FUSCOM AGENDA

O'Shea's Royal Hotel, Goondiwindi
Tuesday 8th and Wednesday 9th of August 2017

FUSCOM provides a great opportunity for researchers, interested consultants and growers to keep up to date on the progress of current plant disease related projects in the Australian cotton industry. It also provides the chance to co-ordinate the efforts of researchers, extension officers and industry personnel to achieve the same goal of successful crop protection.

Registration is free to attend FUSCOM, but important for catering purposes. Please contact Sharna Holman if would like to attend (both days/or which day and sessions) and any dietary requirements by Friday 4th of August.

Tuesday 8th August 2017

TIME	**PRESENTATION**
10am	MORNING TEA
10:30am	Welcome – Sharna Holman (FUSCOM Chairperson)
Session 1 – 10:35am	Disease surveys – Chairperson: Annabel Twine (CottonInfo REO Darling Downs)
10:40am	Linda Smith (QDAF) and Aphrika Gregson (NSW DPI) - National disease surveys – an update
11:10am	Discussion
Session 2 – 11:20am	Fungal pathogens/soil health – Chairperson: Warwick Stiller (CSIRO)
11:25am	Gupta Vadakattu (CSIRO) – How different are soil fungal communities in different cotton growing regions
11:35am	Oliver Knox (UNE) – Filling in the gap on cotton border cells **PRESENTATION AVAILABLE**
11:45am	Linda Smith (QDAF) – Reniform nematode update
11:55am	Hannah Hartnett (CSD) – Overview of trials, including V and F rank update
12:05pm	Discussion
12:30pm	LUNCH
Session 3 – 1:30pm	Verticillium – Chairperson: Susan Maas (CRDC)
1:45pm	Linda Scheikowski (QDAF) – Verticillium trial work at Getta Getta
2.00pm	Jerome Leray (WA Mapping) – Utilising drones in finding and analysing Verticillium (Getta Getta) **PRESENTATION AVAILABLE**
2:15pm	Carlos Trapero (CSIRO) – Can we solve the Verticillium disease problems through plant breeding? **PRESENTATION AVAILABLE**
2:30pm	Sean Boland (Gwydir Cotton Growers Association) – Verticillium – a grower's perspective **PRESENTATION AVAILABLE**
2:50pm	Discussion
3pm	AFTERNOON TEA
Session 4 – 3:30pm	Verticillium forum – Chairperson: Susan Maas (CRDC)
3:35pm	Toni Chapman (NSW DPI) – Managing Verticillium risk for cotton update
3:50pm	Elizabeth Aitken (UQ) – The use of the green fluorescent pigment gene to study plant pathogen infection processes
4:05pm	Linda Smith (QDAF) – Diagnostic kit for Verticillium from plant tissue

4:20pm	Discussion
4:45pm	CLOSE OF DAY

Workshop Dinner – Townhouse Inn Restaurant 6.30pm

Wednesday 9th August 2017

Session 5 – 9am	Viruses – Chairperson: Linda Smith (QDAF)
9:10am	Murray Sharman (QDAF) – Virus update
9:20am	Daisy Stainton (UQ) – Invasive and native whiteflies in Australia: cryptic species complex, endosymbionts and Begomoviruses
9:30am	Discussion
Session 6 – 9:40am	Fusarium and Black root rot – Chairperson: *TBC*
9:45am	Maria Manjarrez (Microbiology Laboratories Australia) - Development of a quantitative diagnostic test with predictive potential to detect T. basicola (Black root rot) **PRESENTATION AVAILABLE**
9:55am	Noeleen Warman (QDAF) - The movement of *Fusarium oxysporum* f.sp. *cubense* Sub-tropical Race 4 through entire banana plants observed using GFP-transformed isolates.
10:05am	Duy Le (NSW DPI) – Novel products, innovative solutions to cotton diseases
10:20am	Discussion
10:30am	MORNING TEA
Session 7 – 11am	Issues and Gaps – Chairperson: Sharna Holman (QDAF/CottonInfo REO Central Queensland)
	Issues from the floor • Manufacturing of FarmCleanse
	Cotton Industry Gaps
12:30pm	Meeting closure – Sharna Homan (FUSCOM Chairperson)
	LUNCH
3 – 5.30pm	Verticillium Information Session

FUNDED BY COTTON RESEARCH AND DEVELOPMENT CORPORATION

VERTICILLIUM INFORMATION SESSION

CottonInfo will also being holding an information session for growers and consultants focusing on the local impacts of Verticillium and trial results that deliver practical solutions on Wednesday afternoon (3 – 5.30pm) Please RSVP by Monday 7th of August to Sally Dickinson, 0407 992 495 or sally.dickinson@cottoninfo.net.au

ABSTRACTS FOR FUSCOM

SESSION 1: DISEASE SURVEYS

National Disease Surveys – An Update

QDAF, NSW DPI and CottonInfo

Aim of research

Commercial cotton crops across New South Wales and Queensland were inspected 4 to 6 weeks after planting in 2016 and late season before defoliation in 2017. The incidence and severity of those diseases present were assessed and field history (e.g. ground preparation, cotton variety, planting date, fertiliser, seed rate etc.) was recorded for each of the fields that were surveyed. Data collected was stored in a nationally shared database. Analysis of this data will potentially highlight practices that are conducive or suppressive to disease development. This information will be used to develop strategies to better manage cotton diseases. Additionally, particular attention was given to surveying fields for the presence/absence of exotic diseases.

What was found?

Different regions are being affected by different pathogens; however there are commonalities across regions. The environmental conditions strongly influenced disease development as expected. The cooler than average September and October was conducive to seedling diseases such as Black Root Rot (BRR); particularly in Southern NSW. Historically BRR is not a pathogen of concern in Queensland, however in early planted cotton BRR infection was concerning. A common issue early season was leaf damage due to high insect pressure, as well as insect damage of roots. Poor emergence, seedling diseases and establishment issues were reflected in the high seedling mortality observed on some fields.

The periods of extreme heat from January through to April suppressed Verticillium development/expression of symptoms; however the cool change that followed enabled rapid development. Regions in northern NSW had significant Verticillium incidence, with some fields as high as 84% infection. Fusarium wilt was evident, particularly in the Gwydir, where an incidence of 9% was recorded. In late September reniform nematode was detected in one new field, on a farm known to have this pest. Reniform nematode was not detected outside of Central Queensland. Mealy bug in Queensland was generally low, however significant damage was recorded in some fields in Central Queensland. It was encouraging however to see the natural predators of mealy bug having a significant impact on this pest.

What does it mean for industry?

There are numerous pathology challenges that cotton growers face requiring an integrated approach to manage.

Future research/extension

Respond to growers concerns, investigate cause of problems, conduct research to better understand management of diseases and deliver effective management strategies.

QDAF – Linda Smith, Linda Scheikowski and Tim Shuey

NSW DPI – Aphrika Gregson and Duy le

CottonInfo – Annabel Twine, Sharna Holman, Sally Dickinson, Amanda Thomas, Janelle Montgomery, Geoff Hunter and Kieran O'Keeffe

SESSION 2: FUNGAL PATHOGENS AND SOIL HEALTH

How different are soil fungal communities in different cotton growing regions?

Gupta Vadakattu (CSIRO)

Fungi are an important component of soil biota playing important roles in a number of plant essential functions. Previous research has shown that the diversity and abundance of soil fungal community in cotton soils varied significantly by cropping history. The aim of this research is to determine the composition and abundance of soil fungal communities in soils from different cotton growing regions. We analysed surface soils collected from cotton fields (as part of disease surveys) for fungal community composition, microbial activity and chemical properties. Results have shown significant differences in the population abundance, diversity and genetic composition of fungal communities in cotton soils both at the field level and between the 5 different cotton growing regions analysed. The region based similarity in genetic composition of fungal community is seen at the species (OTU) and genus levels and to some extent at Family level. The presence of a region-based fungal community suggests a region-based specificity of management impacts on fungal community and implications to disease incidence.

This research will be repeated in 2017-18 cotton season to validate the findings and to quantify seasonal variation in order to determine the significance of the stability of region based fungal community composition. We will also analyse all the data to identify links between soil properties, management history and fungal community composition and abundance. Data will also be evaluated for relationships with disease incidence as measured in field surveys done by Linda Smith and group.

Filling in the gap on cotton border cells

Oliver Knox, Gupta Vadakattu, Martha Hawes and David Backhouse

POWERPOINT PRESENTATION AVAILABLE

Border cells are found at the root tips of most plant species where they interact with the biotic and abiotic aspects of the soil the plant encounters. In 2007 we demonstrated that there was a moderate relationship between cotton border cells and F rank. However, there was a large gap in the data set with most varieties at that time having between 3000 to 5000 border cells, whilst Sicot189 was out on its own at 12000 border cells per root tip. We have recently assessed the border cell numbers on the current Bollgard3® varieties and the numbers fell between 6000 and 16000 border cells per root tip. The relationship of this on disease rank and more recent work on the exogenous DNA that is associated with the root cap and its mucilage will be discussed.

Reniform Nematode – An Update

Linda Smith, Linda Scheikowski, Tim Shuey (QDAF)

Aim of research

Reniform nematode (*Rotylenchulus reniformis*) is an important pest in cotton production in Central Queensland. No cotton cultivars are commercially available that have resistance to reniform nematode, however rotation to resistant or non-host crops has the potential to contribute to the management of this nematode. This research aims to determine the effect of cropping practice on soil populations of reniform nematode over time. It is important that the rotation crops are effective against reniform nematode, without increasing other harmful nematodes in the soil, hence all plant-parasitic nematodes present were counted. Cotton yield was obtained to build on data previously collected for determining damage threshold of reniform nematode.

What was found?

Reniform nematode populations are increasing over time in those fields planted predominately to a host crop. In the 2016/17 season some fields had populations exceeding the estimated damage threshold of 1000

Rr/200mL of soil post-harvest (10% yield reduction). The reniform nematode has a very wide host range, including mung beans. A field planted to mung beans following an early cotton crop in CQ had increased populations of both reniform and root-knot nematode (*Meloidogyne* spp.) when sampled in May 2017 compared to the previous season.

Even though wheat is an effective crop against reniform, it is susceptible to lesion nematode (*Pratylenchus* spp.). One field sampled on the Darling Downs early season had a population of lesion nematode above the damage threshold for wheat.

Reniform nematode was not detected outside of CQ**.**

What does it mean for industry?
Reniform numbers are reduced in the soil when deprived of a suitable host crop. This does not mean that nematode densities are reduced indefinitely, but a successful crop rotation can reduce nematode levels enough so that a following susceptible crop will produce sufficient yields. Success of rotation to a non-host depends on the population at planting, because when planted back to cotton, reniform numbers are significantly increased.

It is important to monitor the population of all plant - parasitic nematodes because there is potential to increase the population of one type of nematode pest whilst attempting to manage another. Given that there are few non-host options to manage reniform nematode in CQ, monitoring of all plant-parasitic nematodes is essential.

In February 2012, *M. incognita* was identified in cotton in Ayr. This species of root-knot causes significant yield reductions (26%) on susceptible cotton cultivars without any chemical control measures in the USA. Mung bean is also a host of *M. incognita*, as well as other root-knot species that do not infect cotton, hence it is important that species identification be conducted. Nematologist Jennifer Cobon QDAF suggested that soil samples be sent to SARDI for species confirmation. Given that root-knot nematodes prefer lighter soils, this nematode may only cause significant crop damage in soils with a high percentage of sand.

Future research/extension
Currently there are no chemicals available in Australia for managing reniform in cotton. Seed treatments will be trialled in the future to determine effect on reniform nematode. Adama will be providing a fluensulfone seed treatment formulation for testing.

There needs to be a discussion around collaborating with SARDI to develop a molecular test for *R. reniformis*. There are already quite a few tests that would be useful for cotton (or would quantify the pathogens of crops grown in rotation with cotton). The nematode tests currently available include *Meloidogyne* spp., *Pratylenchus thornei* and *Pratylenchus neglectus*. Beneficials such as the AM fungi and free-living nematodes can also be quantified. From a nematological perspective, we are losing the expertise required to count nematodes manually, so most diagnostic work will be done with DNA in the future.

The Come Clean Go Clean principles of good farm hygiene are just as important for limiting the spread of plant-parasitic nematodes as they are for other soil-borne pathogens. Unaided populations will not disperse more than a few metres each season so rapid spread on and between farms will be as a result of water or soil movement via implements, vehicles etc. Eradication of an established population is generally not viable, so the best management approach is prevention**.**

F-rank, V-rank and Diseases Trials Update

Hannah Hartnett (CSD)

Aim of research
Disease resistance is an important consideration for growers when selecting a variety of cotton planting seed. Resistance to Fusarium and Verticillium wilt are currently rated using the F-rank and V-rank respectively. The purpose of this system is to provide growers with a measure of the relative wilt resistance or

susceptibility of new or existing varieties. Trials are conducted by CSD and CSIRO at numerous sites each year with the aim of increasing the number of comparisons made for each variety against a standard, thereby building on the reliability of the ranks assigned. In the past season CSD has also initiated additional trials examining Verticillium wilt tolerance in relation to yield.

What was found?

Recent trials were hindered by the relatively low disease pressure experience in the 2016-2017 season with a number of trials exhibiting insufficient disease incidence to generate usable data. However, due to the substantial number of trials conducted, there were still numerous successful trials enabling the ranks to be updated.

What does it mean for industry?

Increasing the accuracy and reliability of the disease ranks improves the grower's ability to make an informed variety selection when planting into a disease affected field.

Future research/extension

- Continue yearly disease rank trials to update the ranks of current varieties and to assign ranks to new varieties nearing commercialisation.
- Investigate the need to conduct strain specific V ranks.
- Continue Verticillium tolerance trials.

SESSION 3: VERTICILLIUM

Verticillium wilt – an update

Linda Scheikowski, Linda Smith, Tim Shuey

Aim of research

Verticillium wilt continues to be a major disease problem for cotton production. The aim of this research was to investigate the effect of growing rotation crops on soil inoculum levels of *Verticillium dahliae*. A field trial is being conducted at “Getta Getta”, North Star investigating the effect of corn, sorghum, bare fallow and cotton on disease. Initially in 2015/16 replicated trials were side by side, one trial treated as dryland and the other irrigated. In the 2016/17 season the dryland section was again planted to corn, sorghum, cotton and bare fallow with the irrigated section oversown to cotton. Both trials were irrigated. Where the cotton was oversown across the previous crops we investigated disease progress from mid-February. Time-lapse cameras were set up to capture disease development which will be linked to soil and air temperature, rainfall and irrigation data. Flagging tape was applied to plants to monitor severity of external disease symptoms over time. Prior to harvest flagged plants were cut, assessed for internal symptoms and plated out to confirm the presence of the pathogen. The field trial was also surveyed by Jerome Leray using Drone technology, to determine whether Drones (UAVs) can be used to detect Verticillium wilt in cotton with high spatial accuracy.

Additional rotation research is being conducted in collaboration with CottonInfo (Geoff Hunter) who is collecting soil from six sites in northern NSW to assess soil levels of *Verticillium dahliae* following different rotation crops. Soil populations are being assessed via plating onto semi-selective media and also sending the soil to SARDI Molecular Diagnostic Centre who have a commercial molecular test for estimating the amount of *Verticillium dahliae* DNA/g soil.

What was found?

The intense hot summer was beneficial in keeping external symptoms of Verticillium masked until later in the season. Following rainfall and a drop in temperatures disease developed rapidly mid-late March with considerable disease noted in the field trial. The percentage of flagged cotton plants with internal vascular discolouration at the end of the season was 84% in cotton-cotton, 77% in fallow- cotton and 74% in both sorghum and corn-cotton rotations. Despite the high level of infection the cotton still yielded well, largely as a result of the later onset of cooler weather. Strain 2A had been identified initially from the trial site, however with extensive plating of stems 1A was also detected. A very low incidence of Fusarium wilt was also present.

What does it mean for industry?
Planting rotation crops is a good tool to be integrated into a disease management strategy for Verticillium. As more results are gathered about a wider range of crops a more complete understanding will be gained for suggested timeframes required to reduce soil inoculum levels.

Future research/extension
Soil populations will continue to be monitored from the field trial and CottonInfo rotation sites. Additional trial sites may be sourced for further rotation trials. The potential of green manuring forage sorghum will also be evaluated, as this practice causes a shift in the soil microflora composition and may aid in disease management.

Soil solarisation of hot spots will be examined over the summer fallow with the aim to reduce inoculum levels in the upper 40cm of soil.

A quick diagnostic kit for *Verticillium dahliae* from plant tissue based on LAMP technology has been developed by Enbiotech in Italy. The kit is in its final stages of EPPO validation. Once complete there is potential to test the kit in Australia.

Can we solve the Verticillium disease problems through plant breeding?

Carlos Trapero and Warwick Stiller (CSIRO)

POWERPOINT PRESENTATION AVAILABLE

Verticillium wilt (VW) is currently a major concern in many of the major Australian cotton growing regions. The aim of our project is to increase the level of the resistance to VW in CSIRO cotton varieties. In order to do that, we are evaluating plant material currently being generated by the core breeding program in the field, as well as identifying and introgressing new sources of resistance to be incorporated to the program. New trial sites infested with VW and covering a range of different isolates and levels of virulence have been selected to evaluate all the plant material. Another important focus of this project will be the development of screening methodologies in controlled conditions capable of reliably predicting the level of resistance to VW observed in the field. Temperature, isolates, and different methods of inoculation will be some of the factors taken into account to achieve this objective. Additionally, molecular markers for VW resistance which would be essential to speed up the breeding process are being developed by Iain Wilson (CSIRO, Canberra), and will be validated in this project. The long-term outcome will be the development of cotton varieties more resistant to VW, increasing the yield where VW is already present in low or moderate levels and allowing cotton growing in areas where VW is present in higher levels.

Verticillium – a growers' perspective

Sean Boland (Gwydir Cotton Growers Association)

POWERPOINT PRESENTATION AVAILABLE

The Gwydir Cotton Growers Association is presenting a growers' perspective on the impacts of Verticillium Wilt on their cotton production system to get a better understanding of the incidence, severity, impact and management being conducted on a grass-roots and farm level.

SESSION 4: VERTICILLIUM

Managing Verticillium risk for cotton – Update

Toni Chapman (NSW DPI)

Research has identified multiple strains of Verticillium wilt in New South Wales and Queensland, including a defoliating strain which is considered a high priority pest in the Cotton Industry Biosecurity Plan. This project aims to help growers reduce the impact of Verticillium wilt by developing a greater understanding of the disease system and delivering key parts of a management decision tool kit.

Aim of research

Research efforts will focus specifically on Verticillium wilt and developing a fee for service diagnostic tool for growers that will quantify the inoculum levels of *Verticillium dahliae* in soil. New knowledge developed during the life of this project combined with 30 years of NSW DPI survey data will allow the team to use their extensive expertise in cotton pathology to develop key components of decision matrices which will allow growers and managers to assess the risk of where and when to plant.

The key focus will be on the defoliating form of Verticillium wilt, which is the highest priority pest in the Cotton Industry Biosecurity Plan. The presence of this pathogen was detected by NSW DPI in 2015 and it is essential that a greater understanding of this pathogen and its role in the Australian landscape is realised.

The key to combating the pathogen that causes Verticillium wilt is an understanding the genetic makeup, how diverse the isolates are in this country and discovering more about their alternative hosts. The work undertaken in this project will focus on transmission pathways through the quantification soil assay, a highly anticipated tool for use in cotton disease management that will be developed in this project.

After successfully developing a fast and accurate diagnosis to VCG type, the development of the protocol will allow for the quantification of the pathogen in soils will allow for effective management of this pathogen in cotton.

This presentation will provide an update on the following project objectives:

- Culture collection
- Establishing a soil sampling protocol
- Minimum inoculum levels for disease symptoms – baseline thresholds
- Long term rotation cropping and effect on inoculum levels
- PhD student update

Benefits to the industry

- Industry will have access to a diagnostic service for *Verticillium dahliae* in cotton
- Industry is informed on pathology trends and biosecurity issues and has the capacity to respond to emerging issues
- Growers are able to implement practices to reduce the impact of Verticillium wilt, assisted by access to a tool that can quantify the inoculum levels of Verticillium dahliae in soils.

Future research

- Continue optimising the soil sampling protocol
- Potential links with satellite imagery for sampling decisions
- Continue optimising/validating real time PCR tool
- Further pot trials on minimum levels of inoculum pending results of this current experiment
- International laboratory exchange for PhD student

Extension

- Recommended sampling strategy to be published in FACT SHEET
- Effect of long term rotation published in Spotlight article
- PhD thesis published on completion
- Phylogenetic trees comparing Australian and overseas isolates published

The use of the green fluorescent pigment gene to study plant pathogen infection processes

Elizabeth Aitken (School of Agriculture and Food Sciences, The University of Queensland)

Using conventional light microscopy to study the infection processes of plant pathogens requires clearing of plant tissue and then the use of fungal specific stains. Clearing of tissue is particularly problematic when the tissue is highly lignified and/or possess high levels of phenolic substances. Interpretations can also be complex when trying to distinguish the plant pathogenic structures from those of endophytes or other beneficial fungi that may be present in the tissue. By transforming the pathogenic fungi with the jellyfish green fluorescent pigment (GFP) gene, it is possible to visualise the fungal hyphae as they penetrate through the plant tissue, particularly when used in conjunction with a confocal microscope. The confocal microscope builds up a series of images taken at different depths allowing a three-dimensional overview of the fungus as it moves through the plant tissue. To achieve this, we first need to transform the fungus with the GFP. Transformation with GFP has previously bene done in our lab with *Fusarium oxysporum* f.sp. *cubense* (Foc) which causes Fusarium wilt on banana. We know from the literature that Verticillium is also amendable to transformation. This is important, as some fungi can be recalcitrant to transformation. Once we have GFP Verticillium we can study the infection process not just on cotton but on other species that we believe may harbour the Verticillium pathogen out of season.

Quick diagnostic kit for *Verticillium dahliae* based on LAMP technology

Linda Smith (QDAF)

A quick diagnostic kit for *Verticillium dahliae* from plant tissue based on LAMP (loop-mediated isothermal amplification) technology has been developed by Enbiotech in Italy. LAMP technology is a robust and sensitive method to detect a plant pathogen in the field. LAMP uses constant temperature and pathogen-specific primers to amplify the genetic data in a field sample, enabling the detection of specific DNA patterns, such as those of the target pathogen. The kit is in the final stages of validation with the European and Mediterranean Plant Protection Organisation (EPPO) and this is expected to be completed in September 2017. Once validation is finalised there is potential to utilise LAMP technology on cotton for identification of *Verticillium dahliae* in field. The technology will provide a fast (1 hr), excellent alternative to plating and nested-PCR assays for the early, sensitive and low-cost detection of *V. dahliae* in the field. It will also allow differentiation between *Fusarium oxysporum* f. sp. *vasinfectum* and *Verticillium dahliae*, two of the primary causal agents of wilt in cotton, which are difficult to differentiate as symptoms of the disease are very similar. This diagnostic will not identify strain of *V. dahliae*.

SESSION 5: VIRUSES

Diversity of *Cotton bunchy top virus* in Australia and *Cotton leafroll dwarf virus* in Timor-Leste

Murray Sharman, QDAF (Ecosciences Precinct, Brisbane)

Aims of research

1. Characterise the CBTV strains associated with symptomatic and non-symptomatic infections

2. Determine genetic relatedness of Thailand and East Timor CLRDV to the “atypical” strains from South America known to overcome CRLDV-resistance

What did we find?

CBTV. We have known for several years that there are two strains of *Cotton bunchy top virus* (CBTV-A and –B) and it appears that only CBTV-B causes bunchy top disease in cotton. We have now used NGS to characterise the complete genomes of CBTV-A and –B. The resulting consensus sequences were approximately 5,900 nt in length and covered all six coding regions (open reading frames) of the genomes. The CBTV strains share only 77 % nucleotide (nt) identity across the whole genome. They are more closely related to each other than to other poleroviruses but this is a similar level of identity shared between other distinct species such as CABYV and MABYV or TuYV and BWYV. For the complete coat protein amino acid sequence, the CBTV strains share only 70 % amino acid (aa) sequence identity and CBTV-A has a higher sequence identity to Chickpea chlorotic stunt virus. For the complete P0 protein, the CBTV strains share only 50 % aa sequence identity. This new genome sequence data for the CBTV strains, in combination with the differences in expressed disease, provides strong evidence that they are distinct polerovirus species. We need to decide on some logical names to distinguish them.

CLRDV. Some recent reports from Brazil and Argentina (da Silva et al. 2015; Cascardo et al. 2015; Agrofoglio et al. 2017) showed that the PO gene is the most variable region of the *Cotton leafroll dwarf virus* (CLRDV) genome and shows the greatest differences between the standard and resistant-breaking strains of CLRDV. The P0 protein is reported to control the ability of the virus to overcome plant resistance. I have now sequenced the partial PO gene from 12 isolates of CLRDV from Thailand, Timor-Leste and Uzbekistan, and compared these to published sequences of standard and resistance-breaking strains from Brazil and Argentina. The standard (typical) and resistance-breaking strains from Brazil and Argentina form their own clades which share about 90-91% nt identity. By contrast, the PO gene sequences from Uzbekistan, Thailand and Timor-Leste show greater diversity. In fact, the diversity between CLRDV samples from two sites in Timor-Leste separated by only 21 km (Lospalos and Luro), share about 86 % nt identity, which represents greater diversity than that between the standard and resistance-breaking CLRDV samples from across vast distances and the two countries of Brazil and Argentina. Further diversity is also present in the CLRDV samples from Thailand and Uzbekistan which both form their own clades.

What do findings mean for industry?

Until we find evidence that CBTV-A is involved in any disease of cotton, most future surveillance or resistance breeding may be best focused towards CBTV-B. These two distinct poleroviruses are well established in most cotton growing regions and indicate that we need to be on the lookout for other poleroviruses that may move into cotton, particularly if the industry is expanded in Northern Australia.

This new data shows a wide diversity of CLRDV PO gene sequences across Uzbekistan, Thailand and Timor-Leste and may suggest that SE Asia is a more likely centre of origin (and diversity) for CLRDV than South America. Based on this data I think it is most likely that CLRDV was an incursion into South America. This genetic bottle neck means there is little diversity between CLRDV samples across Brazil and Argentina but still enough diversity to select a resistance-breaking strain in the presence of cotton containing a CLRDV resistance gene. There may be enough diversity in the CLRDV strains present in Timor-Leste to overcome a resistance gene in cotton. The industry should remain vigilant to minimise the risk of incursion or spread of CLRDV.

Future research and extension

- An unsuccessful attempt at next generation sequencing (NGS) was made for an isolate of CLRDV from Thailand. It is possible that the irradiation required for import of quarantine material may have affected the downstream NGS reaction. I am planning to attempt NGS again for CLRDV from Thailand and Timor-Leste.
- I have detected new polervirus species in a range of hosts in Northern Australia and Timor-Leste. I plan to characterise them further and determine how common they are in important alternative hosts.

- I plan to publish the findings of high diversity of CLRDV from Asia.
- I plant to publish two separate journal papers about CBTV to report: 1. the characterisation of the complete genomes of CBTV-A and –B; and 2. the host range, diagnostic assays, geographic distribution and genetic diversity studies for viruses associated with cotton bunchy top in Australia.

Invasive and native whiteflies in Australia: cryptic species complex, endosymbionts and Begomoviruses

Daisy Stainton[1], Sharon van Brunschot[2], James Hereward[1], Gimme Walter[1]
[1] School of Biological Sciences, The University of Queensland, Brisbane, Australia
[2] Natural Resources Institute, University of Greenwich, Kent, UK

Cotton leaf curl disease (CLCuD) is caused by a number of viruses, which are spread by the silverleaf whitefly *Bemisia tabaci*, a major pest of cotton in its own right. Epidemics of CLCuD overseas have reduced cotton yield and resulted in large financial losses. Symptoms include upward leaf curling, vein thickening and leaf-like growths on the underside of the leaves (enations). None of the viruses which cause CLCuD are present in Australia. However, a number of whitefly species are present here, including native Australian species, and these may have the potential to transmit CLCuD should an incursion occur.

Many insects, including *B. tabaci,* contain bacteria within their cells. These endosymbionts can influence the biology of the insects and may potentially play a role in their ability to transmit plant viruses. Little is known about the endosymbionts that are present in Australian whiteflies. This project is investigating the identity of the whiteflies present in Australia, the endosymbionts they harbour, and the ability of these whiteflies to transmit the viruses that cause CLCuD.

Genetic characterisation of the species present in Australia has identified a number of *B. tabaci* in Australia, including a species not previously known to be present here. Next-generation sequencing tools have been used to gain further insight into the whiteflies present in Australia and the endosymbionts that are hosted by these insects. The endosymbiont profiles harboured by the different species are being determined. Overseas experiments associated with this project, and within special containment facilities, are investigating which whitefly species are able to transmit the viruses that cause CLCuD.

Although the viruses that cause CLCuD are not present in Australia, epidemics of CLCuD overseas have reduced cotton yield and resulted in large financial losses. Within Australia there are a number of *B. tabaci* species, so understanding the ability of CLCuD to establish in Australia requires knowledge of the potential vectors that are present here. At the completion of this project, we will not only have gained in depth knowledge of the *B. tabaci* whitefly present in Australia, but will also have enhanced our understanding of the threat of CLCuD establishing in Australia should an incursion occur.

SESSION 6: FUSARIUM AND BLACK ROOT ROT

Developing a quantitative diagnostic test with predictive potential to detect *T. basicola*

Maria Manjarrez (Microbiology Laboratories Australia)

POWERPOINT PRESENTATION AVAILABLE

After a comprehensive survey of worldwide scientific literature related to detection and quantification of black root rot in cotton (*Thielaviopsis basicola*), a plate-based method used by the Extension Service of The University of California was the method showing the most promising results to be used in Australian soils. However, an improvement to the method was needed to undoubtedly identify T. basicola. DNA sequencing of the isolated colonies that grew after 30 days of incubation allowed for the detection of black root rot levels under different agricultural management practices. The test, which may be capable of detecting inoculum levels that could cause severe disease symptoms, is intended to be use as a tool to help cotton farmers to make informed decisions before the planting season. So far, by using this method, we were able to separate soils that were showing high levels of the disease against those that were not showing any symptoms. A useful threshold was set up to indicate when a soil may allow to crop cotton or better use a rotation. However, there were soils that showed high levels of black root rot, which did not present high levels of *Thielaviopsis* basicola but rather other pathogens such as Alternaria and *Cylindrocarpon*, indicating that black root rot may be the result of more than a single pathogenic species. The soils screened so far also included flooded soils and soils under different crop rotations. However, a clear trend between different agricultural practices cannot be drawn without using several sites and replicates and different degrees of infective propagules to recreate levels of severity in glasshouse and in the field. The development of this "predictive" test may be used to help farmers with pre-plant decisions. However, the use of this tool is only one step out of the entire management practices pool that may interfere with the severity of the disease.

The movement of *Fusarium oxysporum* f.sp. *cubense* Sub-tropical Race 4 through entire banana plants observed using GFP-transformed isolates

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The fungus Fusarium oxysporum f.sp. cubense (Foc), is the causal agent of Panama disease, one of the most destructive diseases in banana crops worldwide. Current control methods are based on exclusion and monitoring, therefore an understanding of the movement of the pathogen within an entire plant is essential to ensure these methods are effective. Under strictly controlled conditions, Cavendish 'Williams' (Musa AAA) and Lady Finger (Musa AAB) banana plants were inoculated using Foc Sub-tropical Race 4, which had been modified with a Green Fluorescent Protein (GFP). Every 10 days for 80 days, the plant tissue was examined using confocal laser microscopy allowing the visualisation of the movement of Foc. Through the use of this technique, the fungus was observed infecting the roots and moving through the xylem of the corm into the lower areas of the plant. This was observed prior to external symptoms being expressed. As the disease progressed, Foc was observed in both intact and discoloured xylem vessels. As leaf sheaths began to senesce, the fungus moved into surrounding air chambers and through stomata onto the outer surface of the sheaths. Here it formed macroconidia from sporodochia, and long-lived chlamydospores. Visualisation of the movement of Foc through the banana provides an improved understanding of the disease progression, assisting in the advancement of future Panama disease management strategies for banana growers.

Innovative solutions to cotton diseases

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The development of effective disease management tactics to control soilborne pathogens such as Verticillium dahliae (Verticillium wilt), Fusarium oxysporum f. sp. vasinfectum (Fusarium wilt) and Thielaviopsis basicola (black root rot) will support the ongoing success of the Australian cotton industry.

NSW Department of Primary Industries and the Cotton Research Development Corporation are investing in research to identify suitable new/novel products for cotton disease management. New product research will involve identification of potential solutions through efficacy screening, including assessment of technical feasibility. Products of interest will include commercially available fungicides currently registered for use in other commodities but not on cotton, and experimental chemical and/or biological control agents.

Where potential disease management solutions are identified steps will be taken to progress these products toward registration or commercialisation for use in cotton. As part of this process, scientifically rigorous screening procedures will be developed with a view to these methodologies contributing to a registration data package and/or supporting future commercialisation processes.