

Allen S, Scheikowski L, Gambley CF, Sharman M, Maas S (2009-10) Cotton Pest Management Guide. In ' Common diseases of cotton'. (Ed. CCE Team) pp. 120-123.

Allen S, Scheikowski L, Gambley CF, Sharman M, Maas S (2010-11) Cotton Pest Management Guide. In 'Integrated Disease Management'. (Ed. CIDaD Team) pp. 115-116.

Allen S, Scheikowski L, Gambley CF, Sharman M, Maas S (2010-11) Cotton Pest Management Guide. In 'Common diseases of cotton'. (Ed. CIDaD Team) pp. 117-120.

Gambley CF Case Study - Cotton leaf curl disease. In 'Biosecurity in Agriculture and the Environment. '. (Ed. S McKirdy). (CABI: Canberra, Australia) unpublished.

Gambley CF (2011) The biosecurity threat posed to Australian cropping systems by silverleaf whitefly-transmitted viruses. In 'Northern Farming Systems IPM Researchers Forum'. Toowoomba. (Ed. K Charleston). (Department of Employment, Economic Development and Innovation)

Gambley CF (2011) 'Draft Contingency Plan for Cotton Leaf Curl Disease.' Department of Employment, Economic Development and Innovation, Brisbane.

Gambley CF, Wilson L, Sharman M, Allen S (2011) Prepare for biosecurity threats: exotic cotton viral diseases exposed. In 'Cropping Solutions Seminar '. Moree. (Ed. F Anderson). (Crop Consultants Australia Incorporated)

Smith LJ, Gambley CF, Scheikowski L (2008) Can you identify these exotic diseases? In 'Australian Cotton Conference'. pp. Poster. (Queensland Department of Primary Industries and Fisheries: Gold Coast)

Taylor S, Maas S, Gambley CF, Wilson L, Kauter G (2009-10) Cotton Pest Management Guide. In 'Cotton Industry Biosecurity Plan'. (Ed. CCE Team) pp. 24-28

Taylor S, Maas S, Gambley CF, Wilson L, Kauter G (2010-11) Cotton Pest Management Guide. In 'Cotton Industry Biosecurity Plan'. (Ed. CIDaD Team) pp. 131-135.

(c) for future research.

Further evaluation of the susceptibility of Australian native gossypiums and hibiscus to the CLCuD-associated pathogens is unlikely to provide significantly valuable information in relation to risk mitigation for CLCuD. The evidence presented in the results section of this report is sufficient to indicate these species are highly likely to be a risk as potential alternative hosts for the disease if introduced into Australia. The presence of resistance genes to the viruses and/or DNA-β satellites within the native species is possible and this could be a useful research avenue to explore. However, each different begomovirus/DNA-β satellite combination would require testing separately, as results for one combination may not successfully extrapolate to others. The project leader has contacts in Pakistan, India and Egypt which would be applicable if further work in this area is desired.

The knowledge on susceptibility of gossypium and hibiscus to CLCuD is also sufficient to warrant periodic survey of native and crop populations of these species in northern Australia. The potential risk of establishment and transfer of the disease from countries to the north of Australia still exists until it is determined there are either no CLCuD pathogens present in these countries or that there are no pathways open for transfer of the disease.

Networks established through the DAFF funded projects will allow progress on evaluating weed species for susceptibility to CLCuD pathogens and other related begomoviruses that infect *Malvaceae* species. Further research could be completed in Egypt and India as virologists in these countries have expressed a desire to work in collaborative projects with Australia.

Commercial cotton crops require continued monitoring for endemic and exotic virus diseases. This should be done through the annual disease surveys and occasional virus specific surveys.

Preliminary results from projects DAQ0001 and DAQ1201 have identified genetically distinct strains of CBTV causing disease in cotton in NSW and QLD. Significant further work, beyond the scope of the current objectives, will be required to characterise the complete genome of these strains, further optimise existing or develop new strain-specific assays and also to investigate if biological differences may also exist for these strains such as host range. Further clarification of the range of symptoms caused by CBTV is also warranted.

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)

No publications from this research project are yet completed. Possible publications include:

- Expansion of the host range of Cotton leaf curl Gerzira virus to include melon and capsicum
- Identification of two Australian native *Gossypium* speices as hosts of CLCuD

In collaboration with Mr Murray Sharman and project DAQ1201:

- The genetic diversity of Cotton bunchy top virus strains and their distribution in Australian cotton (pending the publication of CBTV identity by CSIRO staff which is in preparation)

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

http://www.cottoncrc.org.au/content/Industry/Tools/Symptoms_Identification_Tool/Cotton_Symptoms/Exotic_Cotton_leaf_curl_disease.aspx

References

Cannon RM, Roe RT (1982) 'Livestock disease surveys. A field manual for veterinarians.' (Department of Primary Industry Bureau of Rural Science: Canberra)

Part 4 – Final Report Executive Summary

Worldwide, virus diseases, especially Cotton leaf curl disease (CLCuD) cause serious economic losses to cotton production. For example, CLCuD cost the Pakistan industry an estimated US$5 billion between 1992 and 1997. CLCuD is not known to occur in Australia and is a significant biosecurity risk for the industry. The disease is caused by a complex of different begomoviruses and DNA-β satellite molecules. Okra, hibiscus and papaya are alternative hosts of the CLCuD complex. However, the survival of the disease between cotton seasons is not fully understood and other alternative weed and crop hosts may also harbour the disease. The CLCuD complex is transmitted by the silverleaf whitefly, which is widespread and abundant in many Australian cotton production areas. In countries to the immediate north of Australia, there is a range of plant species infected with various begomoviruses and DNA-β satellites, many of which may have the potential to cause CLCuD. Therefore, establishment of CLCuD, in native and endemic *Gossypium* and *Hibiscus* species present in northern Australia is a risk.

The major outcome of this project was a draft contingency plan for CLCuD. The plan contains details on how to detect, contain and eradicate or manage the disease if introduced to Australia. The plan is available for comment by the industry, thus providing opportunity for industry input on what aspects are included in an emergency response for CLCuD. Furthermore, the industry was provided with training on the symptoms of the disease and how to find it which will assist in early reporting and subsequently improve the chance of containment and eradication or management of the disease if introduced.

Alternative CLCuD hosts were clarified within the project including the identification of two new crop hosts, melon and capsicum, and two new native gossypium hosts, *Gossypium sturtinanum* and *Gossypium robinsonii*.

Surveys of commercial cotton and native gossypium provided confidence that CLCuD is not present in Australia. It also allowed a thorough evaluation of endemic viral diseases and confirmation that the endemic strain of TSV is not causing economic damage to Australian cotton. CBTD was regularly detected at low levels but during the 2011 season was causing economic damage on a number of properties. This recent outbreak allowed opportunity to evaluate the disease under highly conducive conditions and thereby enhance knowledge on its management. Multiple samples were collected to confirm ratoon cotton as an important disease reservoir.

Preliminary results on characterisation and epidemiology of the polerovirus(es) causing CBTD has highlighted the complexity of the disease. Symptomology, causal agents and disease reservoirs all require clarification. Some of these issues will be addressed in the current CRDC project DAQ1201.

Appendices

Appendix 1 - National Diagnostic Standard for CLCuD
Attached as a separate document.
Appendix 2 – Draft contingency plan for CLCuD
Attached as a separate document.