

Monitoring the threat from cotton-infecting viruses

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VIRAL diseases are a significant constraint to cotton production in many parts of the world. In Australia, only one virus disease, cotton bunchy top (CBT), caused by cotton bunchy top virus (CBTV), has been reported to cause economic losses which have generally been sporadic and localised but occasionally widespread. For example, in the 1998–99 season, CBTV caused losses of approximately \$74 million across the Australian industry. Severe, but less widespread outbreaks occurred again in the 2010–11 season.

But many of the most economically damaging virus diseases of cotton are exotic to Australia and pose a significant biosecurity threat, such as:

- Cotton leaf curl disease (CLCuD) which is transmitted by silver leaf whitefly; and,
- Cotton leafroll dwarf virus (CLRDV; cotton blue disease) which is transmitted by cotton aphid.

For example, CLCuD caused losses in Pakistan of approximately US\$1 billion per year from 1992 to 1997. CLRDV caused an industry limiting disease in Brazil in the early 2000s and resistant varieties and control of the aphid vector are still required.

Cotton Bunchy Top

Two genetically distinct polerovirus species associated with CBT disease (CBTV-1 and -2) have been identified from almost all growing regions in Australia. From a wide range of samples across most growing regions, there is low genetic diversity within CBTV species -1 and -2. But these CBTV species are about as genetically different to each other as they are to Cotton leafroll dwarf virus (CLRDV – cotton blue disease). CBTV-2 is closely associated with typical CBT symptoms in cotton causing stunted growth, pale to red mosaic patterns around margins of leaves and down curling of leaves (Photo 1). CBTV-1 can infect cotton in the absence of CBTV-2 but it does not appear to induce obvious symptoms.

A total of 15 host species have been identified for one or both of the CBTV species. The most commonly identified hosts of CBTV in and around cotton growing areas are volunteer/ratoon cotton and *Malva parviflora* (marshmallow weed) in some areas. Off-farm roadside volunteers, greater than one season old were



Photo 1: Typical cotton bunchy top symptoms in Australian cotton causing stunted growth, pale to red mosaic patterns around margins of leaves and down curling of leaves. Some of these symptoms are easily confused with the exotic cotton leafroll dwarf virus, except the for the mosaic pattern around leaf margin which is distinctive for cotton bunchy top.



Photo 2: Dr Murray Sharman inspecting *Gossypium barbadense* in the southern region of Timor-Leste as part of the joint surveillance activity with plant health staff from the Australian and Timor-Leste governments. (PHOTO: M. Sharman)

commonly found with CBTV which indicates that they pose a significant threat as a long term source of virus (and aphids) that can move into cropping areas. This knowledge provides an opportunity to target and eliminate the source of the disease to

reduce the risk of CBTV and aphid vectors persisting between seasons. It also provides vital information about likely key hosts of the related exotic virus, cotton leafroll dwarf virus (CLRDV).

Threats from the north

As part of CRDC-funded project work, several surveys for virus threats were done in emerging cotton production areas of northern Australia and also in Timor-Leste from 2016–18. Timor-Leste is a small country about 500 km north of the Australian mainland. It has a tropical climate and diverse, largely subsistence agriculture across a range of landscapes (Photo 2). Surveys were made possible with the collaboration of the Australian and Timor-Leste governments as part of the International Plant Health Program. Samples were initially tested for viruses in basic conditions in hotel rooms during surveys and returned to Australia under quarantine permit for further lab testing for poleroviruses.

Cotton leaf roll dwarf virus

Over 500 samples were collected from a wide range of host species from over 70 sites in eastern, central and western Timor-Leste. About 100 *Gossypium* samples (37 *G. arboreum*, 40 *G.*



Photo 3: Possible mild symptoms of cotton leafroll dwarf virus on *Gossypium arboreum* in Timor-Leste. (PHOTO: M. Sharman)



Photo 4: Typical symptoms of standard cotton leafroll dwarf virus from Brazil including severe stunting and down curling of leaves. (PHOTO: M. Sharman)



***Gossypium* species have a significant role in Timorese culture for ceremonial Tais clothes.**



Initial testing is in motel rooms.

barbadense, 25 *G. hirsutum*) were collected from 27 different sites and CLRDV was found to be relatively common in all three *Gossypium* species from seven sites. *Gossypium* plants were only ever present as scattered plants in home gardens but they have a significant role in Timorese culture for ceremonial Tais cloth and so were present in low numbers in most towns visited. While these *Gossypium* species were only present in very low numbers across the landscape, the virus was present in more than 25 per cent of *Gossypium* plants tested. This may indicate that another more common host of CLRDV is present in the landscape that we are not yet aware of. But it is likely that volunteer and ratoon cotton may be important hosts for CLRDV, just as they are for cotton bunchy top in Australia.

During the surveys, it became apparent that symptoms of CLRDV in Timor-Leste were mild or not at all obvious (Photo 3). This was in stark contrast to the severe symptoms seen in cotton from South America and Thailand which typically include severe stunting of the plants, down curling of leaves and greatly reduced yield (Photo 4).

In recent years, an atypical form of CLRDV has become a concern for cotton production in South America because it is able to cause disease symptoms in the CLRDV resistant varieties being grown. Symptoms of atypical CLRDV are not as severe as standard CLRDV but include reddening and folding down of leaves, and reduced yield (Photo 5). A key question from our work was whether CLRDV from Timor-Leste or Thailand may be able to overcome the CLRDV resistance genes in cotton in the same way that the atypical CLRDV has done in South America.

Further investigation into the diversity of CLRDV from Timor-Leste has provided some clues to explain the differences in symptoms. Partial virus genome for CLRDV isolates from Thailand



Photo 5: Symptoms of a typical cotton leafroll dwarf virus from Brazil including reddening and folding down of leaves.
(PHOTO: M. Sharman)

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and Timor-Leste was determined for more than 12 samples from two regions of the virus genome. The analyses of these partial genome regions indicate that CLRDV isolates from both Thailand and Timor-Leste have much greater diversity compared to CLRDV from across Brazil and Argentina. In fact, CLRDV samples from valleys separated by about 20 km in Timor-Leste, displayed greater genetic diversity than reported samples from across a range of about 2000 km in Brazil and Argentina.

These results indicate that south east Asia may be the centre of origin for CLRDV. It is possible that the strains of CLRDV found to date in Timor-Leste may not pose a significant risk of causing disease in commercial cotton – similar to the endemic CBTV-1 which infects cotton, but does not appear to cause a disease. But caution is still warranted because the high diversity of CLRDV in Timor-Leste may be hiding a strain that does cause disease like that seen in South America.

Another significant finding from the surveys in Timor-Leste was that CLRDV was confirmed in ornamental hibiscus which is a commonly grown garden plant in many tropical regions. It is also vegetatively propagated and moved long distances, which may pose a risk of moving CLRDV into new countries or regions. Within Australia, we found that ornamental hibiscus was commonly infected with an undescribed polerovirus (does not infect cotton) from northern New South Wales to Darwin and Kununurra. We also found CBTV-1 in ornamental hibiscus. These results indicate that this commonly grown garden plant may be an important long-term reservoir for poleroviruses, some of which infect cotton.

During surveys in Timor-Leste, at least another three new polerovirus species were found in various host species. A couple of these aphid-transmitted polerovirus species were also detected in northern Australia, suggesting a wind-borne pathway for aphids may exist between Timor-Leste and Australia. Such a pathway may change the risk profile for a possible movement

of cotton leafroll dwarf virus, or other insect-borne threats, into northern Australia, which is of particular concern with the expansion of cotton production in far northern regions. CLRDV and other related poleroviruses also infect grain legume crops such as chickpea. Little is known about the role these grain viruses may play in disease outbreaks in cotton in the same farming systems. There are also a number of weedy legume species in tropical areas that could potentially host these poleroviruses.

No cotton leaf curl disease found

Thankfully, the surveys in Timor-Leste did not find evidence of the major exotic threat of cotton leaf curl disease (CLCuD), caused by begomoviruses. But the vector of CLCuD, silverleaf whitefly, is commonly found in most cotton growing regions of Australia. While CLCuD has not yet been found in Timor-Leste, SLW and several begomovirus species are established there. Typical symptoms of CLCuD in cotton include severely stunted growth, greatly reduced yield, severe curling of leaves, thickened leaves and raised leaf veins. Sections of leaf veins often appear darker from underneath due to thickening which sometimes also develops into leaf-like growths from bottom of leaves known as enations (Photo 6).

Surveillance for exotic virus threats in emerging cotton production regions is continuing in a new multi-industry project co-funded by CRDC. This project will also aim to develop improved rapid diagnostics to assist in virus identification in suspect samples to provide early detection in the case of an incursion.

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Photo 6: Typical symptoms of the exotic cotton leaf curl disease (CLCuD) include severely stunted growth, severely curled leaves that are thicker with raised leaf veins. Sections of raised leaf veins appear darker from underneath due to thickening and also sometimes develop into leaf-like enation growths from bottom of leaf. (PHOTO: Dr Cherie Gambley, DAF)