# FINAL REPORT

For Public Release

## Part 1 - Summary Details

**CRDC ID:** RRDP1724

**Project Title:** Improving the management of cotton diseases in Australian Cotton farming systems

**Project Start Date:** 1/07/2016 **Project Completion Date:** 31/08/2019

**Research Program:** 1 Farmers

## Part 2 – Contact Details


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**Date submitted:** _____

## Part 3 – Final Report

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

### *Background*

**1. Outline the background to the project.**

Soil-borne diseases are a major constraint to sustainable cotton production in Australia. This project will help reduce the impact of diseases and nematodes on cotton production, using an integrated approach, through improved understanding of the pathogen, cultural practices that reduce disease severity and disease suppressive microbial community, ensuring a more sustainable, profitable and competitive industry in Australia.

The project will deliver key diagnostics, surveillance, and response capacity for cotton pathology in Qld and NSW. R&D will focus on Verticillium wilt and reniform nematodes using analytical laboratory, glasshouse and small plot field trials, combined with the extension of outcomes. The project will focus on developing practical solutions for these issues and aim to have 50% of growers adopting practices that reduce disease incidence.

In this project, areas of continued research include disease surveys to monitor diseases of cotton in Qld and NSW. The collection of disease incidence and farm practice data will form a key factor for development of IDM strategies and enable better targeting of efforts to manage this pathogen complex.

The distribution and density of reniform and associated yield loss were recently defined by DAF Qld. This project will continue to build on this work by monitoring reniform distribution as well as impact in ongoing experiments that aim to develop integrated management tactics for this pest.

This project will evaluate the disease suppressive potential of typical soils from fields in different cotton growing regions. It will characterise the structure of fungi and beneficial microbial communities, quantify their abundance and activity, assess management impacts and link them with disease incidence/suppression. Improving soil based biological suppression would help develop an IPM style disease management program.

### *Objectives*

**2. List the project objectives and the extent to which these have been achieved, with reference to the Milestones and Performance indicators.**

**Objective 1. How is the incidence and severity of cotton diseases changing?**

*1.1 Conduct review of current disease survey methodologies and processes conducted in collaboration between DAF Qld, NSW DPI and CottonInfo and develop survey protocols including communication, technology and use of data and geospatial database established in agreement with industry and partners.*

Protocols for disease surveys were developed and agreed upon by industry and project partners. Protocols were developed to establish clear roles and responsibilities for project and CottonINFO team. Rules around privacy and use of survey data were established in agreement with industry and partners. Any changes were agreed upon prior to implementation. ACHIEVED.

*1.2 DAF Qld, NSW DPI and CottonInfo to conduct annual early and late season disease surveys of endemic and exotic plant pathogens on commercial cotton farms in Qld & NSW, monitoring incidence and severity of diseases of cotton and recording presence/absence of exotic pests.*

Disease surveys and summary of findings were completed for Qld and NSW and communicated to industry. An additional communication of results as of 2017/18 season was the presentation of regional survey updates by CottonInfo as part of the CSD roadshow. ACHIEVED.

*1.3 Design and develop Geospatial database.*

A review of suitable digital approaches for collecting and collating geospatial data across commodities was undertaken. The Fulcrum app. was used and designed specifically for in-field collection of data. A Microsoft Excel document was designed specifically to collate raw data for analyses. Relevant data was included for 2016 – 2019. ACHIEVED

*1.4 Geospatial database developed and kept up to date, joint ownership of IP (refer IP schedule 2).*

A geospatial database was developed in Microsoft Excel and kept up to date. Obtaining additional data from growers was time consuming and often difficult to obtain, hence at present there are many gaps in the database. Three years of field assessed cotton pathology data and management practices has been collected. Multivariate analyses of survey data were performed to test for any effect of previous crop and cotton trash present on early and late season diseases. Further correlation network analyses were performed on the data to identify relationships between diseases and yield and diseases themselves in the whole data set and in each state. Composition and abundance of microbial communities has been analysed for soils collected from different regions with different cropping histories and varying disease incidences. A laboratory based pathogen suppression potential assay provided a quantitative measure of a cotton soils ability to support or inhibit soil-borne fungal pathogens such as *Verticillium dahliae*. These analyses have assisted to address systems questions on disease management. ACHIEVED.

*1.5 Develop and implement disease survey template for growers to assist in collection of field data. This might include nutrition, previous crop history, irrigation schedules, significant events, yield.*

A data collection template for growers was developed and distributed to growers. Pathologists and CottonInfo followed up on grower surveys. Grower survey data was entered into the database. ACHIEVED.

*1.6 Determine the influence of environment, disease epidemiology, and cultural practices (e.g. row spacing, seed rate, sowing date, irrigation practices etc.) on disease through analysis of database supported by additional information and trials where required.*

Analysis of survey data completed and implications for integrated disease management published. MOSTLY ACHIEVED.

Survey data has been analysed to some degree, however further analyses can be conducted once modifications are made to the database as per Adam Sparks recommendations and when a more complete data set is achieved. Due to the time required to obtain data from growers over the three years of the project, to collate into dataset and analyse, the implications for disease management have not yet been published.

*1.7 Survey team (QDAF, NSW DPI, and CottonInfo) meet quarterly.*

Survey Team regularly discussed research progress either via telephone conference call or physically. ACHIEVED, however discussions were not always quarterly.

*1.8 Develop a sample diagnostic flow chart/chain of custody procedures for all diseases and virus samples that are either collected during surveys or submitted for diagnostics.*

All samples from NSW initially sent to EMAI. All samples from QLD were sent to ESP. A flow chart and chain of custody procedures was developed for all Australian cotton pathology inquiries. A clear point of contact and process for growers and consultants was communicated to industry by CottonInfo. ACHIEVED.

*1.9 Process diagnostic cotton samples (including samples collected during surveys), isolate pathogen and conduct VCG and/or PCR to characterise pathogen.*

Samples processed and identification and distribution of results in accordance with agreed procedures (2.1). MOSTLY ACHIEVED. Some Fusarium and Verticillium isolates still pending.

**2. Research question: What is the host range of Vd, is Vd seed-borne in cotton, and what is the temperature sensitivity of strains?**

*2.1. DAF Qld to conduct glasshouse trials (Tor Street and ESP) to determine pathogenicity of different strains of Vd under different environmental conditions on commercial cultivars of selected rotation crops e.g. Chickpea, cereals, corn.*

Some crops commonly rotated with cotton, namely mungbean, chickpea, wheat and barley are actually symptomatic hosts being systemically colonised by two different strains of *V. dahliae* in glasshouse studies. ACHIEVED.

Crop rotation trials at "Getta Getta" has provided valuable data on rotations to non-host crops and their potential as a management strategy for Verticillium. ACHIEVED.

*2.2. Collect seed from Vd infected plants/receive seed from CSIRO trials at Narrabri nursery, surface sterilise and plate onto semi-selective medium. Incubate plates and observe for growth of Vd, isolate, single-spore and characterise using PCR and VCG analysis.*

The recovery of *V. dahliae* (VCG 1A) from 0.025% of acid-delinted seed identifies that the pathogen can be seed borne in cotton. ACHIEVED.

*2.3. DAF Qld - Test different Vd strains on cotton at different temperatures under controlled environmental conditions to determine temperature sensitivity on virulence. Conduct growth tests of isolates at different temperatures to determine growth curves of each strain.*

ACHIEVED. There was variability among isolates within each strain, highlighting that individual isolates rather than specific VCG groups, have the potential to grow differently and potentially be more of a threat in certain regions.

*2.4. Communicate research outcomes.*

ACHIEVED. Research findings from this project have been presented at grower meetings, conferences and industry meetings throughout the project. Data is currently being drafted, aiming for inclusion in a publication dedicated to Verticillium management in the journal *Plant.*

**3. Research question: What is the economic impact of reniform nematode and how do we manage it?**

Please note, all research related to reniform nematode is reported in the final report for CRDC funded project DAQ1803. This is due to movement of milestones into a separate reniform project that commenced on 11 Nov 2017. Final report was submitted on 31 Dec 2019.

**4. Research question: What is the disease suppression potential of cotton soils from different cotton growing regions.**

*4.1 Determine the composition and abundance of soil fungal communities in surface soils from different cotton growing regions.*

ACHIEVED. Fungal community network analyses were conducted. Observations were made on the changes in the microbial diversity and activity in a short-term rotation experiment. Genetic analysis was conducted of fungal community in soils from three seasons.

This study was mostly a descriptive genomics-based investigation identifying the nature of the community in Australian cotton soils and influencing factors and it should be extended to understand the functional role of the different groups present to understand the functional importance of the specific members of microbial community.

*4.2 Evaluate the impact of management on total microbial catabolic activity and beneficial microbial community composition in different soil types.*

ACHIEVED. Cotton soils from farmer fields in different cotton growing regions and under varying management practices were analysed for the total microbial activity (catabolic activity and diversity) and relationships determined.

*4.3 Test disease suppressive potential of soils from fields under different management history.*

PARTLY ACHIEVED. A laboratory 'soil plate assay' to quantify pathogen suppression potential (PSP) of cotton field soils was developed. There were delays in the development of a soil suppressive bioassay due to issues with pathogen inoculum viability and consistent infection of pathogen in host plant.

*4.4 Communicate research outcomes.*

ACHIEVED. A total of eight presentations given at the cotton industry meetings, cotton research conferences, national and international scientific meetings.

Manuscript 1 - The refereed conference paper on 'Disease suppression: soil fungal community diversity and interactions' presented at the 10th Australasian Soilborne Disease Symposium held in Adelaide in 2018 is being finalised as a scientific paper to be submitted to the Journal 'Phytopathology' by March 30th, 2020.

Manuscript 2 - Information on the diversity, genetic and catabolic composition of soil bacterial and fungal communities in the rotation experiment at NorthStar, presented under Milestone 4.1.2 is being converted into a manuscript titled "Crop rotation effects on composition of soil microbial communities, microbial activity and potential implications to biological suppression" (abstract accepted) for submission to the Journal MDPI Plants – Special issue on Management of Verticillium disease, Abstract accepted manuscript to be submitted by April 30th, 2020.

**5. Research question: What percentage of growers adopted practices that reduce the incidence of diseases and pests on their farms at project completion?**

*5.1 Adoption pathways and measurement of adoption*

Discussions held with Warwick Waters, manager of CottonINFO Team, and adoption pathway(s) outcomes including monitoring of adoption agreed and reported to CRDC. PARTIALLY ACHIEVED. The method chosen to measure adopted pathway was via a simple evaluation survey based on a template provided by Sharna Holman, emailed to growers and consultants. Some responses have been received and these are reported. However, the majority of responses is pending.

## *Methods*

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

**1. Research question: How is the incidence and severity of cotton diseases changing?**

**1.1 Conduct review of current disease survey methodologies and processes conducted in collaboration between DAF Qld, NSW DPI and CottonInfo and develop survey protocols including communication, technology and use of data and geospatial database established in agreement with industry and partners.**

Review of current disease survey methodologies and processes

The approach to meet this milestone was for participants to engage in face-to-face meetings, phone conferencing and communication via email. Shortly after project commencement, a face-to-face meeting was held (27 September 2016) at the EcoSciences Precinct to discuss how surveys in NSW and Qld cotton growing regions would be conducted. Participants included Rod Jackson, Annabel Twine, Linda Smith, Linda Scheikowski, Susan Maas and Lisa Bird. Discussions covered how information was to be collected, what templates were needed, potential databases for data storage and sharing of data, confidentiality, coding of farms for database for confidentiality and the roles and responsibilities of all parties involved. Follow-up meetings were held regularly and as required to finalise methodologies and processes.

*Database to Store Disease Survey Data*

The aim was to use a database that was accessible by both NSW DPI and DAF simultaneously, for ease of updating and viewing by staff from both departments. If this were not doable, a database shared electronically would be considered.

Three potential programs considered were 1). Kathmandu, a program used by the DAF wheat-breeding team; 2). SharePoint, which is a document management and collaboration tool developed by Microsoft which can be used as a central portal for exchange of information and is available through DAF; and 3). Microsoft Excel, which is a widely used spreadsheet program used within both departments.

Discussions were held between NSW DPI and DAF to determine suitability of each program and the required format of the database.

*Training of CottonInfo and Pathology Teams within NSW DPI and DAF*

Training of participants of surveys from CottonInfo and pathology teams in NSW DPI and DAF was required to ensure all participants understood expectations of participation and knew how to conduct survey. Training was also required on how to use the Fulcrum app to collect geospatial data. Training was through face-to-face meetings, conference calls and on the ground training during surveys. Regular conference calls and communication through the project was conducted to provide an opportunity for participants to offer their views on what worked well and what didn't work so well, so that the process could be improved. An important part of the training was the process for sample collection and where to send samples for diagnostics.

**1.2 DAF Qld, NSW DPI and CottonInfo to conduct annual early and late season disease surveys of endemic and exotic plant pathogens on commercial cotton farms in Qld & NSW, monitoring incidence and severity of diseases of cotton and recording presence/absence of exotic pests.**

Cotton disease surveys

Commercial cotton crops across New South Wales and Queensland were inspected in October – December (2016, 2017, 2018) and in March – April (2017, 2018, 2019), with the assistance of CottonInfo. The incidence and severity of those diseases present were assessed and field history, ground preparation, cotton variety, planting date and seed rate were recorded for each of the fields surveyed in the 2016/17, 2017/18 and 2018/19 seasons. Survey methodology as per milestone 1.1 in RESULTS section.

### 1.3 Design and develop Geospatial database

David Larson (ACRI, NSW) developed a mobile app, Fulcrum, in consultation with the pathology team, specifically for cotton disease surveys. The national disease survey project uses Fulcrum to collect field data for a centralised database accessible to Linda Smith (Project Leader) and Aphrika Gregson (as administrator).

Use of the app is by invitation only. Once an invitation email was received, set-up was straightforward.

1. Download Fulcrum app on your mobile
2. Attempt to sign in using your email address and the password: xxxx
3. Make a dummy record wherever you are, filling in each field in the form 'Transect Record' (especially photos)
4. Sync using the red circular arrows

Aphrika Gregson prepared a How-to guide to assist new users of the Fulcrum app and as a refresher for previous users (Figure1).

# TRANSECT RECORD
# FULCRUM APP USER GUIDE

*The use of Fulcrum mobile app in addition to traditional field forms facilitates a convenient, standardized and real-time digitization of Disease Survey data recorded in-field, whilst simultaneously contributing to a geospatial database. Records are visible only to the person responsible for creating them, with the exception of the lead pathologist/s and Fulcrum administrator. In this way data privacy concerns are met, whilst still allowing a large team to coordinate and contribute data.*

## Scope

To capture geospatial data, create a digitised copy of physical field form and effectively communicate sample data to pathologist.

A new entry (or Record) is used at each transect. Records start and end the boundary of transect.

## Workflow

1. **Field assessors enter new transect and create a new record** (GPS auto logged)
2. Enter basic data: Date collected, Region Farm Field and Transect number , Disease detected (simple), any additional comments, photograph of the accompanying field form and a **photo looking back over transect**
3. Sync data when an internet connection is available
4. Pathologist/Administrator uses Fulcrum desktop to view latest field surveys

**For accuracy of GPS data collection, it is important that each new record is initiated at the start of transect. It is equally important to capture a photo looking back over the transect, as this simultaneously provides information on general crop development and a final GPS location.**

## Other notes

- Compatible with both iPhone and Android operating systems
- Does not require internet connection at sampling time
- Adjust camera settings to medium/high resolution before using app (approx. 200kb per photo)
- Suggested user have access to a charger/ external battery for their device (GPS/Internet connection/Camera use)
- GPS coordinates variable 5m + on iPhone 4S, this is improved with the use of a BadElf GPS Pro kit*
- Field users will only see their own data

**Please get in touch with Aphrika if you have any questions, if something isn't working or can be improved! ☺**

Authors: A.Gregson, D.Larsen
Ver. 2.0

NSW Department of Primary Industries

11/10/2017

**Figure 1.** How-to guide for Fulcrum app

### 1.4 Geospatial database developed and kept up to date, joint ownership of IP (refer IP schedule 2).

An allocated administrator at NSW DPI manages the Fulcrum app. Administration moved from David Larson to Aphrika Gregson once the app was up and running effectively.

### 1.5 Develop and implement disease survey template for growers to assist in collection of field data. This might include nutrition, previous crop history, irrigation schedules, significant events, yield.

The design of a disease survey template for growers to assist in the collection of field data was discussed between NSW DPI, CottonInfo and DAF. The template developed is a word document that can be emailed to growers/consultants and can be filled out by printing a hard copy, writing by hand, scanning and retuning by email, or completed on the computer and returned by email. The information received was added to the disease survey excel database.

### 1.6 Determine the influence of environment, disease epidemiology, and cultural practices (e.g. row spacing, seed rate, sowing date, irrigation practices etc.) on disease through analysis of database supported by additional information and trials where required.

This analysis was performed by Dr Adam H. Sparks (USQ, Toowoomba) to perform multivariate analysis on Queensland and New South Wales cotton production survey data for the seasons 2016-2017, 2017-2018 and 2018-2019.

The data were compiled into four spreadsheets, one for early and one for late observations for Queensland and New South Wales, respectively in Microsoft OneDrive. Data were then imported into R (R Core Team 2019) for analysis. Data were visualised using the ggplot2 (H. Wickham 2016) package and analysed using MCMCglmm (J. Hadfield 2010) and glm models. MCMCglmm provides a Bayesian linear mixed model capable of dealing with data that often do not meet assumptions for traditional linear analysis methods. Network models were constructed using corrr (Ruiz et al. 2019) to show relationships between diseases and yield and clusters of disease that frequently co-occur. The analysis was performed entirely in R and all files were written as R Markdown files to create a HTML book for sharing the results using bookdown (Xie 2016, 2019).

The analysis focused on interpreting the graphs of data showing diseases by area in each state and the amount of trash in each paddock by each area in each state and on the effect of previous crop on disease incidence and lastly on the effect that trash had on disease incidence. Previous crops were binned into broad categories to help with small sample sizes and cotton was used as the baseline previous crop.

1. R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
2. H. Wickham. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.
3. Jarrod D Hadfield (2010). MCMC Methods for Multi-Response Generalized Linear Mixed Models: The MCMCglmm R Package. Journal of Statistical Software, 33(2), 1-22. URL http://www.jstatsoft.org/v33/i02/.
4. Edgar Ruiz, Simon Jackson and Jorge Cimentada (2019). corrr: Correlations in R. R package version 0.4.0.https://CRAN.R-project.org/package=corrr
5. Yihui Xie (2019). bookdown: Authoring Books and Technical Documents with R Markdown. R package version 0.16.
6. Yihui Xie (2016). bookdown: Authoring Books and Technical Documents with R Markdown. Chapman and Hall/CRC. ISBN 978-1138700109

### 1.7 Survey team (QDAF, NSW DPI, and CottonInfo) meet quarterly.

The aim of this milestone was for the survey team to meet as regularly as possible to discuss progress. Options included face-to-face at a specifically designated location, at industry meetings (eg. FUSCOM, CCA meetings) and conferences (e.g. The Australian Cotton Growers Conference, AACS Australian Cotton Research Conference).

**1.8 Develop a sample diagnostic flow chart/chain of custody procedures for all diseases and virus samples that are either collected during surveys or submitted for diagnostics.**

The aim of milestone 1.8 was develop a sample diagnostic flow chart/chain of custody procedures for all diseases and virus samples to ensure that the diagnostic process was consistent between NSW DPI and DAF. A process for diagnostics was discussed and developed between pathology teams in NSW DPI and DAF, and CottonInfo. Once decided upon, information was disseminated to all concerned. Updates and changes to the process were made as required, after discussion and agreement from survey participants.

**1.9 Process diagnostic cotton samples (including samples collected during surveys), isolate pathogen and conduct VCG and/or PCR to characterise pathogen.**

**Methods for pathogen identification NSW DPI** (Prepared by Duy Le)

**Pathogen isolation**
Samples collected or received during the survey seasons were process as follows for pathogen recovery. The isolation was initiated with surface decontamination of the diseased plant tissue with 70% ethanol for around 10 s and blotted dry with paper towel. Under aseptic conditions, small sections of diseased tissue were excised and embedded into potato dextrose agar (PDA Difco) amended with 100 ppm streptomycin sulfate (Sigma Aldrich) (sPDA). The dishes were sealed with parafilm and incubated at 25 °C in darkness for two to three days. Colonies emerging from infected tissue were individually sub-cultured onto new sPDA dishes. Pure cultures were then incubated at 25 °C in darkness for at least 7 days before small plugs (0.5 $cm^2$) were excised from the colony margins, submerged in sterile water and stored at room temperature.

**DNA extraction**
Genomic DNA was obtained using the Wizard® Genomic DNA Purification Kit (Promega). Fungal myycelia (10-100 mg) were scraped off culture dishes and transferred into a 1.5 mL tight-lock Eppendorf tube. The extraction steps were as followed by the manufacture's recommendations.

**Methods for pathogen identification DAF**

Diseased cotton samples were received at ESP, Dutton Park, Qld and DAF, Tor Street, Toowoomba, Qld for assessment during 1 July 2016 – 30 August 2019. Stem sections with vascular discolouration were surface sterilised with 70% ethanol. Small pieces of discoloured vascular tissue were cut from the stem and plated onto ¼ strength PDA/S medium and incubated at room temperature. Fungal cultures were examined using a light microscope for *Verticillium dahliae* and *Fusarium oxysporum*. For Verticillium, fungal cultures were examined for verticillate spore-bearing structures on conidiophores, typical of *Verticillium dahliae*. Concentric rings formed by microsclerotia may be observed around the isolated plant tissue. For Fusarium, fungal cultures were examined for microconidia in false-heads on short monophialides, and typical feathery growth on the edge of the culture and white fluffy aerial mycelium with purple colour on underside of culture plate. If present, fungus is subcultured onto growth media to generate a clean culture.

*F. oxysporum* is analysed using Vegetative Compatibility Group (VCG) analysis to determine the strain of the pathogen. The results are communicated back to the sender of the initial sample.

*V. dahliae* is analysed using PCR to determine strain (defoliating and non-defoliating) of the pathogen. Isolates were subcultured onto both Czapeck Dox media and ¼ PDA/Strep media to indicate pathotype.

*Vegetative Compatibility Group (VCG) Analysis*
Each isolate of *F. oxysporum* f. sp. *vasinfectum* (Fov) was characterised using Vegetative Compatibility Group (VCG) analysis (Correll et al. 1987).

*PCR detection of Australian* Fov *with specific primers*

A gel-based PCR was conducted using specific PCR primer FovSIX6-F2 (CTTCACGGCAGACCCG), together with SIX6-R1 (CAAGACCAGGTGTAGGCATT) as described by Chakrabarti et al. 2011.

*Cultures for DNA extraction*
Cultures were initiated with a 4.5-mm-diameter mycelial plug taken from the leading edge of a monoconidial colony previously grown for one week at room temperature on ¼ PDA/S. The fungal growth was scraped off the culture plate using a sterile spatula/scraper and placed in a labelled 1.5 ml Eppendorf tube.

*DNA extraction method*
The method used was the same as those reported in CRDC 2016 DAQ1402 Final Report. In short, genomic DNA was obtained using QuickExtract as per manufacture's recommendations.

*Specific Polymerase Chain Reaction (PCR) from* Verticillium dahliae *Cultures*
A gel-based PCR was conducted using specific PCR primer set DB19/DB22/espdef01 as described by Mercado-Blanco et al. (2003).

1). Mercado-Blanco, J., Rodríguez-Jurado, D., Parrilla-Araujo, S., and Jiménez-Díaz, R. M. 2003. Simultaneous detection of the defoliating and nondefoliating *Verticillium dahliae* pathotypes in infected olive plants by duplex, nested polymerase chain reaction. Plant Dis. 87:1487-1494.

**2. Research question: What is the host range of Vd, is Vd seed-borne in cotton, and what is the temperature sensitivity of strains?**

**2.1. DAF Qld to conduct glasshouse trials (Tor Street and ESP) to determine pathogenicity of different strains of Vd under different environmental conditions on commercial cultivars of selected rotation crops e.g. Chickpea, cereals, corn.**

Verticillium field rotation trials "Getta Getta", North Star

Two crop rotation field trials commenced in the 2015/16 season in adjoining areas of the same field at "Getta Getta", North Star. The field had a high level of Verticillium present.

Crop treatments included cotton, corn, sorghum or fallow. In the first season (2015/16), one trial (Trial 1) was flood irrigated and the other (Trial 2) was grown dryland. Both trials were fully irrigated thereafter. In Trial 1, cotton was planted across all treatments in the second and final year of the trial (2016/17). In Trial 2, the same crops were sown back into the same plot in the second year (2016/17) before cotton was planted across all treatments in the following season (2017/18). End of season disease incidence was determined in the final season of each trial by assessing the amount of cotton stems with vascular stem browning and thus providing data on the effect on Verticillium of either one or two years of crop rotation compared to back to back cotton. Verticillium infection was confirmed by isolation of the pathogen from stem sections back in the laboratory.

To determine soil population levels of *V. dahliae,* soil samples were collected every season pre-planting and post-harvest from all treatments and sent to SARDI Molecular Diagnostic Centre in South Australia who provided a quantity of *V. dahliae* measured as pgDNA/g of soil using their PreDicta-Pt test. While this test has not been validated, it provided indications of increasing or decreasing amounts of Verticillium following the different rotations and complemented disease incidence data. One hundred soil cores were collected per plot to a depth of 15cm, thoroughly mixed and a subsample sent to SARDI for analysis.

Trial 1: One year of rotation crops
2015/16 season: The four crop/fallow treatments were imposed in December 2015 and completed in April 2016. The trial was irrigated and disease incidence in the cotton treatments measured in March 2016 was 71%.

During the 2016/17 season when cotton was over sown across the entire trial, the development of foliar symptom expression and severity was determined from marked subplots within each plot (ten plants in

each of two adjoining rows assessed in ten subplots per plot) and related back to environmental conditions during the season.

The appearance and severity of visual foliar disease symptoms were rated on three dates (February 17th 2017, March 3rd 2017 and March 29th 2017) based on the following scale:

1 = no evidence of disease
2 = interveinal chlorosis
3 = interveinal chlorosis & necrosis of the leaves < 30 % of the plant
4 = interveinal chlorosis & necrosis of the leaves 30-60% of the plant
5 = interveinal chlorosis & necrosis of the leaves > 60% of the plant
6 = dead or defoliated plant

This data was then used to determine an average disease intensity index which takes into account disease severity and incidence of foliar symptoms at a given sampling time and is expressed as a percentage of the maximum possible disease.

Soil and air temperature and rainfall data were obtained from the CSD FastStart™ Fund Soil Temperature Network, which was located on farm in a nearby field.

The trial was planted to Sicot 746B3F (V-rank 100) on the 12th November 2016 and watered up on the 14th November 2016. Irrigations occurred as follows: 1st January 2017; 16th January 2017 (with 100 kg urea); 1st February (with 50 kg urea): 12th February; 26th February 2017. The trial was picked using a commercial picker on May 17th and 18th 2017. Only an overall trial average yield was obtained from a commercial picker.

Disease incidence was determined in the marked subplots at the end of the season by cutting the stems at ground level and noting the presence or absence of vascular stem browning. Pathogen presence and strain identification was confirmed through isolations back in the laboratory. The number of bolls per plant was also collected.

Trial 2: Two years of rotation crops
2015/16 season: The four crop/fallow treatments were imposed in December 2015 and completed in April 2016. The trial was dryland the first season only and disease incidence in the cotton treatments measured in March 2016 was 63%.

2016/17 season: Treatments (three reps) were intended to be over-sown on the same plot, however an error at planting occurred with a slight mix-up with corn and sorghum resulting in the following rotation sequences; two replications of corn-corn and one replication of sorghum-corn; one replication of sorghum-sorghum, one replication of corn-sorghum and one replication of fallow-sorghum. Data was analysed where either corn or sorghum were consistently the second year crop. Planting dates were as above for Trial 1.

2017/18 season: Sicot 714B3F cotton (V-rank 111) was planted on rainfall moisture across all treatments on October 17th 2017. Irrigations occurred on December 7th 2017, January 2nd, January 13th, January 24th, February 15th 2018 (0.8ML/ha). The trial was defoliated March 20th and April 2nd 2018 and picked on April 17th 2018 using a commercial John Deere CP690 picker with in-built scales, providing a yield estimation. Climatic data was collected for the 2017/18 season. Soil temperature was determined from a Tiny-Tag placed at 10cm in a hill in the field. Air temperature and rainfall was obtained from the growers' weather station located on farm.

Disease progress was monitored in the later part of the season by destructive sampling to determine disease incidence at several times during the season in the cotton following two years of rotation crops. Assessment dates were January 17th, February 8th, March 13th and April 12th 2018 and occurred in the western half of the trial. At each sampling time, fifty plants per plot were cut at ground level, assessed for presence of vascular browning and stem pieces then plated back in the laboratory on isolation media to confirm pathogen presence/absence and identify strain. An overall end of season disease incidence

was measured across the entire trial area also. Boll numbers per plant were measured from pre-marked subplots.

Soil samples were collected and sent to CSIRO, Adelaide for analysis of changes in microbial populations (e.g. changes in soil fungi and bacteria composition and overall microbial catabolic capacity) under the different rotations. This is reported under milestone 4.

2018/19 season: New rotation trial
A new trial commenced in the 2018/19 season with forage sorghum planted where the corn treatments had been planted two years previously, with the intention to incorporate the forage sorghum and monitor any microbial population changes. The trial was planted on November 29th 2018 to cotton cv. Sicot 748B3F (V-rank 103), sorghum cv. Sentinel and forage sorghum cv. Super Sweet Sudan. There were three replications per treatment. The trial was watered once to aid establishment and then, due to drought ran out of water. Apart from soil collection (November 12 2018 and May 22nd 2019) only a disease incidence estimation was attempted in February 2019.

The trial was mulched, offset and left fallow until further rainfall enables the trial to continue in some capacity.

**Soil Solarisation**
To manage Verticillium wilt the initial soil inoculum level needs to be reduced which will influence the development of disease. As well as crop rotation, soil solarisation has the potential do to this. Soil solarisation has been reported to successfully control Verticillium wilt in cotton, olive, fruit trees, eggplant and globe artichokes. In Spain, solarisation of the soil has been used to reduce the microsclerotia population before planting a host crop. The method was effective in soils with up to 60 % clay and beneficial microflora were not affected. Soil preparation is very important for this approach to be effective and needs to be cultivated sufficiently to break down clods to a fine tilth and watered to field capacity to enable heat penetration at depth. In cotton trials in Spain, soil solarisation for six to ten weeks reduced the population of *V. dahliae* in the 0- to 40-cm soil layer to undetectable or very low levels. Soil temperature had to be at 35°C for six weeks and if temperature was lower, then a longer solarisation time was required. Under these criteria the beneficial effects lasted three years.

Given the potential of soil solarisation to lower Verticillium the opportunity arose to initiate trials to determine if solarisation may be a possible management strategy in the Australian cotton system. This was being targeted for particularly bad “hot spots” of Verticillium rather than entire fields. Two solarisation trials were initiated in two different seasons.

**Soil Solarisation Trial near Mungindi (2017/18)**
A small soil solarisation trial was set up on a property near Mungindi in a field which reportedly had Verticillium wilt previously, to determine the effect of solarisation on the soil population of *V. dahliae*. Treatments were either plastic or no plastic, randomly replicated six times. The grower had worked the soil to a fine tilth by working it and rolling it approximately five times. Some trash was still present. The hills of four rows x thirty metre length were covered with 12.5 µm biodegradable clear plastic on 28th November 2017 using a tractor with the plastic rolls attached (Figures 2 and 3). Soil probes were placed at ten centimetres under both plastic and control (no plastic) treatments on 30th November 2017 to record soil temperature. Approximately 40 mm rain fell in a short period of time on the 2nd December 2017 washing soil away from the hill holding the plastic. The plastic blew away from the hills to varying degrees from then until 15th December when the sections that had blown off were re-laid by shovelling soil back onto the edges. By six weeks (11th January 2018) much of the plastic had degraded.

Soil samples were taken from the top 10 cm of soil prior to laying the plastic from control plots and then post-experiment to depths of 0-20 cm and 20-40cm from both treatments. Soil samples were sent to SARDI to get an estimate of the amount of *V.dahliae* DNA present.

Since it could not be confirmed when cotton would next be planted into the field, due to weather conditions, twenty soil cores per plot were collected to a depth of 10 cm using bulb planters on November

14th 2018. This soil was used to set up a bioassay under glasshouse conditions where small pots were planted with six seeds/pot of Sicot 746B3F (V-rank 100), and assessed thirteen weeks later for symptom expression. Plants were also plated out to determine if *V. dahliae* could be recovered.

**Figure 2.** Laying of biodegradable plastic on 28th November 2017


**Figure 3. A)** Plastic on hills, 28th November 2017 and **B)** close up of plastic 15th December 2017

**Soil Solarisation Trial at "Getta Getta" (2018/19)**

A soil solarisation trial was set up in a small area of one of the previous continuous cotton plots in the "Getta Getta" rotation trial in the summer of 2018/19. 12.5 µm biodegradable clear plastic was laid by hand on the hills of six by ten meter rows on November 29th 2018 (Figure 4). A further six by ten meter rows were marked out as controls without plastic. The soil was moist at the time of set up. Soil temperature probes were placed at 10 cm and 20 cm depth under both treatments. Soil cores to a depth of 20 cm were collected from each treatment plot and then again on March 14th 2019. The plastic had degraded within six weeks. A subsample of soil was sent to SARDI for a PreDicta-Pt test to determine the quantity of *V. dahliae* in the soil pre- and post- solarisation. Additional soil was collected on March 14th to a depth of 10 cm and set up in a glasshouse bioassay in small pots to try to determine disease incidence in the two treatments. Sicot 746B3F (V-rank 100) cotton was planted into the pot assay. The cotton struggled, exhibiting signs of chemical effects, not growing normally and was assessed 7 months later, still quite stunted, for signs of disease.

**Figure 4.** Plastic laid to solarise soil at "Getta Getta", November 29 2019

**Millet vs Incorporation of Forage Sorghum and the effect on Verticillium, 2017/18 (North Star)**

The incorporation of green manure crops is often used to aid in plant disease control and has been used for Verticillium. For example the control of Verticillium wilt in cauliflower with the incorporation of broccoli residues was previously thought to be due to the production of volatiles toxic to the pathogen (i.e. biofumigation). More recent research has shown, however that the mechanism of control may actually be caused by a shift in the soil microflora responsible for disease suppression. Changes in the microbial activity and community may affect the pathogen population through competition, parasitism, predation or antagonism. Ideally, soil must be moist to enable microorganisms to become active and break down vegetation. It was suggested to DAF staff (Prof. Subbarao, personal communication) that forage sorghum as a green manure crop may have the same potential as broccoli to alter soil microflora to manage disease in cotton. For Australian cotton growers, forage sorghum would be a more suitable crop than broccoli to include in the cropping regime and so was investigated for its effectiveness in controlling Verticillium wilt. Forage sorghum was included in the "Getta Getta" field trial last season for this reason but due to drought could not be maintained and grown as initially planned.

A small trial was set up, near North Star, in part of a pivot with a history of high Verticillium wilt to look at the effect of incorporating forage sorghum on disease, compared to growing millet which was planted in the rest of the pivot. Four replicated strips of millet and sorghum were assessed. Soil samples were taken 30th November 2017 when both crops were approximately two weeks old. Due to planting issues, however both the millet and sorghum were sprayed out and replanted shortly afterwards. The forage sorghum was incorporated on January 17 2018 when approximately 1m tall (Figure 5). The millet was grown and baled for hay. Post experiment soil samples were taken on 16th April 2018. All soil samples were sent to SARDI for determining the amount of *V. dahliae* DNA present. Soil collected post-harvest was analysed as part of milestone 4, but unfortunately was unable to be analysed for bacterial and fungal changes. Cotton was planted into the pivot in the 2018/19 season and six hundred plants in both treatments were assessed for disease on February 28th 2019.

**Figure 5.** Chopping and incorporating forage sorghum

**Host range studies (glasshouse trials)**

Crop rotation is a potential tactic to aid in the control of Verticillium wilt however, some rotation crops may be infected by *V. dahliae* and contribute to soil inoculum. The scientific literature from overseas largely suggests that cereals such as wheat, barley and oats are asymptomatic hosts of the Verticillium wilt pathogen, with colonisation of the roots only occurring. There has been one report from Idaho in 1985 (Mathre 1986) where symptomatic barley plants were observed growing in a previously cropped potato field. Symptoms included longitudinal chlorotic leaf stripes and brown vascular bundles. *V. dahliae* was recovered and pathogenicity tests on this isolate showed it could infect and cause symptoms in barley, wheat and oats. In their study, based on symptom development, oats were the most susceptible, followed by barley with wheat showing fewest symptoms.

In greenhouse studies by Krikun and Bernier (1987) isolates from wilt infected pea and potato were used to inoculate wheat, barley and oat which were all able to be systemically infected by at least one of these isolates. *V. dahliae* was able to be isolated from leaf tissue of all three cereals despite their being no foliar symptoms associated with the colonised tissue in this study. There were differential reactions reported between the two wheat cultivars to infection: one of the wheat cultivars tested was infected by both the pea and potato isolate whereas the other wheat cultivar was not infected by either. Similarly the pea isolate was able to be recovered from barley but not the potato isolate. Whereas in oat this was reversed. This highlights the variability among *V. dahliae* isolates on their pathogenicity on different hosts and cultivars within hosts.

Pulses including mungbean, faba bean and chickpea have all been reported as hosts of Verticillium overseas. In Australia the susceptibility to *V. dahliae* of some commonly rotated crops in the cotton farming system is largely unknown. In previous Queensland DAF growth chamber studies grain sorghum was confirmed as a non-host (cv.s MR Apollo, MR Buster, MR Scorpio, Mr Taurus), whereas faba bean and chickpea (cv. Genesis, Gully and HatTrick) were all symptomatic and susceptible to infection by *V. dahliae*. These results were previously reported in final report DAQ1402. A glasshouse experiment was set up in this project to determine the susceptibility of some cultivars of mungbean (green and black gram), wheat, barley and chickpea (desi and Kabuli type) to two isolates (VCG 1A and VCG 2A) of *V. dahliae.* The pathogenicity of the two isolates was determined on different cultivars of the different crops listed in Table 1. Cotton cultivars were included to ensure that the technique was working.

**Table 1.** Crops and cultivars assessed to determine host susceptibility to two isolates of *V. dahliae*

| **Crop tested** | **Cultivar** |
|---|---|
| Mungbean (green) | Berken |
| | Celera II-AU |
| | Crystal |
| | Jade-AU |
| | Satin-II |
| Mungbean (black gram) | Onyx-AU |
| Wheat | Dart |
| | Mitch |
| | Wallup |
| Barley | Compass |
| | Rosalind |
| | Flinders |
| | Commander |
| Chickpea (Desi) | HatTrick |
| | Drummond |
| Chickpea (Kabuli) | Monash |
| Cotton | Sicot 754B3F |
| | Sicot 746B3F |
| | Sicot 748B3F |
| | Sicot 707B3F |
| | Sicot 714 B3F |

Seeds were sown into sterilised M-Mix (potting mix) and maintained in the glasshouse until inoculation. Inoculum of the two strains (VCG 1A and VCG 2A) of *V. dahliae* was prepared by growing the isolates on Czapek-dox plates at room temperature for three weeks. Spore suspensions were prepared by adding a small amount of sterile distilled (~15ml) water to each plate and gently scraping the cultures with a scalpel. The resulting suspension was passed through a small sterile sieve to remove excess hyphae and adjusted to approximately $10^6$ conidia per ml. Two week old seedlings were gently removed from the potting mix and inoculated by dipping the roots into the relevant spore suspension for five minutes. Four plants per pot (three for chickpea) were re-potted into M-mix. For non-inoculated controls seeding roots were dipped into sterile water for five minutes, prior to re-potting. Plants were grown under glasshouse conditions for five to six weeks. The average temperature was 21.5°C with a range of between 14.5°C and 31.4°C for the duration of the experiment.

After incubation disease incidence was determined and growth parameters such as plant height and fresh shoot weights were recorded for each plant. Vascular discolouration in the stem/shoot was noted when present.

Mathre, D.E (1986) Occurrence of Verticillium dahliae on barley. *Plant Disease* 70: 981.
Krikun, J. and Bernier, C.C (1987) Infection of several crop species by two isolates of Verticillium dahliae. *Canadian Journal of Plant Pathology* 9: 241-245.

**2.2. Collect seed from Vd infected plants/receive seed from CSIRO trials at Narrabri nursery, surface sterilise and plate onto semi-selective medium. Incubate plates and observe for growth of Vd, isolate, single-spore and characterise using PCR and VCG analysis**.

Testing cotton seed for *V. dahliae* infection

*V. dahliae* is seed-borne in some hosts (e.g. spinach, lettuce and radish). Göre *et al*. (2011) has shown that *V. dahliae* is seed transmitted in cotton-seed from Turkey. Studies from China also provide evidence of long-distance transmission of Verticillium wilts by the presence of the pathogen on either the seed surface or inside the seed coat. To determine if Australian cotton-seed is infected with *V. dahliae*, Dr Warwick Stiller (CSIRO) collected seed from Verticillium infected fields from three different fields in the

nursery at Narrabri. Acid-delinted seed was sent to QDAF for isolations. All seed was initially rinsed in sterile water then surface sterilised in a 1.2% bleach solution for 5 minutes, rinsed in sterile water twice (for three minutes and two minutes) before laying to dry in a laminar flow cabinet. Each seed was cut in half and plated onto ¼ PDA amended with streptomycin, incubated for three weeks and checked frequently for signs of fungal growth. A total of 3976 seeds (Field 2: 1464 seeds, Field Old 2: 1276 seeds, Field L1: 1236 seeds) were plated out.

Göre, M.E, Erdoğan. O., Altin, N., Aydin, H., Caner, Ö.K., Filizer, F. and Büyükdöğerlioğlu, A. (2011). Seed transmission of verticillium wilt of cotton. *Phytoparasitica* 39: 285-292.

**2.3. DAF Qld - Test different Vd strains on cotton at different temperatures under controlled environmental conditions to determine temperature sensitivity on virulence. Conduct growth tests of isolates at different temperatures to determine growth curves of each strain.**

Temperature sensitivity of strains

There are reported differences in optimal growth of the different VCG groups. In order to increase the knowledge on the potential of the different strains of *V. dahliae* to cause disease under different environmental conditions the mycelial growth rate of isolates from two strains (VCG 1A and 2A) were conducted *in vitro*. Agar plugs of twelve isolates of VCG 1A and twelve isolates of VCG 2A were sub-cultured from plate culture onto Czapek-Dox Agar (CDA) and incubated in the dark at 5°C, 10°C, 15°C, 20°C, 24°C, 27°C, 30°C or 32°C (+/- 1°C) for 14 days. There were five plate replicates per isolate per temperature. The diameter of the actively growing mycelium was measured at intervals during the fourteen days and an average daily growth rate was calculated for each isolate at the completion. This experiment was only done once and needs to be repeated.

**2.4. Communicate research outcomes.**

Results have been presented at various conferences, industry and grower meetings.
Data is being finalised and collated for journal submission, aiming for publication in a special issue of the journal Plants on “Management of *Verticillium* Wilt Disease” with a 30 April 2020 deadline.

**3. Research question: What is the economic impact of reniform nematode and how do we manage it?**

Reported in final report for DAQ1803.

**4. Research question: What is the disease suppression potential of cotton soils from different cotton growing regions.**

**Biological suppression of soil-borne pathogens:**

Biological suppression generally refers to the reduction of the incidence or severity of a plant disease even in the presence of a pathogen, favourable climatic conditions and the susceptible host plant for the disease. This type of suppression can be the result of either the suppression of the pathogen or the incidence of disease or both. Pathogen suppression occurs when soils become inhospitable to the pathogen itself, whereas lack of disease incidence can be the result of the inability of the pathogen to cause disease due to the pathogen-microbe-plant interactions and/or from changes to the plant’s resistance to the pathogen. In the rain fed grain-cropping systems, adoption of conservation agricultural practices has influenced microbial C turnover and developed agronomically useful levels of disease suppression in cropping soils (Gupta *et al.* 2011). High levels of disease suppression, that can result in minimal or no disease constraints to plant growth and productivity, have been reported from a variety of cropping systems worldwide including in farmer fields and experimental sites in Australia (Gupta *et al.* 2011).

The successful control of many soil-borne plant pathogens involves management of the pathogen at a combination of different microsites (*e.g.* inoculum source and rhizosphere) in soil and at different time

periods (pre-season or in the presence of the susceptible plant). Therefore, *in situ* enhancement of natural disease suppression may be more effective than adding inoculants.

Biologically suppressive soils are differentiated into two categories i.e. (i) 'General suppression' which refers to the inhibition of pathogenic populations, and is related to either the activity of the total microflora or diverse microbial-faunal interactions and (ii) in contrast 'specific suppression' refers to the activity of specific groups of microorganisms (antagonists), Recent research has shown that the level of disease suppressive activity against soil-borne fungal diseases in grain crops in rain fed cropping regions in South Australia and Western Australia is a function of the population, activity and composition of the microbial community (Gupta *et al.* 2011, Penton *et al.* 2014). Management practices that supply higher levels of biologically-available C over long periods (>5-7 years) and maintain higher levels of microbial C turnover can result in changes to the composition and activity of the soil microbial community and consequently increase suppression.

The disease suppressive potential of a field soil is generally deduced from long-term (multiple season) history of disease incidence in susceptible crops although the presence of pathogen is somehow known. Controlled environment-based plant bioassays involving (i) disease response curves to multiple pathogen levels and (ii) transfer of suppression using sterile soil media (Roget et al. 1996) can provide the disease suppression potential of field soils. These types of assays can provide a semi-quantitative or quantitative information representing disease suppression potential of a soil. However, these do not separate the different phases of suppression phenomenon i.e. suppression of pathogen and/or suppression of actual disease assay. Currently such assays have not been standardised for cotton soils and diseases. It is known that interactions between soil-borne plant pathogens and general members of the soil microbial and other biota can affect the survival of the pathogen in the absence of host plant and its growth towards a host plant. Such information can help identify pathogen suppressive soils, quantify the level of disease suppression potential of field soils and evaluate various soil and crop management practices for their ability to promote biological disease suppression.

### 4.1 Determine the composition and abundance of soil fungal communities in surface soils from different cotton growing regions.

**Objective 1. Disease suppressive microbial communities – Soil fungi: long-term cropping system experiments at ACRI, Narrabri**

Fungi are an important component of biota accounting for >60% of microbial abundance in agricultural soils including under irrigated cotton farming systems in Australia. Fungi can form hyphal networks allowing them access to diverse and multiple microsites thereby overcoming spatial constraints in terms of C and nutrient availability and redox potential gradients (1, 2).

Soilborne diseases such as Fusarium wilt, Black root rot and Verticillium wilt have significant impact on cotton production. Currently the management of disease impacts is through the selection of genetically resistant cultivars (where available), agrochemical application and rotation with non-host crops. But even in our current high *F-rank* (resistant) cultivars significant losses can occur from disease such as Fusarium under the right environmental conditions. Biological disease suppression mediated by soil microorganisms including soil fungi can assist farmers in reducing the impact of diseases on cotton production through crop management (2).

Methods:
Studies about interactions among different microbial species/populations play a critical role in ecological research, especially about the complexity and (functional) stability of communities and their role in broad-scale ecosystem functions, e.g. plant health, decomposition, nutrient cycling (1). Plant associated fungal communities can function as an important line of defence against fungal pathogens e.g. species-rich communities are more resistant to pathogen invasions. New developments in molecular ecological network analysis together with NextGen sequencing data can help elucidate community diversity, spatial scale network interactions and responses to environmental changes, an important factor in ecological research. The objective of this study was to interrogate the data on the fungal community composition in fields with differences in long-term cropping practices for community structure as influenced by

cropping history and their links to disease incidence and suppression. For this the data on genetic composition of fungal communities from the surface (0-10 cm) soils (collected prior to 2013 and 2015 planting) in ongoing field experiments at Australian Cotton Res Institute, Narrabri (experiments at F6E & D1 blocks; KK-disease block) was used as the fields had varying history of disease incidence (Karen Kirkby, Nilantha Hulugalle, Ian Rochester, personal communication) which were used to categorize the fields either disease suppressive (F6E and D1 fields) or conducive (KK). The data for the fungal community genetic composition was obtained using 28S LSU rRNA or ITS region sequencing based techniques. Also, results for diversity indices showed significantly greater diversity in the long-term crop rotation experiment (F6E) where limited or no significant disease was observed over several seasons compared to that in the KK-field suggesting a potential role of general soil fungal community in disease occurrence in these fields. These results confirm observations in rainfed grain cropping soils in South Australia showing the key role soil fungal communities can play in the suppression of soilborne fungal diseases in cereal crops. To decipher microbial community co-occurrence patterns, molecular ecological networks, based on statistical correlations, for bacterial and fungal communities were constructed using the amplicon sequence data. Networks were constructed using the Molecular Ecological Network Analysis (MENA) Pipeline8 (Deng et al., 2012). MENA was implemented with random matrix theory (RMT) based methods to automatically identify the appropriate similarity threshold, the minimum strength of the connection between each pair of nodes, prior to network construction. Full details of methods used are described in Gupta et al. (2019).

Methods:
Surface (0-10 cm) soils from the crop rotation experiment at Northstar (Getta Getta) in NSW collected early in the cotton seasons during 2017-18 and 2018-19 were analysed for composition of soil bacteria and fungi, abundances of bacteria, fungi and pseudomonds) and catabolic diversity of total microbial community were analysed.

**Objective 3: Genetic composition and diversity of soil fungal communities in cotton fields**
Research from the previous project showed a location based (soil type and environment) variation in fungal communities in cotton soils i.e. diversity and abundance of soil fungal community varied significantly in experimental plots located at ACRI, northern NSW and Qld. This has implications to the development of management options to manipulate both beneficial and pathogenic fungi.

Methods:
Surface (0-10 cm) soils were collected using a systematic protocol at the time of disease incidence survey during the cotton seasons 2016-17, 2017-18 and 2018-19 i.e. a total of 414 soils from 117 locations analysed. Briefly, soil samples were collected at each of the ten stops during the disease survey within each plot/field within a ~200M row using a ~60mm dia soil corer and bulked to obtain a single sample. This was replicated three times in each field. Soil samples were transported to Adelaide laboratory for microbial and chemical analysis. Samples were processed using standardized protocols (described previously) and analysed for soil fungal community and abundance, microbial activity, catabolic diversity, mineral N and dissolved organic carbon levels. Subsamples were analysed for detailed chemical properties. Data were analysed for bioinformatics analysis and statistical significance using Primer E and Genstat programs. Full details for methods used to determine the abundance and genetic composition of fungal community, bioinformatic and statistical analyses are similar to those described in Gupta et al. (2019).

### 4.2 Evaluate the impact of management on total microbial catabolic activity and beneficial microbial community composition in different soil types.

**Milestone 4.2 Catabolic diversity and microbial biomass**
Soilborne plant pathogens interact with general soil microbial community both during the non-crop phase affecting their survival, growth and their ability to reach host plant root. Additionally, interactions between general microbial community and plant can impact plant nutrition and ability to tolerate biotic and abiotic stress thereby influencing the impact of the disease, its severity and overall plant health. Cropping practices that affect soil habitat structure (e.g. Tillage), availability of food sources (C) from crop residues (e.g. residue retention and rotation) and agrochemical application are known to influence the total

microbial biomass (MB) and its ability to response to favourable conditions. For example, the size of total microbial biomass and activity are significantly influenced by soil physico-chemical properties (Gonzalez-Quiñones et al. 2011; Gupta et al. 2019). While soil MB, the living component of SOM, comprises a small percentage of total soil organic matter, it serves as the engine for all biological functions. Previous research has shown that microbial catabolic diversity (based on carbon substrate utilization profiling) was significantly different between disease suppressive and conducive soils (Gupta et al. 2009).

**Methods:**
All the soil samples collected both from farmer fields and experiments (e.g. at ACRI, Northstar) were analysed for the size of MB and the ability of microorganisms to utilize carbon substrates (catabolic diversity and potential). Microbial catabolic response ($CO_2$ production to the addition of specific C-containing substrates) and diversity was measured through carbon substrate utilization profiles of soil microbial communities ('community-level physiological profiles,' CLPP) using a modified Microresp R technique (Campbell et al., 2003) adjusted for Australian soils (Knox et al., 2009). The average metabolic response (AMR) reflecting the overall functional capability of soil heterotrophic microbial communities and community metabolic diversity (CMD) was estimated based on substrate induced respiration and number of substrates utilized. Soil samples were also analysed for various physico-chemical properties e.g. pH, organic C, total N, CEC, mineral N (nitrate and ammonium N), dissolved organic N (DON) etc.

**Bacterial diversity and abundance in cotton soils**
Bacterial diversity in cropping soils has been linked to a number of processes key to plant health, nutrition and overall soil quality. Currently little is known about the composition of bacterial communities in cotton soils. The main objective of this project is to determine the genetic composition of soil bacterial communities and abundances of key bacterial groups in cotton fields across different cotton growing regions. For this soil samples from cotton fields from different regions, assessed for disease incidence as part of annual disease survey, were analysed for the diversity and abundance of soil bacterial groups using next generation sequencing and DNA based quantitative assays. Also, the effect of rotation crops on bacterial genetic composition were measured for the field experiment at Northstar (Getta Getta). Results were interrogated to identify the key groups of bacteria present in cotton soils from different regions and their responses to crop rotation with an aim to identify factors that regulate bacterial diversity and any linkages with disease incidence and plant health.

Methods:
Selected samples of surface (0-10 cm) soils collected using a systematic protocol at the time of disease incidence survey during the cotton seasons 2016-17, 2017-18 and 2018-19 were analysed. DNA samples used for fungal community analysis were used for soil bacterial abundance and community composition using qPCR and 16S rRNA amplicon sequencing methods. Full details for methods used to determine the abundance and genetic composition of bacterial community, bioinformatic and statistical analyses are similar to those described in Gupta et al. (2019). Data were analysed for bioinformatics analysis and statistical significance using Primer E and Genstat programs.

**4.3 Test disease suppressive potential of soils from fields under different management history.**

**Milestone 4.3: Disease suppression potential**

**Objective 1**: To develop / standardize a laboratory 'soil plate assay' to quantify pathogen suppression potential (PSP) of cotton field soils.

Surface soil samples (0-10 cm) collected from farmer fields (part of disease surveys) and ongoing experiments, were used in the method development. The different experimental conditions involved in a laboratory bioassay tested include: incubation time, pathogen strain (e.g. defoliating (D) *vs*. non-defoliating (ND) strains of *Verticillium dahliae*), effect of added C substrates, number of zones tested for pathogen growth. Field moist soil samples were prepared to remove large and undecomposed plant residues and stones and passed through a 4 mm dia. sieve, soil moisture adjusted to 55% water filled pore space and pre-incubated at 25 °C for 48-72 hours. Pre-incubated soils were filled into 9 cm dia. sterile petri plates to a depth of 1.5 cm depth (~45g dry soil equivalent per plate). A 2.5mm dia. plug of actively growing *V. dahliae* inoculum is placed at the centre of the plate (Figure 6), soil moisture adjusted

to 55% WFPS, closed with a lid and incubated in a sealed box at 25°C and in darkness. After 7 and 14 days of incubation, soils from either 2 or 3 zones in each plate were used in DNA extraction and quantification of *V. dahliae* abundance using species specific primers (REF) and qPCR method standardized in the project. Preliminary experiments were conducted to test various conditions involved in the qPCR analysis method.

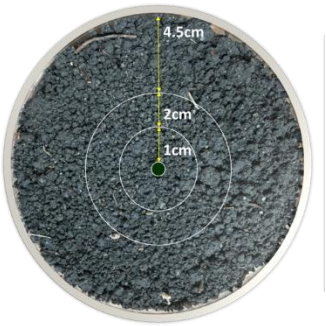



**Figure 6.** Soil plate assay

Abundance of *V. dahliae* using a qPCR method: DNA in each sample was quantified against a DNA standard (λ-phage DNA; $R^2 = 0.98$) using the QuantiT PicoGreen dsDNA assay (Invitrogen, MA, USA). The final extracted DNA was diluted 1:10 to a final volume of 50 µL in molecular grade $H_2O$ and 3 µL was used per 15µL PCR reaction. The amount of *V. dahliae* was quantified using the species-specific primers and conditions outlined below. The primer sequences used for the qPCR analysis are ITS 1F (VER): CTTGGTCATTTAGAGGAAGTAA & ST-VE1R (VER): AAAGTTTTAATGGTTCGCTAAGA giving a PCR product size of 200bp (Lieven et al., xxx)) based on the chemistry from the QuantiTect SYBR Green PCR kit (Qiagen, Vic, Australia) and the PCR was carried out on a Strategene Maxpro3000P qPCR system (Agilent, Vic, Australia). Briefly, the Master Mix included: per reaction 7.5uL of SYBR Green, 0.8uL of each primer and 2.9uL of sterile nuclease free water. The final volume of 15uL was made up of 12uL of Master mix plus 3 uL of target DNA. PCR Conditions are: 95 degrees C for 15' (to activate the Taq), then 45 cycles of (10" at 95 degrees; 10" at 60 degrees; 10" at 72 degrees), then a single 10-minute step at 72 degrees, followed by the Melt Curve from 60 degrees up to 95 degrees.

DNA from the bioassay soil samples was extracted using the Vertisol variant of the Qiagen PowerSoil DNA Extraction method (standardized in our laboratory). A 50uL of subsample of the DNA was further purified using the 96 well MinElute Plate, and placed under vacuum for approximately 2-3 minutes (until all liquid was drawn through). Nuclease free water (50uL) was added to each well, then shaking for 2x2 minutes on a plate shaker. Samples were pipette mixed before being transferred to a fresh, skirt-less 96 well plate. All DNA samples were 10x diluted prior to qPCR analysis, except for the DNA samples from Glencoe which were purified twice and 20x diluted prior to analysis.

**Table 2.** Details of the various experiments conducted as part of the development and standardization of the pathogen suppression potential (PSP) assay.

| Expt # | Location | Treatment | Soil g/plate | *V. dahliae* strain | Incubation time | Additional information |
|---|---|---|---|---|---|---|
| 1 | F6E (suppressive) | N/A | 55 | ND | 2 weeks | |
| | KK | N/A | 55 | | 2 weeks | |
| 2 | Getta-Getta | fallow | 45 | ND | 1 week & 2 weeks | |
| | Getta-Getta | corn | 45 | | 1 week & 2 weeks | |
| | Getta-Getta | Continuous cotton | 45 | | 1 week & 2 weeks | |
| 3 | Glencoe W1 | N/A | 45 | ND | 2 weeks | 1.5% C (Sucrose or ground wheat stubble) |
| 4 | Getta-Getta | fallow | 45 | D | 1 week & 2 weeks | |
| | Getta-Getta | corn | 45 | | 1 week & 2 weeks | |
| | Getta-Getta | Continuous cotton | 45 | | 1 week & 2 weeks | |
| 5 | Boggabilla | Field 6 (High Verticillium) | 45 | D | 2 weeks | |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Boggabilla | Field 9 (Low Verticillium) | 45 | | 2 weeks | |

qPCR was performed against a standard curve of a mixture of *V. dahliae* D and ND strains. Briefly, the standards for the two strains were obtained using the hyphal sample of the defoliating strain of *V. dahliae*-D (from Mungindi, grown on PDA) and spores of a non-defoliating strain of *V. dahliae* (from Getta Getta, NSW). DNA from the two strains was extracted using the Qiagen Plant DNEasy kit. DNA for the two strains was quantified, using a Nanodrop and each DNA strain was individually diluted to 100uL of sample at 200pg/uL concentration. Each DNA strain was then serially diluted, 1:10, to obtain concentrations of 20, 2, 0.2 and 0.2 pg/uL of DNA respectively. 45uL of each strain were then mixed together into a single tube to make the final standards and a standard curve was developed similar to the procedure described in Gupta et al. (2019).

Briefly, soils from the zone of 1 cm around the inoculum plug (i.e. 2 cm dia. circular zone) and the soil from the outer zone (4.5 cm zone) were analysed for the pathogen abundance to determine the rate of growth of the pathogen.

**Objective 2**: To develop / standardize a plant growth assay to quantify pathogen suppression potential of cotton field soils.

While a soil only assay which provides a quantitative measure for interactions between the pathogen and soil microbes, the presence of a susceptible host plant can inform about the actual disease incidence. In addition, for such assays to be relevant for potential responses in field conditions, a plant-based bioassay is better suited. Also, such plant-based bioassays need to reproduce pathogen levels and soil conditions similar to field soils to be able to provide reliable and reproducible results. Controlled environment pot assays with host plants to measure biological suppression potential of soils against fungal pathogens have been reported for diseases in grain crops (Roget et al. 1996; Gupta et al. 1998). As part of standardization of plant-based pot assay to acquire quantitative disease suppression potential measure for cotton diseases, we have tested the effect of factors including pathogen concentration, method/type of pathogen addition, duration of bioassay test etc.

As part of the plant-based pot assay, inoculum of either *Verticillium dahliae* or *Fusarium oxysporum* f.sp *vasinfectum* is required to be added to sterilised soil. Previous work with Fusarium has shown that inoculated millet seed works well in initiating disease. This was tested again for Fusarium as well as Verticillium together with two other inoculum sources - inoculated cornmeal sand mix (CMS) and agar plugs from culture plates (AP). Methodologies and initial results to check the efficacy of the inoculum source and rates required to cause disease are described below.

<u>Millet seed inoculum</u>

*Preparation of seed inoculum*

Untreated millet seed was rinsed in distilled water then covered with distilled water and left to soak overnight. After soaking, excess water was drained; seed was then rinsed with distilled water and placed in a container suitable for steaming. Distilled water was added until just covering the seed, which was then steamed for 30 minutes. The seed was drained of water and 500g of the steamed millet seed was placed into each of 8 x 2L Erlenmeyer flasks, plugged with cotton wool, covered with aluminium foil and sterilised in an autoclave (e.g. on wet cycle at 121°C and 103.4kPa for 20 minutes). Flasks were left to cool overnight and autoclaved a second time before use.

Previously prepared plates of either *Verticillium dahliae* (D and ND strains) or *Fusarium oxysporum* f.sp *vasinfectum* (24500) grown on quarter strength PDA plus Streptomycin (1/4PDA+S) for seven days were used to inoculate the flasks by cutting one plate of culture of the required pathogen into small squares and adding to flask, one plate per flask. The flasks were incubated in the dark at room temperature. To aid uniform colonisation of the seed, flasks were shaken every few days. Once seed was sufficiently colonised the inoculum was spread onto metal trays and air-dried for 10 days at room temperature.

*Pot assay*

Soil and pathogen inoculum was mixed to a weight of 350g. Two inoculum rates, 1g and 4g, were tested. Control pots contained no inoculum. Pots were placed on saucers then watered to approximately field water holding capacity and placed a growth cabinet set at 26°C, 16h light and 8h dark. After one week, three seeds were sown onto the soil surface, covered with 50g soil and then watered. Soil inoculated with *Fusarium oxysporum* f. sp. *vasinfectum* was sown with variety Sicot 707 B3F. Pots inoculated with *Verticillium dahliae* were sown with variety Sicot 754 B3F. Pots were watered to maintain moisture during incubation. Seedlings were observed regularly for external symptoms of disease. After 4 weeks, one replicate was harvested and roots examined for disease symptoms. Plant growth was observed to not to be progressing normally; plants were stunted and leaves were pale, thick and rubbery. This is possibly due to the type of bulbs used for lighting (wavelengths of light) in the cabinet. Pots were moved to a glasshouse with natural lighting. After 10 weeks, remaining plants were harvested and examined for disease symptoms.

Cornmeal sand mix (CMS) inoculum

The CMS was prepared using nine parts coarse sand: 1 part cornmeal: two parts deionised water. The mixture (400 g) was added to 2L Erlenmeyer flasks and autoclaved twice (121°C for 45 min). Previously prepared plates of either *Verticillium dahliae* or *Fusarium oxysporum* f.sp *vasinfectum* grown on Czapek dox or PDA agar plates respectively for seven days were used to inoculate the flasks by adding approximately fourteen small agar plugs of the required pathogen to each flask. The flasks, shaken every few days, were incubated in the dark at room temperature for fifteen days for Fusarium and five weeks for Verticillium. Once colonised the inoculum was air-dried.

Small 400 g capacity olive tube pots using sterilised potting mix were used to test the suitability of the inoculum and determine the best rates to cause disease.

The initial test involved either a) adding a 10 g layer of inoculum just below the soil surface or b) mixing inoculum into the soil at a rate of either 4.3% or 5.7%. Pots were watered and left for one week, to activate the inoculum, before planting either Sicot 707B3F for Fusarium or Sicot 754B3F for Verticillium. Two seeds per plot were planted and watered as needed before being assessed for disease ten weeks later.

Agar plugs (AP) from culture plates

Previously prepared plates of either *Verticillium dahliae* (D and ND strains) or *Fusarium oxysporum* f.sp *vasinfectum* grown on either Czapek Dox or 1/4PDA+S growth plates for seven days were used to inoculate pots. UC potting mix was placed into a 10 cm dia. pot. The soil surface was inoculated with either 1, 3, 5 or 7 plugs of inoculum (Figure 7). A layer of potting mix was placed over the inoculum. Each treatment was replicated twice. Pots were placed on saucers in a glasshouse, watered, and left for one week to allow inoculum to grow out from the inoculation point in the potting mix, before planting either Sicot 707 B3F for Fusarium or Sicot 754 B3F for Verticillium, 3 seeds per pot. Plants were checked regularly for symptoms of disease. Plants were harvested after 10 weeks.

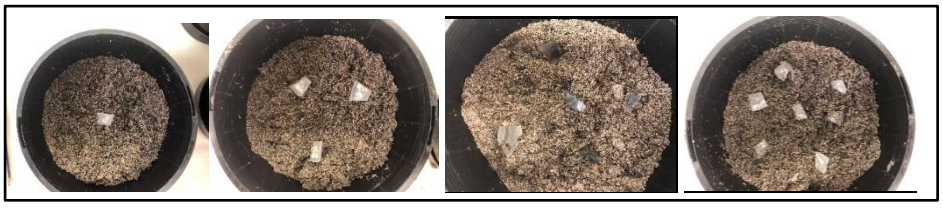


**Figure 7.** Placement of agar plug inoculum at 1, 3, 5 and 7 plugs per pot

**4.4 Communicate research outcomes.**

Results have been presented at various conferences, industry and grower meetings. Data is being finalised and collated for journal submission.

**5. Research question: What percentage of growers adopted practices that reduce the incidence of diseases and pests on their farms at project completion?**

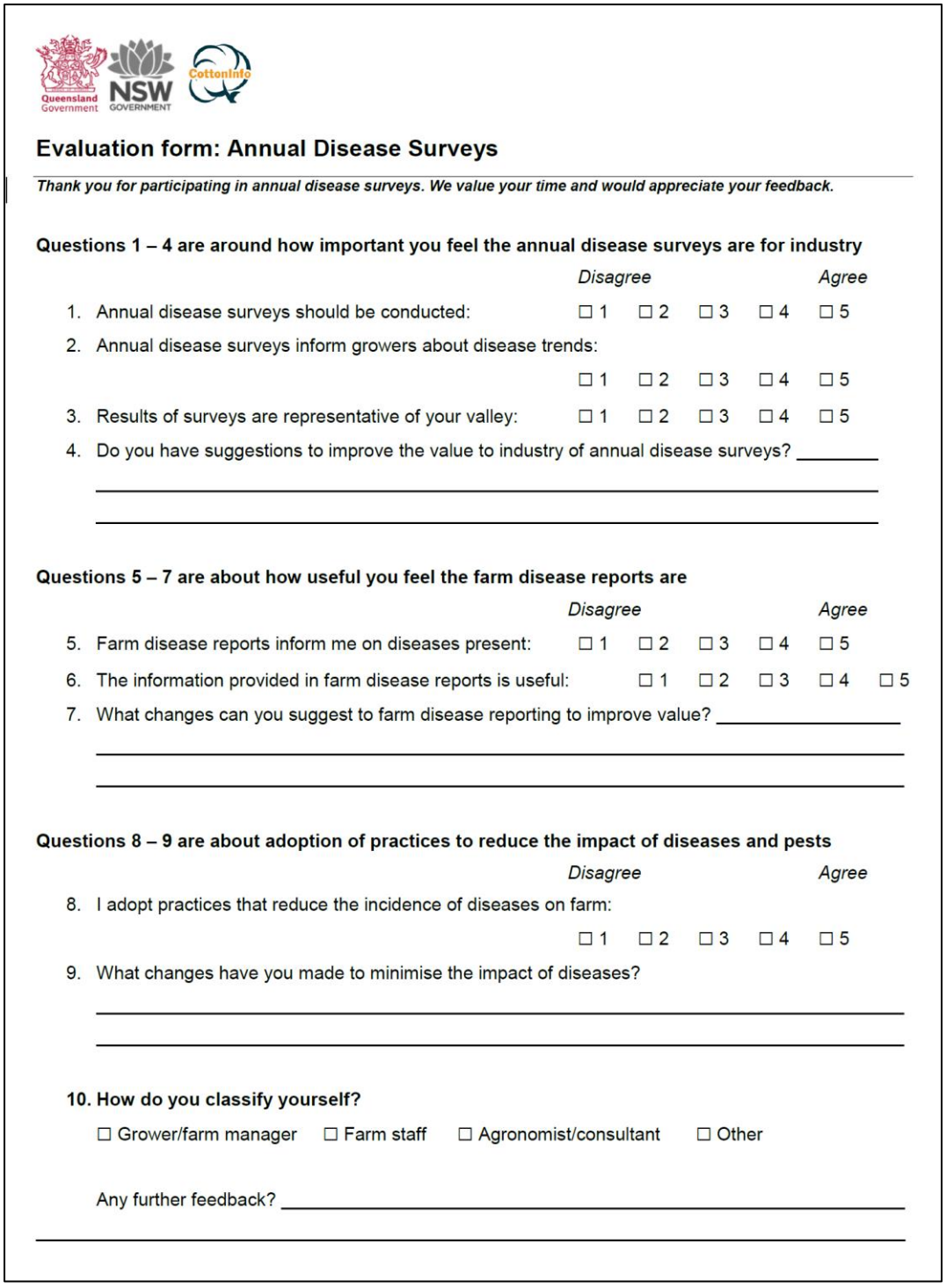
**5.1 Adoption pathways and measurement of adoption**

Various discussions were initiated to determine how to best measure adoption of practices. These include:

- Linda Scheikowski and Linda Smith met with Warwick Waters on 23rd August and 24th September 2016 to discuss Adoption Pathways for this project.
- Contacted Janelle Montgomery and Elsie Hudson on 10th August 2018 to ask their advice on how best to address research question 5. Janelle suggested talking with Warwick Waters.
- Janelle Montgomery and Warwick Waters on 13th August 2018 suggested the Grower Practice Survey conducted by CRDC each year could potentially include some survey questions in the disease section of the survey. At this time, we were too late to be included in this survey, as questions are normally developed in March/April, ready to go out at the end of May. Warwick suggested that if this timing suited for the following year, this would be the preferred way to survey growers rather than a separate survey.
- Elsie Hudson offered to help create a survey through survey monkey to be distributed to growers, if this was supported by Warwick. Out of interest, Elsie asked a few questions on disease in her survey in 2018. She had 28.6% of growers who felt the disease survey did not accurately represent disease incidence in their area. Upon further study of the responses, the same grower/consultant combination responded this way, with the remainder happy with results of surveys.
- A meeting evaluation form used by Sharna Holman following a nematology update in Emerald, was modified, with Sharna's assistance, and formed the basis of an evaluation form for disease surveys on the 25th September 2019.
- It was decided that a survey at the completion of this 3-year National project was best to capture quantitative data.

An evaluation form was developed (Figure 8) and sent to growers involved in annual disease surveys. This form could also be sent to other key people such as agronomists/consultants for their perspective on disease surveys and adoption of management strategies.

The involvement of CottonInfo on surveys has resulted in a successful collaboration in which CottonInfo have a better understanding of cotton diseases and Pathology teams have a better understanding of regional issues directly resulting in quick and effective dissemination of research findings back to the cotton community. This has been through regional newsletters, field day presentations (CottonInfo and Pathologists), research updates at annual general meetings and CCA meetings, CottonInfo E News, industry articles and CottonInfo presentations at CSD roadshows.



## Evaluation form: Annual Disease Surveys

*Thank you for participating in annual disease surveys. We value your time and would appreciate your feedback.*

**Questions 1 – 4 are around how important you feel the annual disease surveys are for industry**

| | *Disagree* | | | | *Agree* |
|---|---|---|---|---|---|
| 1. Annual disease surveys should be conducted: | ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 |
| 2. Annual disease surveys inform growers about disease trends: | ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 |
| 3. Results of surveys are representative of your valley: | ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 |

4. Do you have suggestions to improve the value to industry of annual disease surveys? _____

_____

_____

**Questions 5 – 7 are about how useful you feel the farm disease reports are**

| | *Disagree* | | | | *Agree* | |
|---|---|---|---|---|---|---|
| 5. Farm disease reports inform me on diseases present: | ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 | |
| 6. The information provided in farm disease reports is useful: | ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 | |

7. What changes can you suggest to farm disease reporting to improve value? _____

_____

_____

**Questions 8 – 9 are about adoption of practices to reduce the impact of diseases and pests**

| | *Disagree* | | | | *Agree* |
|---|---|---|---|---|---|
| 8. I adopt practices that reduce the incidence of diseases on farm: | ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 |

9. What changes have you made to minimise the impact of diseases?

_____

_____

**10. How do you classify yourself?**

☐ Grower/farm manager ☐ Farm staff ☐ Agronomist/consultant ☐ Other

Any further feedback? _____

_____

**Figure 8.** Disease survey evaluation form sent to growers/consultants in 2019

## *Results*

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

### 1. Research question: How is the incidence and severity of cotton diseases changing?

### 1.1 Conduct review of current disease survey methodologies and processes conducted in collaboration between DAF Qld, NSW DPI and CottonInfo and develop survey protocols including communication, technology and use of data and geospatial database established in agreement with industry and partners.

Methodologies and processes were finalised following regular meetings and correspondence. A key decision was how disease surveys were to be conducted, as these needed to be consistent across all regions. The methodology for annual disease surveys was developed and agreed upon by all parties. Survey protocol was sent to all participants of surveys.

Annual Disease Survey Protocol

Commercial cotton crops in New South Wales and Queensland are inspected twice each season, in October - December and in January - April. The incidence and severity of those diseases present are assessed. If possible, at time of survey the field history, ground preparation, cotton variety, planting date and seed rate are obtained from grower or agronomist. If not available at time of survey, this information is collected from grower via email or by phone post-survey. Soil is collected for assessment of plant-parasitic nematodes from all fields surveyed. Soil is collected for Gupta Vadakattu as required for microbial assessment (Microbial analyses discussed in milestone 4).

Cotton areas surveyed in Qld include Emerald/Theodore, The Darling Downs, St George/Dirranbandi and Border Rivers. In NSW, cotton areas include Bourke/Walgett, Namoi, Macintyre, Macquarie, Gwydir, Lachlan and Murrumbidgee.

The endemic diseases monitored include Fusarium wilt, Verticillium wilt, Alternaria leaf spot, Tobacco Streak Virus (TSV), Cotton Bunchy Top (CBT) and boll rots, plus anything unusual. Absence data for six exotic diseases are also recorded: exotic Fusarium wilt, exotic Verticillium wilt, Texas Root Rot, Hyperviralent Bacterial Blight, Cotton Leaf Curl and Blue Disease. Other data collected includes rating of herbicide damage and volunteer cotton.

*Survey Protocol* (Figure 9)

Start each field away from edge (approx. 50m).

- Record transect using Fulcrum App.
- Check 10 plants, skip 10 rows, and check 10 plants etc. until 100 plants have been assessed.
    - Move diagonally through the field (stratified walk)
    - GPS end point (after 100 plants assessed)

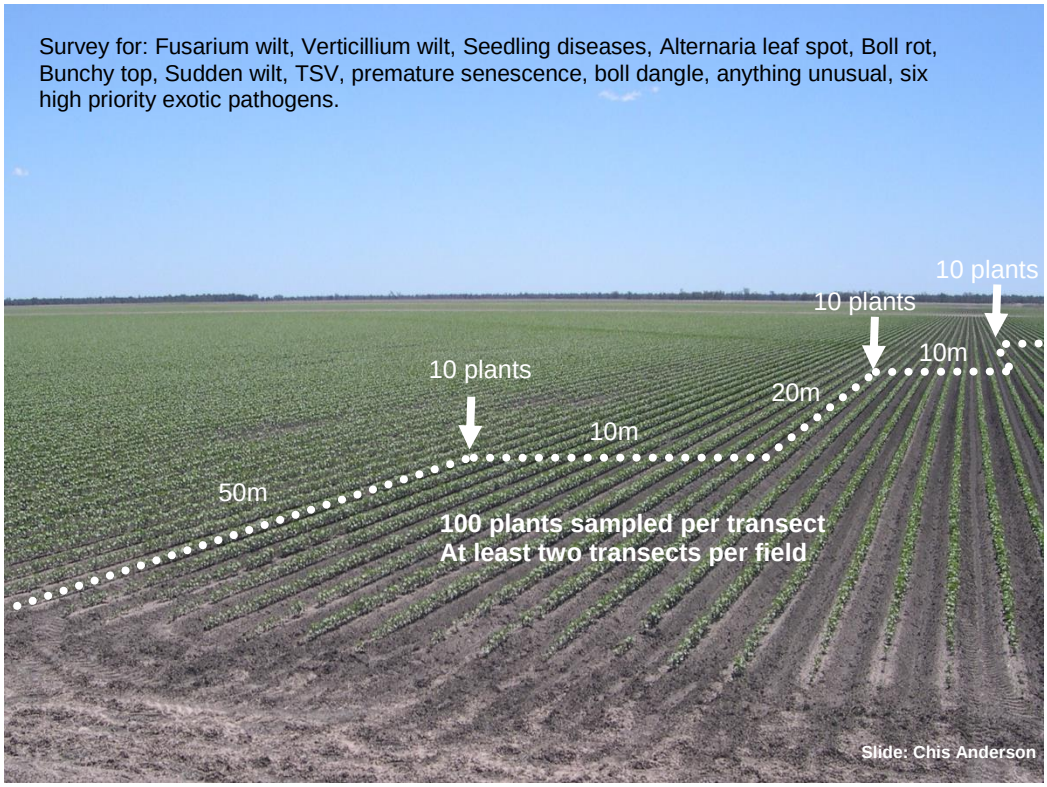



**Figure 9.** Schematic of stratified walk to conduct disease survey of a field

*Early Season*

- Count plants per metre (place a 1 m stick placed along the row), find out from grower the number of seeds planted per metre and variety, previous crop, nutrition as detailed on data form.
- An estimate of the number of seeds planted per metre of row is compared to the number of plants established per metre providing an estimate of seedling mortality. This estimate includes the impact of seedling disease (e.g. *Pythium* sp., *Rhizoctonia* sp., *Fusarium* sp., etc.), seed viability, activity of soil insects (e.g. wireworms), physical problems (e.g. herbicide burn) and effects of adverse environmental conditions.
- Seedling diseases – Number of plants with Pythium (soft rot and stem collapse -bendy root stringy and flexible) and Rhizoctonia (sunken red/brown spots on the lower stem and roots).
- Fusarium – Look for vascular discolouration, wilting, collect sample for diagnosis, note number of plants with Fusarium wilt symptoms.
- BRR -The incidence and severity of Black root rot in the crop can be assessed by a step point transect 4 weeks after planting. Dig up (do not pull) 10 seedlings. Record the number of seedlings with blackened roots. Rate the severity of each seedling taproot on a scale of 0 – 10, where 0 = no blackening and 10 = 100% of the tap root is blackened. Walk up row for 20 meters and across 10 rows. Assess another 10 plants. Continue this until you have assessed a total of 100 plants, as per Figure 9. Express the incidence of disease as a percentage plants infected and severity rated 1-10. Collect samples for confirmation of BRR using microscopy.
- TSV (Figure 10)
- Anything unusual
- Note where volunteer cotton plants are (field, channel etc) and rate 0 = none seen, 1 = < 10 (rare), 2 = 10-100 (common), 3 = > 100 (numerous).
- Look for Bunchy top and vectors in old volunteer plants in channels etc.
- Six high priority exotic pathogens (Figure 13).

*Late Season*

*Alternaria*

- Record proportion of plant affected and approximate leaf area (within that proportion) affected, eg. 25% of leaves on lower third of plant.

- Do not need to record details for every single plant within a group if the level of infection is consistent across a number of plants; proportions are estimated visually.
- Record as ‘trace only’ if only very mild infection.

*Verticillium and Fusarium Wilt*

- Split and cut plants to determine if there is internal discolouration, record number of plants.
- Collect samples for confirmation of pathogen.

*Boll and Seed Rots*

- Assess plant 1 of every 10
- Estimate % of bolls that have boll and seed rot. Separate into boll rot, tightlock and seed rot.
    1. Boll rot - complete collapse of the boll, “boll wall and all”, boll falls apart when squeezed
    2. Tightlock or Hardlock - Failure of locks to ‘fluff out’ when bolls open. Boll wall is firm, however locks remain compact and easily fall out.
    3. Seed rot -A soft brown rot of developing seed within the bolls. May not become apparent until the bolls either drop or open prematurely. Only one or two locks as well as whole boll can be affected.
    4. Do not count rots resulting from fungi gaining entry into boll wall following insect damage – look for ‘bullet hole’.

*Percentage Bolls Open*

- Count the number of bolls open on plant 1 of every 10 checked i.e. a total of 10.

*TSV*

- Count the number of plants with symptoms of TSV (Figure 10).

**Figure 10.** Cotton displaying symptoms of TSV that include purplish brown, necrotic lesions in the leaves

*Bunchy Top*

- Count the number of plants with symptoms of Bunchy Top (Figures 11 and 12).

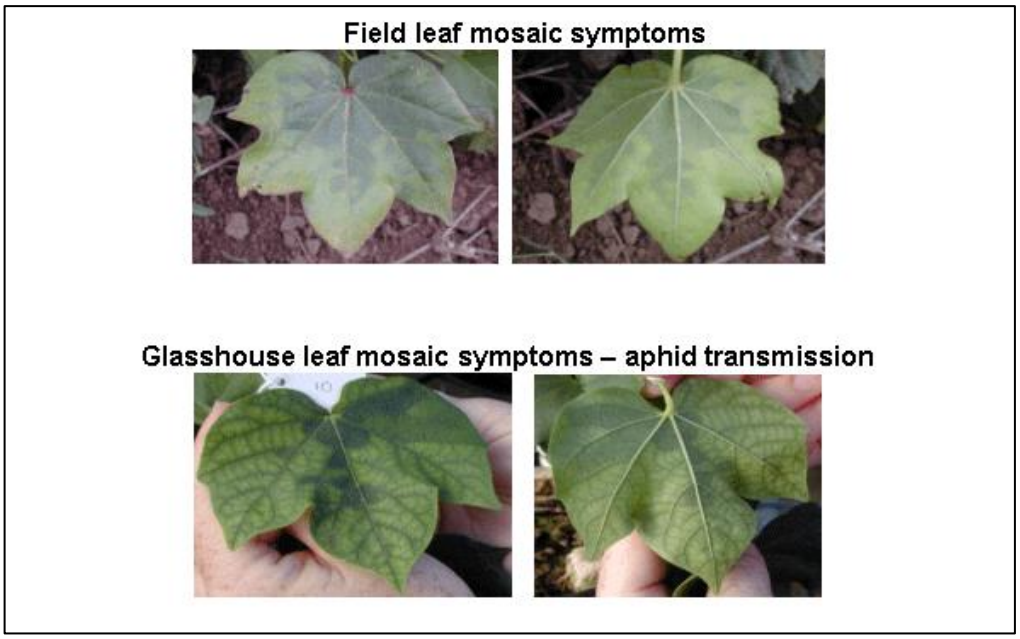



**Figure 11** Symptoms of CBT: mosaic leaf pattern on upper and lower leaf surfaces in the field and leaf mosaic pattern in the glasshouse plants inoculated with aphids from CBT affected plants (provided by Murray Sharman)

**Figure 12.** Symptoms of CBT include small leaves and bolls

*Hormonal damage*

- Rate hormonal damage if observed.

*Volunteer Cotton Plants*

- Note where volunteer cotton plants are (field, channel etc) and rate 0 = none seen, 1 = < 10 (rare), 2 = 10-100 (common), 3 = > 100 (numerous).
- Look for Bunchy Top and vectors in old volunteer plants in channels etc

*Six High Priority Exotic Pathogens* (Figure 13)

- Exotic *Fusarium oxysporum* f.sp. *vasinfectum* (Fov)
- *Verticillium dahliae* (new Strains)
- Texas Root Rot
- Blue Disease
- Hypervirulent Bacterial Blight
- Cotton Leaf Curl

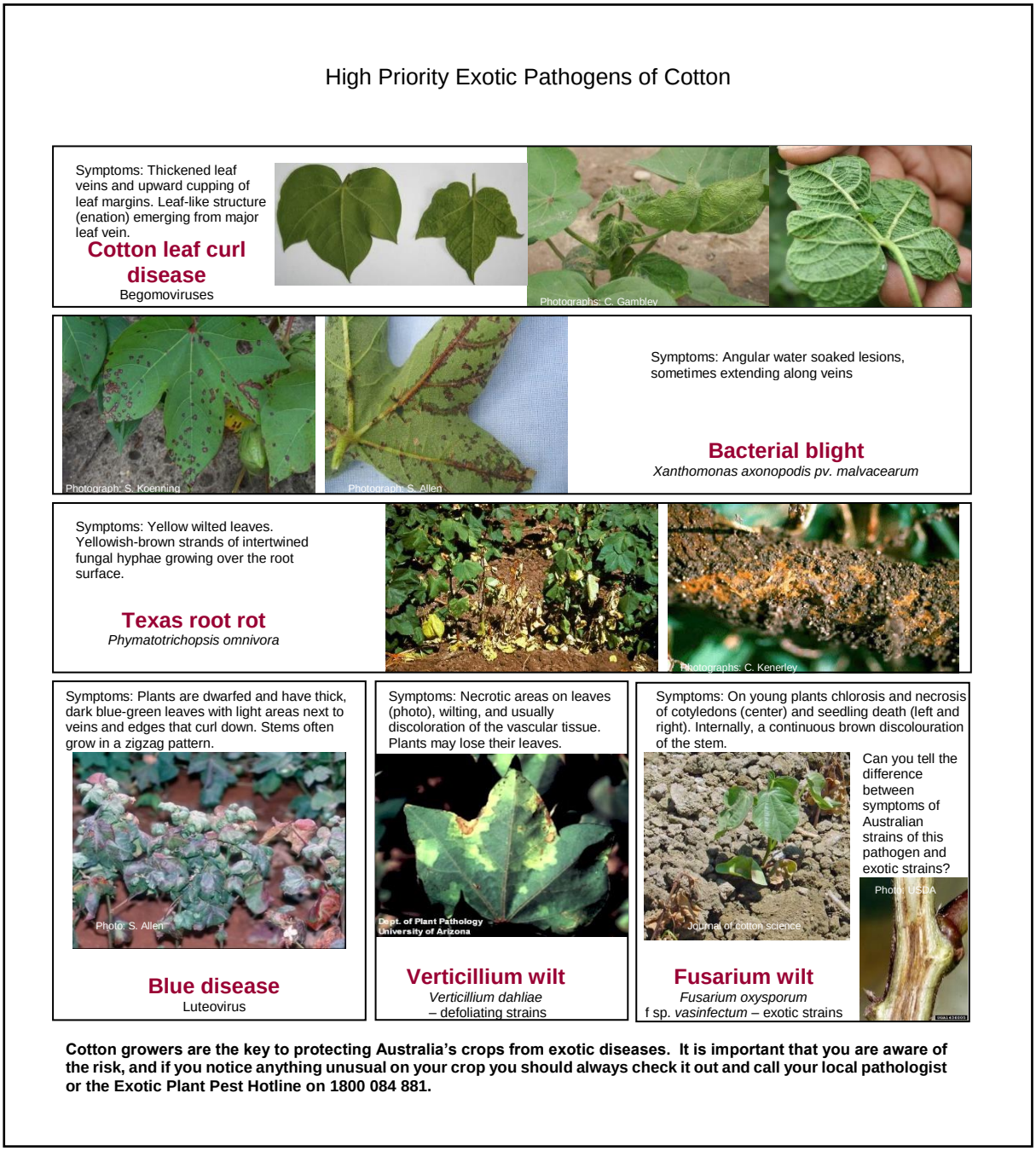



**Figure 13.** Six high priority exotic pathogens of cotton

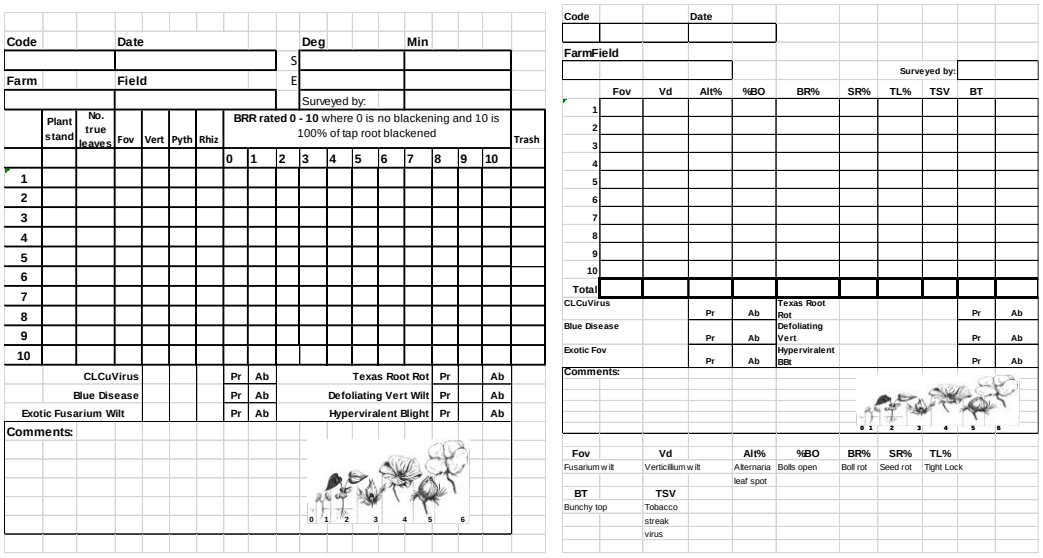



**Figure 14.** Data recording templates for early and late season disease surveys

**Outcomes of meeting to discuss early and late season survey process 27/9/2016**

Discussions were held between NSW DPI, DAF CottonInfo and CRDC to discuss early and late season survey process**.** Outcomes of meeting include:

1). How are farms/fields selected

- Represented of region
- Geographically diverse within a region
- Some farms with similar practices/farming systems and some with different systems
- Previous cropping history
- Dryland cropping
- Some sites - Longitudinal
- Planting dates early/late
- Look at emerging problems as reported
- Irrigation systems (overheads, pipe through bank, surface systems, bankless)
-

2). Data to be collected

- GPS referencing of sample points

3). Roles

- Pathologist on survey select fields on the day based on discussion with CottonInfo. CottonInfo provide suggestion of fields
- CottonInfo to follow up for post survey data (nutrition, yield, irrigation, cultivation practice, variety).

4). Database

- Access, management etc.

5). Confidentiality around growers/sites

6). Coding system that will work

- Code by region, year, farm, field

**Database to Store Disease Survey Data**

On the 4th November 2016, staff met with Michael Hassall, Senior Research Scientist (Bioinformatics) with DAF, to discuss databases that might be suitable to store disease survey data, and that would be accessible to both NSW DPI and QLD DAF. Three programs considered were 1). Kathmandu, a program used by the DAF wheat-breeding team; 2). SharePoint, which is a document management and collaboration tool developed by Microsoft. SharePoint can be used as a central portal for exchange of

information and was available through DAF; and 3). Microsoft Excel, which is a widely used spreadsheet program used within both departments.

It was determined, that to have a database that was accessible by both NSW DPI and DAF simultaneously was going to be unnecessarily complicated due to the IT protection required within each department. NSW DPI and DAF staff agreed that Microsoft Excel is a simple database suitable for our needs and shared safely via email.

Through regular discussion and communication the format of the database was agreed upon by all parties.

**Training of CottonInfo and pathology teams in NSW DPI and DAF**
User accounts for DAF staff were set up on the 11th November 2016 under a Fulcrum organization: NSW Department of Industry. David Larsen provided training 28th November 2016 in Narrabri on using the database Fulcrum that he developed specifically for cotton disease surveys.

DAF pathology team travelled to Narrabri 28th November 2016 to conduct disease survey training with NSW DPI Technical Officer and CottonInfo Regional Officers.

Aphrika Gregson joined the DAF pathology team in central Queensland for the 2016/17 season to learn alongside the pathologists how to conduct early season disease surveys. The Fulcrum app was tested during this survey for ease of use and suitability to purpose.

A conference call with the team was conducted on Monday 23rd January 2017 at 12:00 – 1:00pm (Brisbane time) to discuss progress of project following completion of early season disease surveys. The week prior Annabel Twine attended an REO team meeting and documented comments for discussion. Participants included Lisa Bird, David Larson, Rod Jackson, Aphrika Gregson, Linda Scheikowski, Tim Shuey, Annabel Twine and Linda Smith.

CottonREOs received hands-on training whilst conducting surveys with state pathologists.

Communication, technology and use of data and geospatial database established.

**1.2 DAF Qld, NSW DPI and CottonInfo to conduct annual early and late season disease surveys of endemic and exotic plant pathogens on commercial cotton farms in Qld & NSW, monitoring incidence and severity of diseases of cotton and recording presence/absence of exotic pests.**

## 2016/17 season

Due to late contracting, there was inability to appoint a full team for the 2016/17 season surveys.

Key Findings Early Season
With an unusually wet spring, high insect pressure and a record-breaking heatwave, the 2016/17 cotton season was a challenging one.

Across all surveyed regions in NSW and Qld, seedling diseases - particularly black root rot (BRR) and *Rhizoctonia solani* - were endemic during the early season surveys, most likely resulting from the cool/wet conditions around planting. Thrips damage was also common across all Qld regions early season.

Summary of early season findings for each region (Oct-Dec):

Emerald/Moura/Theodore:
- Significant issues with root development (possibly as a result of bed preparation and moisture issues) causing replants.
- Evidence of some insect activity, with roots appearing chewed.
- Eargwigs, wireworms and symphylids observed.
- BRR was observed in fields at Moura and Theodore.

- Reniform nematodes were observed infecting the roots of cotton in Theodore.

**Figure 15.** Poor root growth observed in CQ

Darling Downs:
- BRR high incidence but low severity.
- Fusarium evident, causing visible symptoms in a few fields.

St George, Thallon, Dirranbandi:
- Fusarium observed on one new field on a farm with known Fusarium.
- BRR low incidence in the region.

Macintyre:
- BRR observed on all six fields examined.
- Verticillium wilt evident in one field.

Gwydir/Upper Namoi:
- Moderate levels of BRR and *Rhizoctonia* sp. detected, along with stunting and stand establishment issues.
- Warmer weather may have hidden symptoms of severe seedling disease.

Lower Namoi:
- Minimal stand losses reported.
- Highest rate of *Rhizoctonia* sp. per field affected 56 per cent of plants.
- *Pythium* spp. only detected in Lower Namoi across several farms.

Macquarie:
- High severity and incidence of *Rhizoctonia* sp., rates as high as 52 per cent per field.
- Stand losses reported.
- Large variation (0-47 per cent) of BRR incidence across surveyed farms.

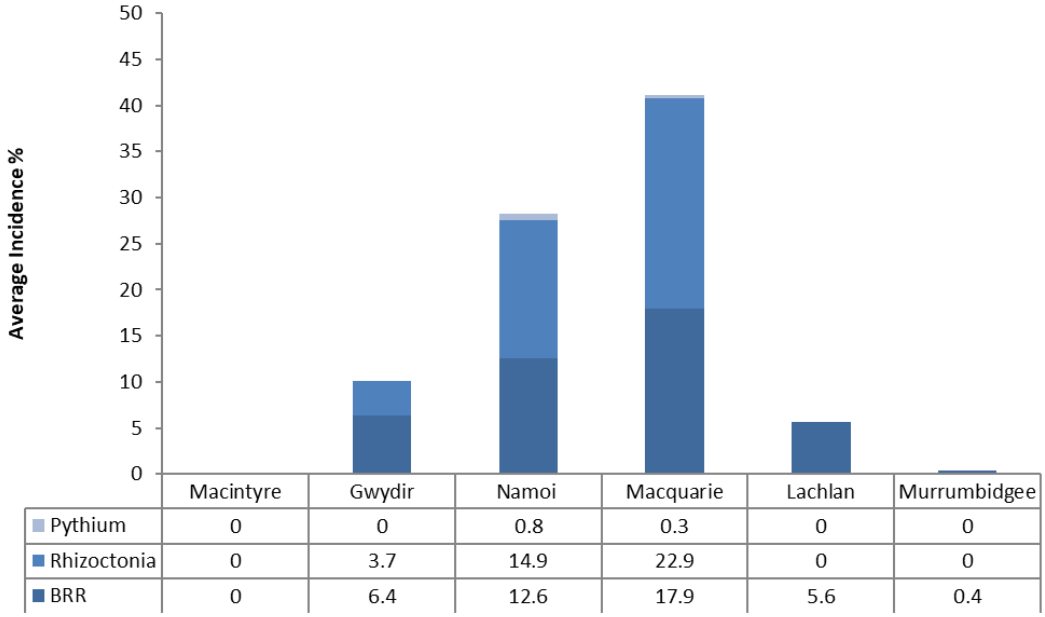
Lachlan:
- Moderate rates of BRR.
- Low severity of other seedling diseases, and minimal stand losses.

Murrumbidgee:
- Lower incidence and severity of BRR.
- Minimal *Rhizoctonia* sp.
- Significant stand losses observed.
- Warmer weather may have hidden symptoms of severe seedling disease.

Figure 16 shows the incidence of seedling diseases observed early season in NSW. It is important to note that the lateness of surveys in NSW meant that conditions were not ideal for detection of Black root

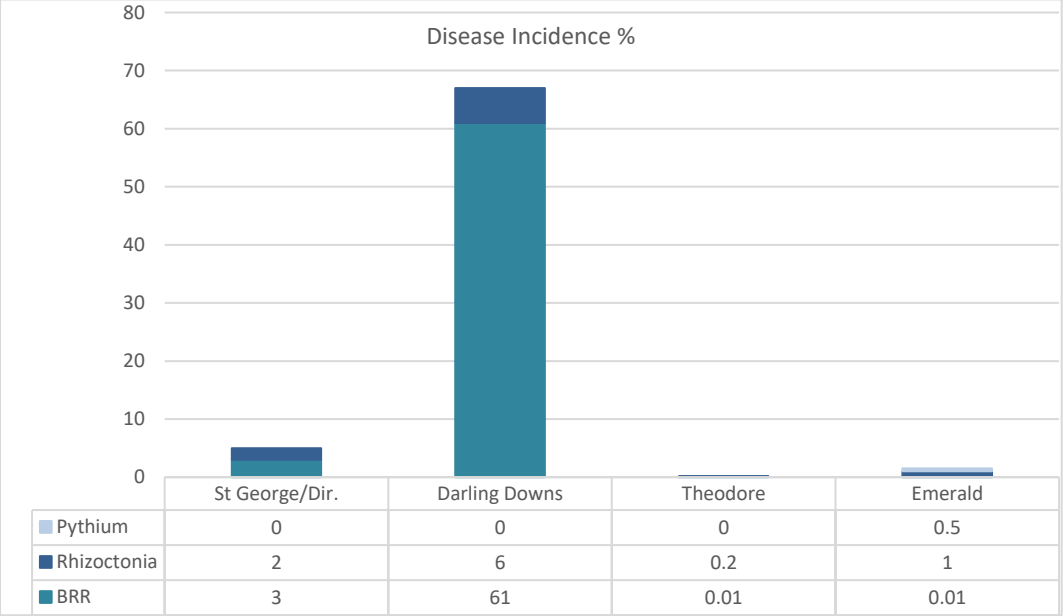
rot/rhizoc (temperature rise in December). The highest incidence of Rhizoctonia in NSW was in the Macquarie followed by the Namoi. A low incidence was detected in the Gwydir, and no detections elsewhere in NSW. The Macquarie region also had the highest incidence of BRR.

In Qld there was a very high incidence of BRR on the Darling Downs, but in general low severity (Figure 17).




| | Macintyre | Gwydir | Namoi | Macquarie | Lachlan | Murrumbidgee |
|---|---|---|---|---|---|---|
| Pythium | 0 | 0 | 0.8 | 0.3 | 0 | 0 |
| Rhizoctonia | 0 | 3.7 | 14.9 | 22.9 | 0 | 0 |
| BRR | 0 | 6.4 | 12.6 | 17.9 | 5.6 | 0.4 |

**Figure 16.** NSW Early season – Average seedling disease incidence (%)




| | St George/Dir. | Darling Downs | Theodore | Emerald |
|---|---|---|---|---|
| Pythium | 0 | 0 | 0 | 0.5 |
| Rhizoctonia | 2 | 6 | 0.2 | 1 |
| BRR | 3 | 61 | 0.01 | 0.01 |

**Figure 17.** Qld Early season – Average seedling disease incidence (%)

Figure 18 shows how seedling mortality is influenced by cool conditions after planting. In the 2014/15 early season surveys in St George, the conditions were dry and hot with few cold days over the planting period. In the 2016/17 however, conditions were cool and wet at the start followed by high heat. Later planted crops suffered less as they established in warmer soils. Planting dates that were followed by cold wet conditions yielded higher seedling mortalities, some of which was due to disease.

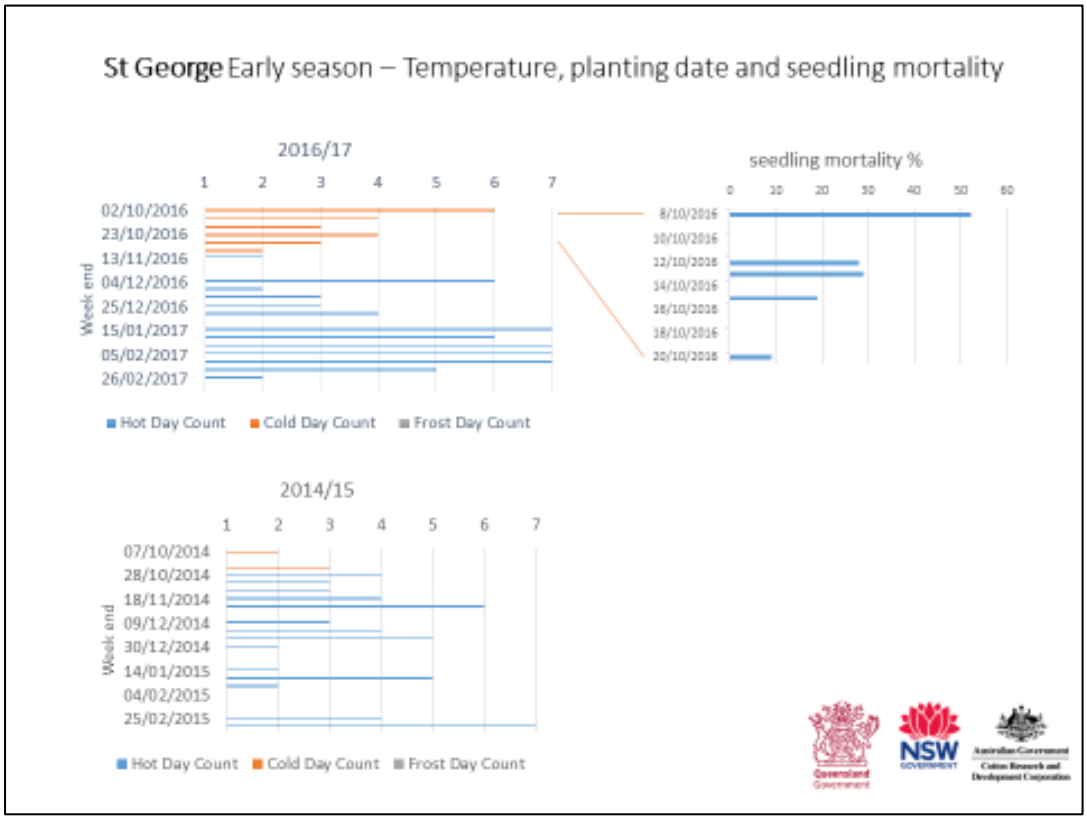



**Figure 18.** Effect of environmental conditions after planting on seedling mortality

Key Findings Late Season

Verticillium wilt was detected in all regions in NSW except the Lachlan Valley (Figure 19), with the Namoi, Macintyre and Gwydir expressing moderate incidences and low incidences observed in the Macquarie and Murrumbidgee.

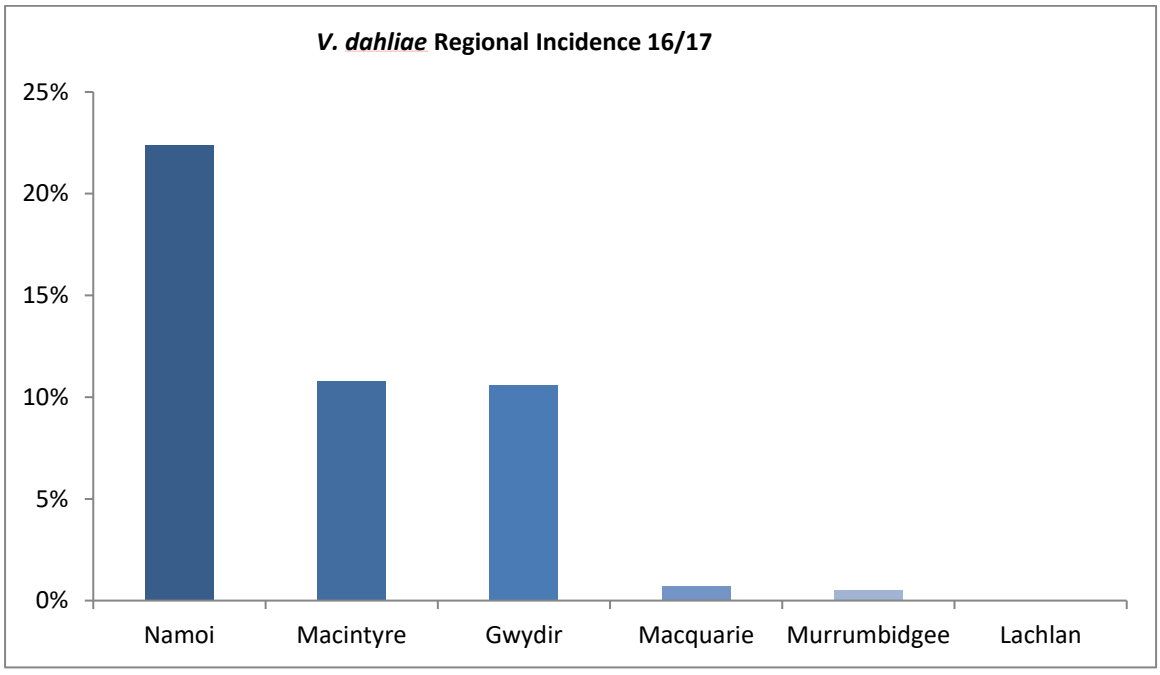



**Figure 19**. Regional incidence of Verticillium wilt in NSW 2016/17 surveys

As recorded from in-field observations of internal (vascular discolouration) and external (wilting, leaf dieback) symptoms. Data based upon surveys of 38 farms; 69 fields across the Macintyre, Gwydir, Namoi, Macquarie, Murrumbidgee and Lachlan Valleys.

In Qld, there has been an increase in the incidence of Verticillium wilt and number of fields with Verticillium wilt (Figure 20).

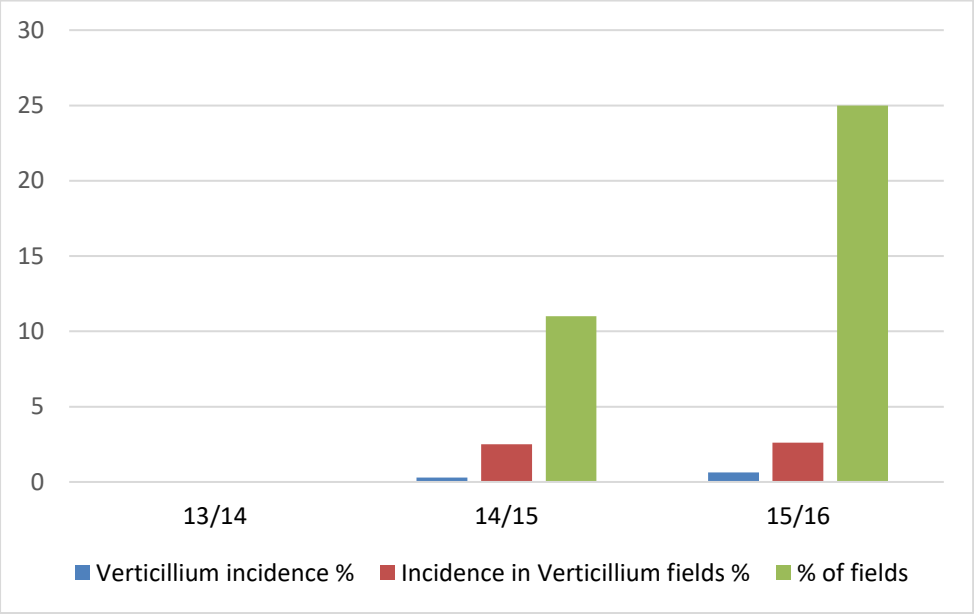



**Figure 20.** Increase in the incidence of Verticillium wilt in Qld over three seasons

Fusarium wilt was detected in all regions of NSW except the Lachlan valley (Figure 21). The highest incidence of 9.1% was in the Gwydir.

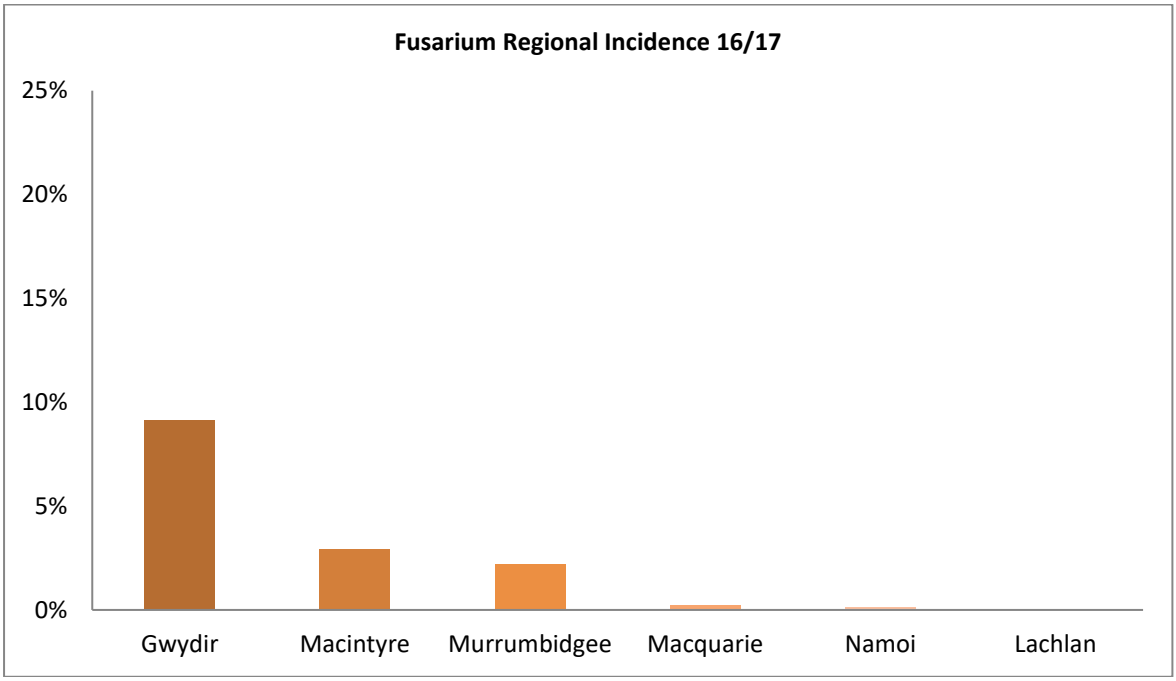



Figure 21. Regional incidence of Fusarium wilt in NSW 2016/17 surveys

Fusarium wilt was detected in all regions of Qld except Emerald (Figure 22). The highest incidence of 10% was in St. George.

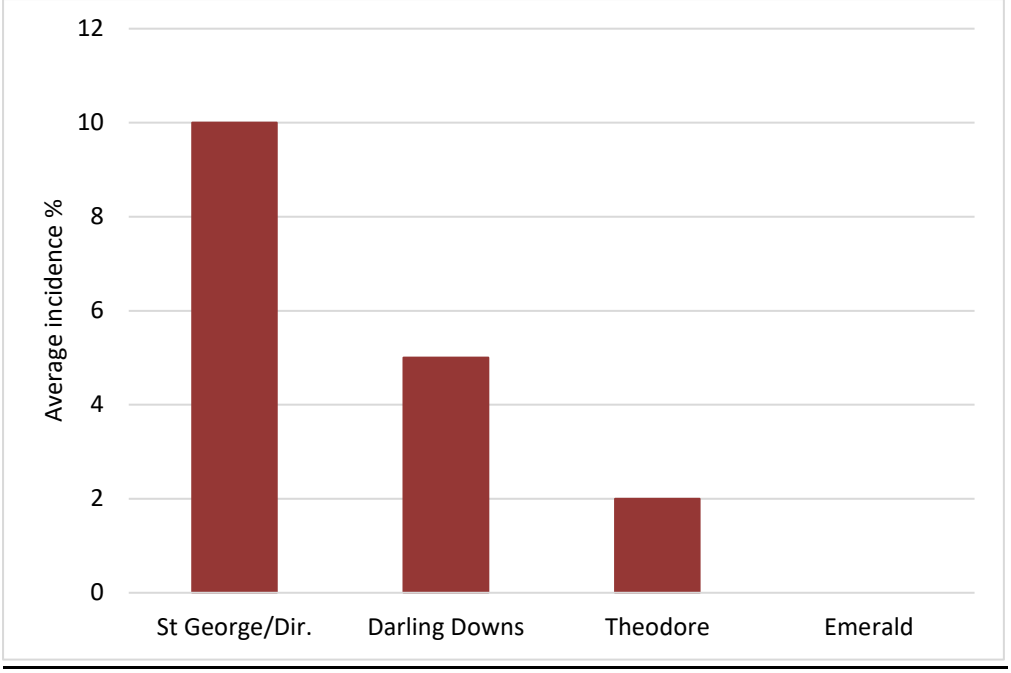



Figure 22. Regional incidence of Fusarium wilt in Qld 2016/17 surveys

Mealy bugs

In general, a low level of mealybugs were observed in CQ( Figure 23). One field however had a high infestation causing large patches of damage to the cotton crop. Natural predators were observed to be present and active.

**Figure 23.** Mealy bugs detected in CQ

Boll rots

In Qld, tight-lock (Figure 24) was generally low with an average incidence of 4%. The incidence of seed rot was 3.4%, Phytophthora boll rot was 0.25% and other boll rots at 4%.

**Figure 24.** Normal cotton vs tight-lock cotton

## 2017/18 season

Key Findings Early Season

Temperature plays many important roles in the growth and development of cotton. Low temperatures after sowing increases the time to emergence and reduces seedling vigour, often leading to poor establishment, poor early growth, and increased risk of seedling diseases. In Australian cotton production systems, events where the minimum daily temperature falls below 11'C is referred to as 'cold shocks'. Growers and advisors use the number of cold shocks in assessing retardation of crops in their areas. Across the industry, the start of the season was cool and wet. Graphs in Figure 25 show minimum temperature and rainfall for Emerald and Narrabri. The red line represents 11C and below this line is the period of cold shock days. After planting there were numerous rain events where cool and cloudy weather could slow down emergence and establishment. Combined, these events are conducive to the development of seedling disease. The timing of crop maturity, yield and fibre quality may also be affected.

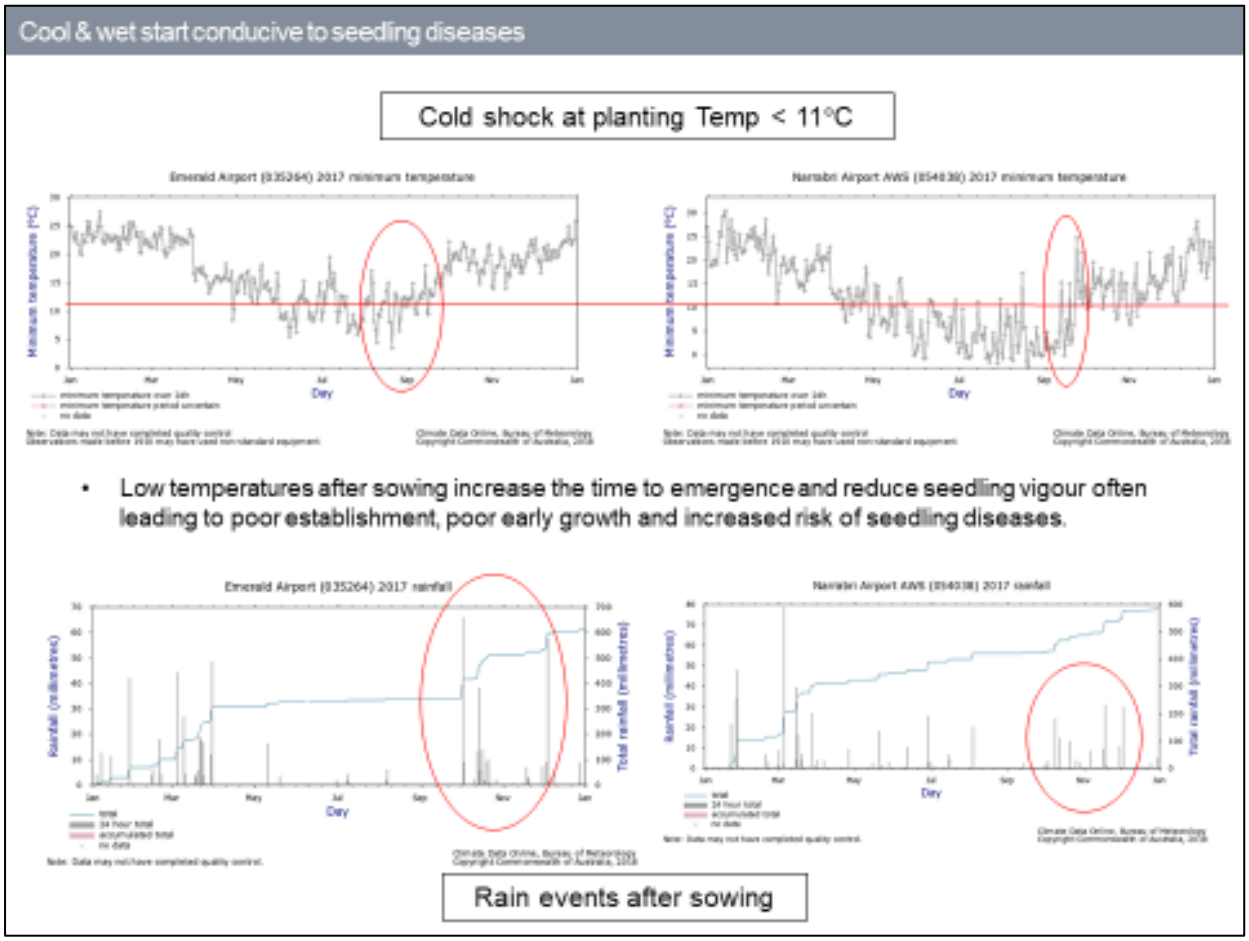



**Figure 25.** Slide presented at FUSCOM showing minimum temperature and rainfall for Emerald and Narrabri. The red line represents 11C and below this line is the period of cold shock days.

RHIZOCTONIA AND PYTHIUM ROTS

The mean incidence of Pythium was very low (<1%) to absent across both NSW and Qld in the 2017/18 season (Figure 26). Rhizoctonia-like symptoms were observed on seedlings in field in all cotton-growing regions in Queensland and NSW (Figure 26). Upon laboratory confirmation both *Rhizoctonia* and *Fusarium* species were isolated from plant tissue. The mean incidence of Rhizoctonia was low across Queensland cotton regions ranging from 0.4 – 2.8%, causing minor concern. In NSW, the mean incidence ranged from 14 – 63% (Figure 26).

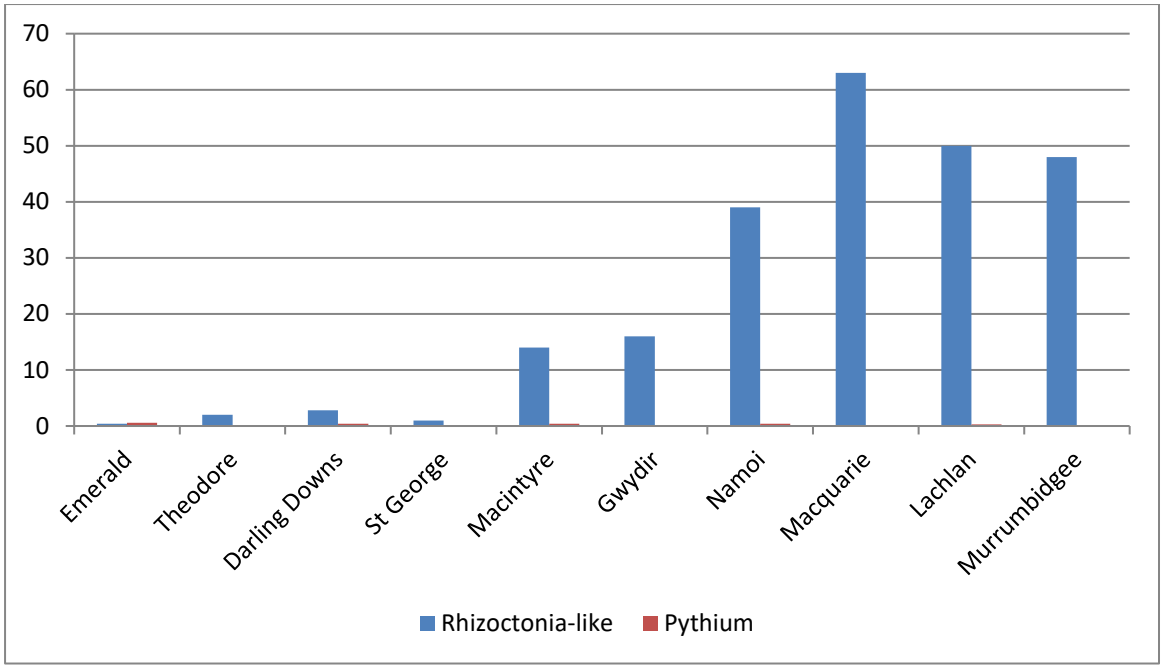



**Figure 26.** Early season disease incidence (%) of Pythium and Rhizoctonia

BLACK ROOT ROT

Black root rot was a significant disease issue for the majority of regions in the 2017/18 season. This disease is a particularly serious problem for southern NSW, because of its cooler climatic conditions which favour the pathogen (Table 3 and Figure 27). However, even in Queensland, significant root damage was observed in all regions due to this pathogen (Table 4), particularly where cool conditions occurred.

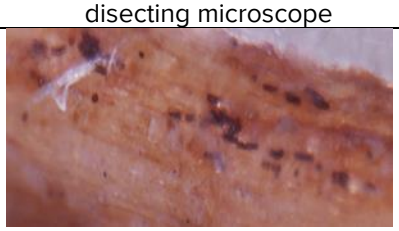
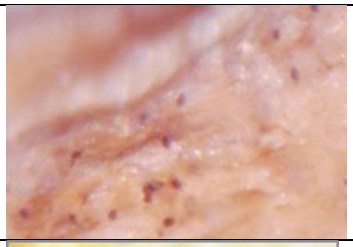
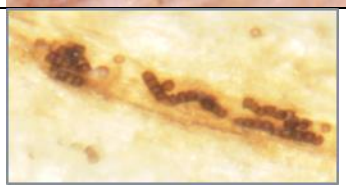
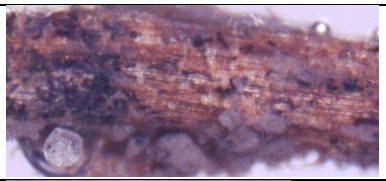
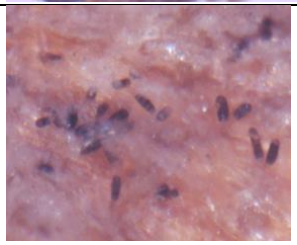
**Table 3.** The average incidence, range of disease incidence and severity of Black root rot in NSW

| Region (NSW) | Incidence | | BRR Severity (%)** |
|---|---|---|---|
| | (%)* | Range | |
| Gwydir | 45 | 9 - 85 | 1.4 |
| Namoi | 37 | 1 - 80 | 9.0 |
| Macquarie | 55 | 12 - 96 | 15.0 |
| Lachlan | 37 | 0 - 98 | 32.0 |
| Murrumbidgee | 15 | 0 - 80 | 10.0 |

*Incidence of disease expressed as percentage of plants infected; **Severity of disease expressed as percentage of taproot blackened, necrotic: severity of 100%=entire taproot black

**Figure 27.** Cotton seedlings displaying Black root rot symptoms from NSW

**Table 4.** Roots of cotton seedlings displaying Black root rot symptoms and spores of BRR observed on the root surface

| Region | Roots | BRR spores observed under disecting microscope |
|---|---|---|
| Emerald |  |  |
| Theodore |  |  |
| St George |  |  |
| Macintyre |  |  |
| Darling Downs |  |  |

ALTERNARIA LEAF SPOT

Alternaria leaf spot was a major disease this season in southern NSW. Serious infection of young seedlings were observed in the Lachlan and Murrumbidgee regions in early December 2017 (Figures 28 and 29). Research is being conducted to better understand the species of *Alternaria* contributing to this leaf spot disease.

**Figure 28.** Severe symptoms observed in early December 2017

**Figure 29.** *Alternaria* affected seedlings in the Murrumbidgee (left) and Lachlan (right) regions

Key Findings Late Season

VERTICILLIUM WILT

Verticillium wilt (caused by *Verticillium dahliae*) was confirmed in the Namoi, Gwydir and Macintyre valleys with an average incidence of 30%, 7% and 5% respectively (Figure 30). Areas with severe symptoms of Verticillium wilt were observed in several fields, with the highest disease incidence recorded in each of these regions of 89%, 56% and 44% respectively. The Namoi was the only region with an increase in the average incidence of disease compared to the 2016/17 season (Figure 30). The significant rain events late January and early February provided conducive conditions for the development of this disease, with external symptoms developing rapidly.

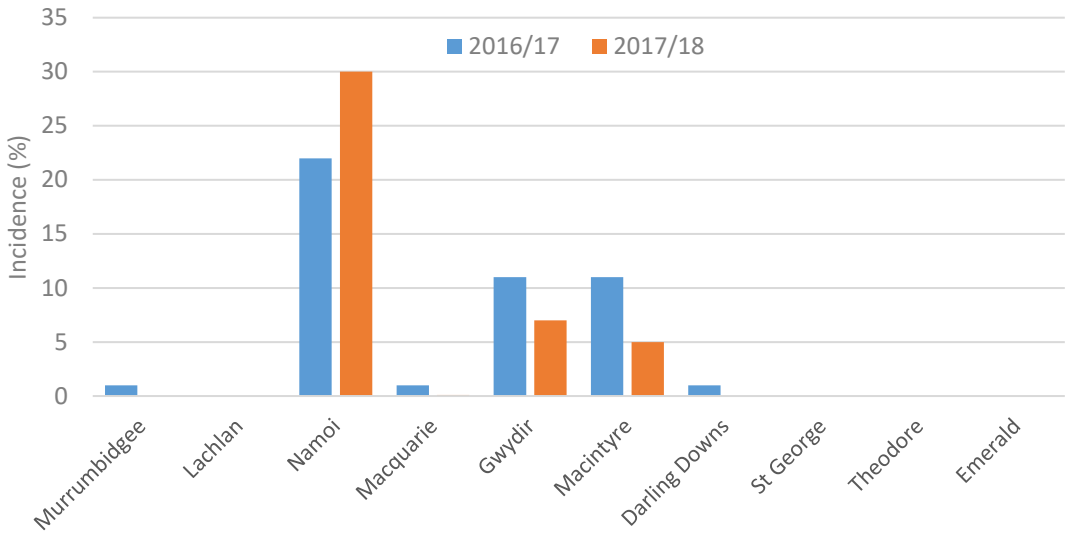



**Figure 30.** The incidence of Verticillium wilt of cotton 2016/2017 and 2017/2018. The disease is present in Queensland, but not detected in the 2017/18 season

FUSARIUM WILT

The average disease incidence of Fusarium wilt for each region in the 2016/17 and 2017/18 surveys is presented in Figure 31, showing that the average incidence of Fusarium wilt increased in the Namoi and Macintyre valleys. It is important to highlight the high incidence of Fusarium wilt detected in some fields this season. Disease incidence was as high as 60%, 46%, 47% and 20% for the Macintyre, St George, Gwydir, Namoi and Darling Downs respectively. There was likely an impact on yield for some of these fields. The significant increase in Fusarium wilt detection in the Macintyre valley (Figure 31) may partly be due to the significant increase in the number of farms/fields and representative area surveyed within this region. The highest incidence recorded in Central Queensland was only 2%. No Fusarium was detected within transects surveyed in the Lachlan or Murrumbidgee, however outside of transects, Fusarium wilt was confirmed in southern regions.

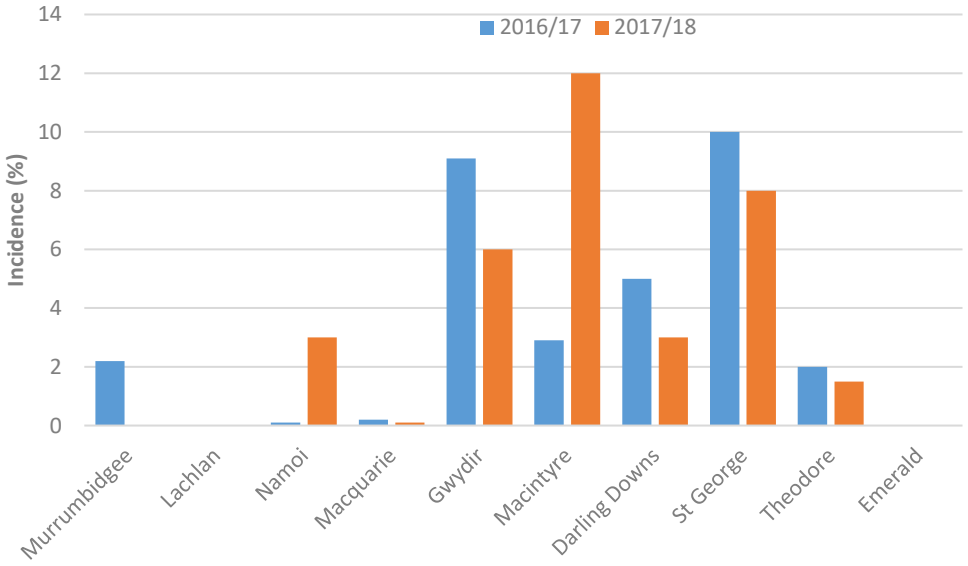



**Figure 31.** The mean incidence of Fusarium wilt of cotton in 2016/17 and 2017/18 season. Fusarium wilt is present in all cotton production areas listed.

BOLL ROTS

Environmental conditions are the biggest factors in determining infection rates of boll rots. Persistent rain, moisture and/or cloudy weather with cooler temperatures are huge contributors to boll rot incidence, especially when occurring at boll cracking. Boll rots were prevalent in some fields in all regions except the Lachlan and Murrumbidgee in the 2017/18 season (Table 5). The average incidence of boll rots was recorded as 2.6% boll rot, 1.5% tight lock and 2.6% seed rot. However, given boll rot development is linked to environmental conditions, timing of disease surveys is important and only captures the disease incidence at that time. The drastic decrease in temperatures plus significant rainfalls during the first week of February, provided conducive conditions for boll rot development in some regions, and in particular tight lock. Significant yield loss this season in some regions has been attributed to boll rots.

**Table 5.** The average incidence and range of disease incidence of boll rot, tight lock and seed rot detected in each region in the 2017/18 season

| Region | Boll Rot (%) | Tight Lock (%) | Seed Rot (%) |
|---|---|---|---|
| Emerald | 2 (0-11) | 5 (2-12) | 2 (1-4) |
| Theodore | 2 (0-11) | 3 (1-12) | 3 (0-7) |
| St George | 2 (1-4) | 3 (1-10) | 5 (0-5) |
| Darling Downs | 1 (0-3) | 3 (0-6) | 1 (0-3) |
| Macintyre | 6 (0-15) | 1 (0-4) | 3 (0-13) |
| Gwydir | 7 (0-40) | 0 | 7 (0-40) |
| Namoi | 3 (0-15) | 0.1 (0-3) | 3 (0-15) |
| Macquarie | 2 (0-12) | 0 | 2 (0-12) |
| Lachlan | 1 (0-5) | 0.1 (0-1) | 0.1 (0-1) |
| Murrumbidgee | 0.2 (0-1) | 0 | 0 |

Note: Range of disease incidence is presented in brackets

**Figure 32.** Examples of boll rots

*Boll rot – Macintyre (Information provided by Chis Teague, CSD)*

The drastic dip in temperatures plus rainfall opened the door for disease in the first week of February (Figure 33). A week later there were 10 days or so of +38C temperatures maxing to 43C at its peak, which then dropped again with rain. Figure 34 shows some of the boll rot and tight lock observed in the Ambassador sites or variety trials this season. Ranges of 8-20 bolls/m were effected by a combination of boll rot and/or tight lock.

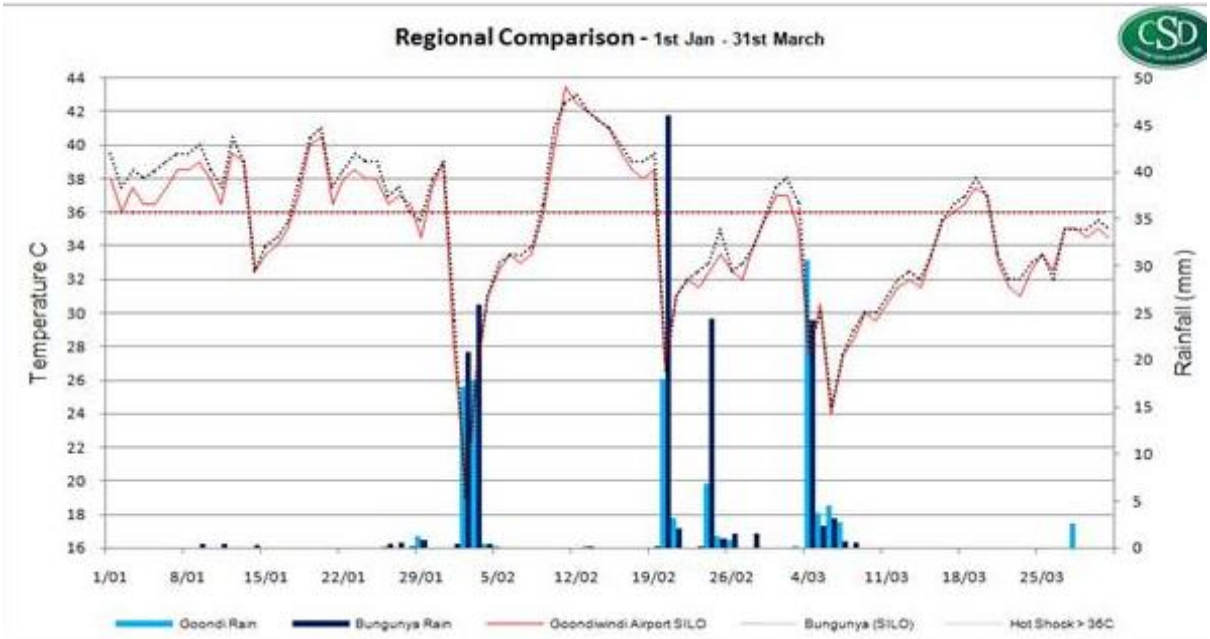



**Figure 33.** Regional comparison of Temperature and rainfall 1st Jan – 31st March

**Figure 34.** Boll rot and tight lock observed in the Ambassador sites or variety trials

MEALY BUGS

Mealybugs were observed on late-planted crops in CQ assessed on the 28th April 2018 causing significant damage, particularly to the tops of plants (Figure 35). Natural predators were observed to be present and active.

**Figure 35.** Mealybugs observed in CQ on late-planted crops

RENIFORM NEMATODE

During the early season cotton disease surveys, soil samples were collected from all fields surveyed for analysis of plant-parasitic nematodes, in particular reniform nematode *Rotylenchulus reniformis*. New farms in Emerald and Moura were confirmed to have reniform nematode. To date, this plant pest has only been detected in central Queensland.

In summary:

- A dry winter, cool start to the season followed by wet and cool conditions in February provided conducive conditions for various diseases to develop throughout the season.
- Planting date would have influenced disease development and incidence.
- Black root rot disease was a significant issue for all regions.
- Alternaria leaf spot is a new and significant disease issue at the seedling stage in Southern NSW.
- Namoi, Gwydir and Macintyre had fields with particularly high incidence of Verticillium wilt.
- High incidence of Fusarium wilt in some fields in the Namoi, Gwydir, Macintyre and St George is of great concern.
- Mixed fields of Verticillium and Fusarium wilts were identified and pose challenging to manage.
- Boll rots were prevalent in some fields in all regions except the Lachlan and Murrumbidgee.
- Yield losses have been attributed to tight lock in several regions.

## 2018/19 season

Key Findings Early Season

BLACK ROOT ROT

Black root rot of cotton (caused by *Berkeleyomyces rouxiae* (= *Thielaviopsis basicola*)) is favoured by cool weather conditions early in the season. The pathogen colonises the root surface, suppresses the development of secondary roots and stunts seedling growth. When temperatures rise, the taproot expands and the blackened root surface is sloughed off and disappears.

Black root rot was confirmed in all regions except Emerald in the 2018/19 season. This disease is a particularly serious problem for southern NSW because of its cooler climatic conditions, which favour the pathogen. In Queensland, significant root damage was observed on the Darling Downs due to this pathogen, associated with earlier planted crops and where cool conditions occurred. In NSW, the highest incidence of BRR occurred in the Gwydir (Figure 36). However, the disease severity was relatively mild; mostly below 15% indicated by the percentage of the taproot surface that was obviously blackened (Table 6). In general environmental conditions were not conducive to BRR, however, in some regions, every field surveyed had BRR and in some fields plants were badly infected (Table 6).

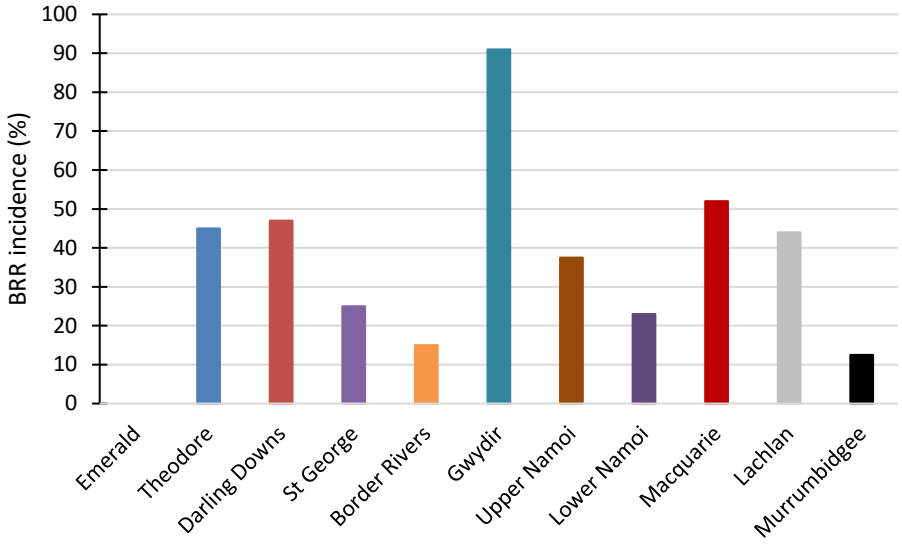



**Figure 36.** Mean Black root rot (BRR) incidence (%) recorded during the early season disease surveys across main growing valleys in Queensland and New South Wales in 2018/19 season

**Table 6.** Mean BRR incidence (%) and severity (%) and percentage of fields surveyed with Black root rot, in cotton growing regions of Queensland and New South Wales from 2016 to 2019

| Region | Mean BRR severity % (Highest incidence recorded) | % of fields surveyed with BRR |
|---|---|---|
| Emerald | - | - |
| Theodore | Trace (0.1) | 45 |
| Darling Downs | 10 (70) | 80 |
| St George | Trace (0.1) | 25 |
| Border Rivers | 19 (76) | 70 |
| Gwydir | <15 | 100 |
| Namoi | <10 | 100 |
| Macquarie | <20 | 100 |
| Lachlan | 24 (75) | 88 |
| Murrumbidgee | 12 (89) | 70 |

Severity = Mean % of tap root surface obviously blackened; Highest BRR severity surveyed for region in parentheses

**Figure 37.** Cotton seedlings from Qld displaying Black root rot symptoms

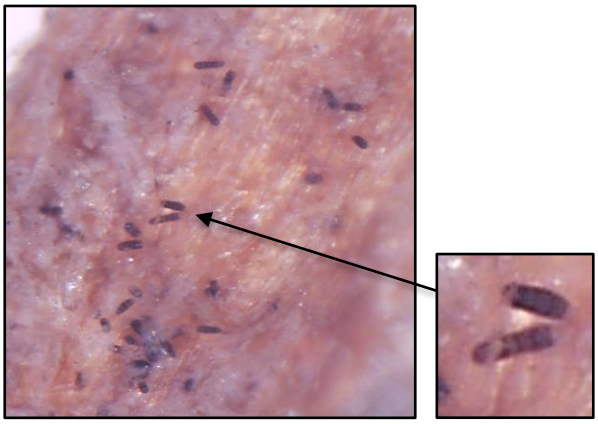


**Figure 38.** Long-lived soilborne spores of Black root rot observed on root

FUSARIUM WILT

Figure 39 shows the mean incidence of Fusarium wilt (FW) detected early season for each region in the 2018/19 surveys. In Queensland, FW remains a key disease for St George and the Darling Downs, detected in 50% and 65% of fields surveyed, respectively. For fields surveyed in St George, the mean incidence was 5.4%; the lowest incidence was 3% and the highest 30%. On the Darling Downs, the mean incidence was 1.4%, with the lowest incidence being 0.5% and the highest incidence 14%. A high incidence of FW was particularly evident following a rain event, reminding us that under conducive conditions, this pathogen can still cause significant disease, and not to become complacent with Integrated Disease Management (IDM). In NSW, the FW pathogen was only detected early season in one growing region, the Border Rivers (Figure 39).

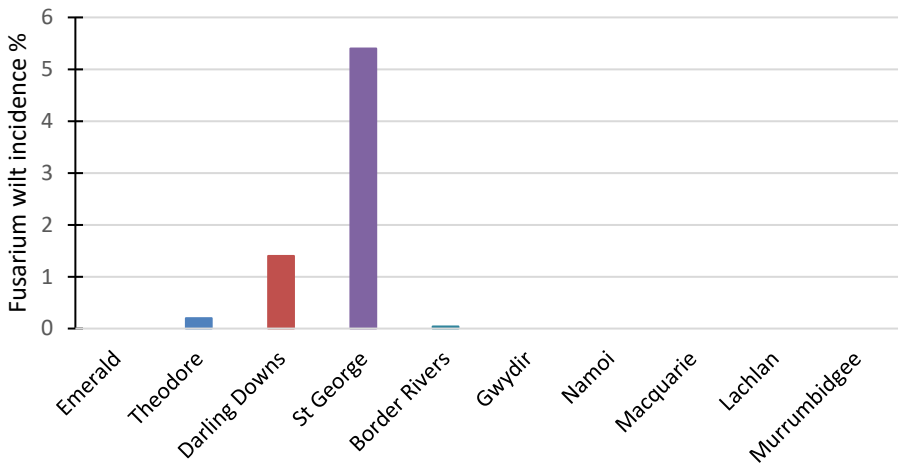



**Figure 39.** Mean Fusarium wilt incidence (%) recorded during the early season disease surveys across main growing valleys in Queensland and New South Wales in 2018/19 season

**Table 7.** Disease incidence range (%) and % of fields surveyed early season with Fusarium wilt detected

| Region | Disease incidence range % | % of fields |
|---|---|---|
| Theodore | 0's, 2.5 | 9 |
| Darling Downs | 0.5 - 14 | 65 |
| St George | 3 - 30 | 50 |
| Border Rivers | 0's, 0.5 | 7 |

**Figure 40.** Seedlings displaying symptoms of Fusarium wilt early season

ALTERNARIA LEAF SPOT

Alternaria leaf spot (ALS) is developing as an early season disease of concern in NSW (Figure 41). Disease severity indicated by percentage necrosis of infected leaves on cotton seedlings was low, however the incidence of young seedlings infected was high in some valleys (Table 8).

**Table 8.** The mean disease incidence and severity of Alternaria Leaf Spot (ALS) detected during early season surveys in NSW

| Valley | Mean ALS incidence (%) | Mean ALS severity (%) |
|---|---|---|
| Gwydir | 59 | <1.5 |
| Namoi | 23 | <2 |
| Macquarie | 8 | <1 |
| Lachlan | 13 | 1 |
| Murrumbidgee | 21.5 | 1.5 |

**Figure 41.** Cotton seedlings displaying symptoms of Alternaria Leaf Spot

RHIZOCTONIA AND PYTHIUM ROTS

Seedling diseases may be caused by numerous pathogens acting alone or in combination that commonly cause 'damping off' (death of seedlings) and reduced plant stands. The main pathogens attacking cotton seedlings are *Rhizoctonia solani*, *Pythium ultimum* and *Fusarium* spp. (not the Fusarium wilt pathogen). *Rhizoctonia* produces a sunken lesion, which girdles the stem, causing the seedling to collapse. *Pythium* infects the seed and radical, causing seed rot and pre-emergence damping off. The seedling stem can also be affected at the soil line, causing post-emergence damping off. Excessive soil moisture and low temperatures predisposes cotton seedlings to *Rhizoctonia* and *Pythium* infection by reducing their rate of growth.

Rhizoctonia-like rot occurred in all cotton-growing regions in Queensland and NSW. The mean incidence was low across Queensland cotton regions ranging from 1% to 2.3% (Figure 42), causing minor concern. In NSW, disease incidence ranged from 20 to 80%. The disease caused minor concern, even where it occurred frequently, due to a low level of severity (Figures 44 and 45). The most severe Rhizoctonia-like rot symptoms were observed in the Macquarie, where the mean disease severity was 30%. Despite the high severity, symptoms were superficial, limited to the collar regions and sloughed off during the crop growth.

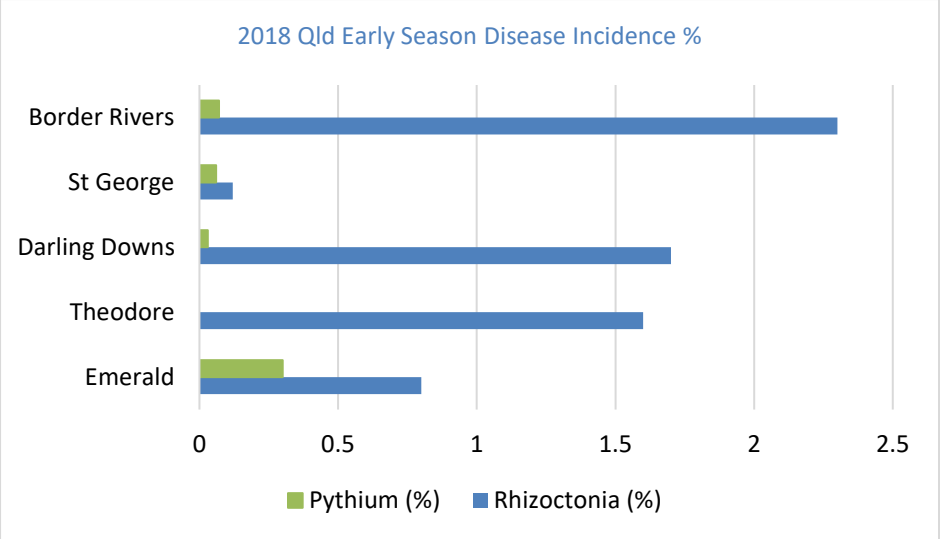



**Figure 42.** Queensland early season disease incidence of Pythium and Rhizoctonia

**Figure 43.** Cotton seedling displaying symptoms of Rhizoctonia infection

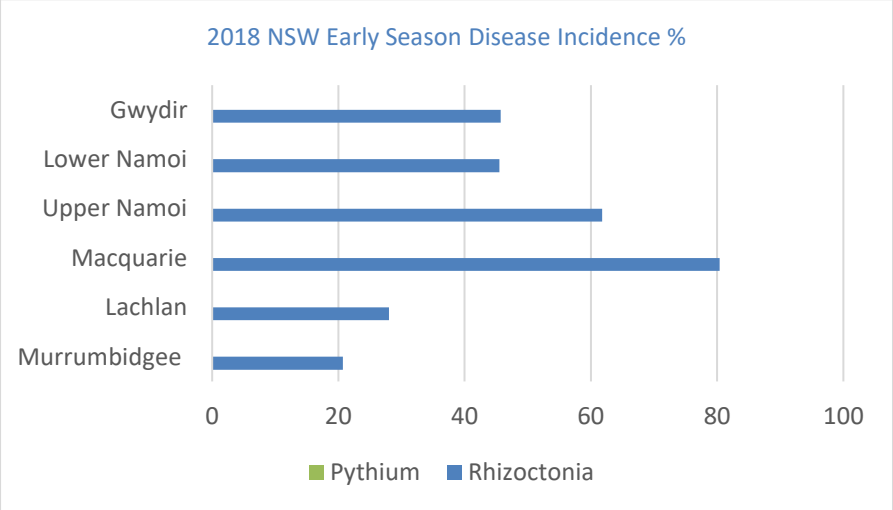



**Figure 44.** NSW early season disease incidence of Pythium and Rhizoctonia

**Figure 45.** Rhizoctonia-like diseased cotton seedlings exhibiting irregular red brown lesions that frequently girdled the collars (red arrows)

RENIFORM NEMATODE

Soil samples were collected from all fields surveyed early season in NSW and Qld for assessment of plant-parasitic nematodes, in particular reniform nematode *Rotylenchulus reniformis*. New farms in Emerald were confirmed to have reniform nematode. To date, this plant pest has only been detected in cotton in CQ (Full report in DAQ1803 Final Report).

Key Findings Late Season

ALTERNARIA LEAF SPOT

Alternaria Leaf Spot was present late season in all cotton-growing regions of NSW and Queensland. Disease severity indicated by percentage necrosis of infected leaves on cotton was low in Queensland, increasing in NSW (Table 9).

**Table 9.** The mean disease incidence of Alternaria Leaf Spot (ALS) detected late season surveys in NSW and Queensland

| **Valley** | **Mean ALS severity (%)** |
|---|---|
| Emerald | 0.1 |
| Theodore | 1.3 |
| Darling Downs | 0.1 |
| St George | 0.1 |
| Border Rivers | 0.1 |
| Gwydir | 2 |
| Namoi | <2 |
| Macquarie | <5 |
| Lachlan | <10 |
| Murrumbidgee | 11.5 |

FUSARIUM WILT

The average disease incidence of FW for each region in the 2016/17, 2017/18 and 2018/19 surveys is presented in Figure 46, showing that the mean incidence of FW increased this season in the Macquarie, St George and Theodore regions compared to the 2016/17 season. In the Macquarie, Gwydir, Darling Downs and Theodore regions, FW increased this season compared to 2017/18 season. It is important to

highlight the high incidence of FW detected in some fields this season. Disease incidence was as high as 58%, 21% and 20% for fields in St George, Darling Downs and the Macquarie respectively. There was likely an impact on yield for some of these fields.

It is imperative not to become complacent of this disease, or relying solely on host resistance. An integrated approach to management is extremely important to manage this disease, as it is with all soil-borne pathogens.

In the Gwydir valley, approximately 30% of the total fields' surveyed had both FW (Figure 47) and Verticillium wilt diseases. This could pose a significant challenge in managing crop residues, crop rotations and cultivar selections to minimise disease impacts.

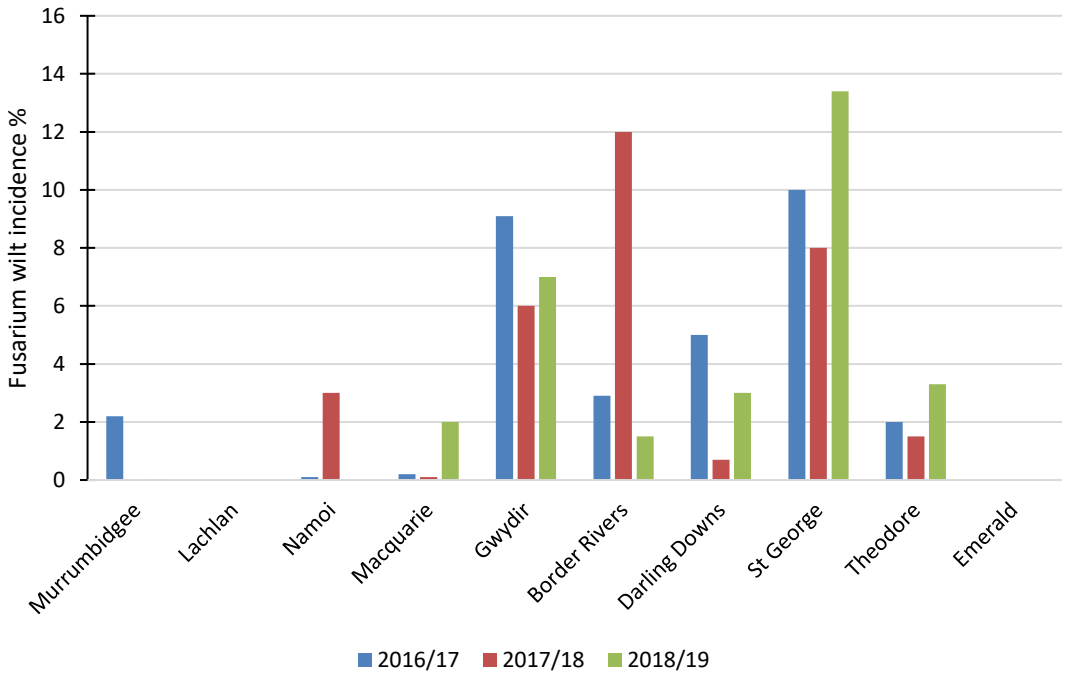



**Figure 46.** The mean Fusarium wilt incidence (%) recorded during late season disease surveys across main growing valleys over three seasons. Fusarium wilt is present in all cotton production areas listed.

**Figure 47.** A typical Fusarium infected cotton plant exhibiting die back symptoms of the main stem and branches

**VERTICILLIUM WILT**

Verticillium wilt (VW) was confirmed in the 2018/19 survey in the Border Rivers, Namoi, Gwydir, Darling Downs and Macquarie regions, with a mean incidence of 21.5%, 20%, 5%, 4.1% and 3.6% respectively (Figure 8). The highest disease incidence recorded in each of these regions was 78.5%, 64%, 47%, 71% and 40% respectively. Areas with severe symptoms of VW were observed in several fields. VW was also detected in the Lachlan and St George regions, however plants were outside of transect, so disease incidence was recorded as zero. The Border Rivers and Darling Downs were the only regions with an increase in the mean incidence of disease compared to the 2016/17 season. There were no significant rain events January/February of 2019, which are generally associated with development of disease symptoms. Alternatively, the high temperatures and dry conditions at this time led to an intense irrigation schedule on farms where water was available, which may have provided a cooling effect in the soil and therefore conditions conducive to the development of VW (Figure 48).

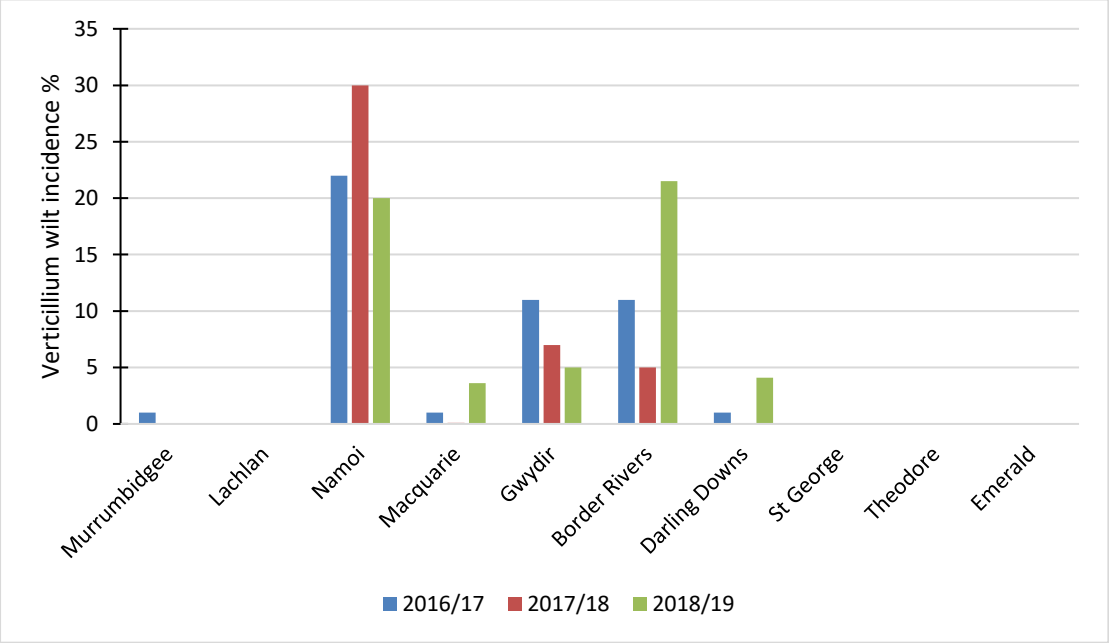



**Figure 48.** The mean Verticillium wilt incidence (%) recorded during the late season disease surveys across main growing valleys over three seasons. Verticillium wilt was detected in 2018/19 survey in the Lachlan and St George, however plants were outside of transect meaning incidence was recorded as zero.

**Figure 49.** A relatively high Verticillium wilt pressure field, where the incidence was recorded up to 50%

BOLL ROTS

Environmental conditions are the key factors in determining infection rates of boll rots. Persistent rain, moisture and/or cloudy weather with cooler temperatures greatly contribute to boll rot incidence, especially when occurring at boll cracking. The dry, hot conditions that generally prevailed this season were not conducive to boll rot development (Table 10). There was one exception however, in one field on the Darling Downs, at time of surveying, 11% of bolls were tight-locked, and 2% with seed rot and boll rot. At harvest, at least 0.5b/ha yield loss was attributed to boll rot on lower lodged branches due to rain (Table 10).

**Table 10.** The mean incidence and range of disease incidence of boll rot, tight lock and seed rot detected in five regions in the 2018/19 season

| Region | % BR | % TL | % SR |
|---|---|---|---|
| Darling Downs | 0.62 (0-3.3) | 2.9 (0-11) | 0.9 (0-3.5) |
| St George | 0.15 (0-0.8) | 0.8 (0-2.2) | 1.1 (0-1.9) |
| Border Rivers | 0.03 (0-0.45) | 0.1 (0-1.95) | 0.4 (0-1.95) |
| Emerald | 0.8 (0 - 1.45) | 1.2 (0 - 1.8) | 0.7 (0 - 1.3) |
| Theodore | 0.62 (0-2) | 1.14 (0-3.8) | 0.9 (0-2.6) |

OTHER ISSUES

*Sclerotium rolfsii*

A high incidence of plant death due to *Sclerotium rolfsii*, which causes a collar rot, was observed in one field under pivot in CQ. Several other fields not included in the survey were also suffering from a high incidence of this disease. The scientific literature suggests that there is an additive effect on crop growth (e.g. soybean and peanut) when both plant-parasitic nematodes and *S. rolfsii* are present in high numbers. The potential of increased disease in cotton when both reniform nematode and *S. rolfsii* are present requires investigation.

Mealy bugs

Mealy bugs were observed on one farm in CQ (Figure 50), colonising the terminal buds of plants, resulting in terminal death.

**Figure 50.** Mealy bugs observed late season in CQ

Cotton Bunchy Top

Cotton Bunchy Top (CBT) caused by a virus vectored by aphids, was detected on two farms in CQ (Figure 51). The mean incidence of disease for the two fields was 3.5%.

**Figure 51.** Cotton bunchy top was evident in CQ

The presence of mealybugs and CBT highlights the need to ensure that ratoon or volunteer cotton and weed hosts are eliminated on farm, particularly over winter. This will assist in breaking the green bridge thereby minimising the risk of CBT and mealy bugs in the following cotton crop.

BOLL ROTS

Environmental conditions are the key factors in determining infection rates of boll rots. The dry, hot conditions that generally prevailed this season were not conducive to boll rot development. One exception was on the Darling Downs. At time of surveying, 11% of bolls were tight-locked and 2% with seed rot and boll rot. At harvest, at least 0.5b/ha yield loss was attributed to boll rot on lower lodged branches due to rain.

SUMMARY

- Cotton is susceptible to many yield-limiting diseases
- To understand the importance of diseases present, surveys are conducted to monitor disease distribution and incidence
- Different regions are being affected by different pathogens
- Conditions generally not conducive to widespread incidence and/or severity of disease
- However, some fields suffered significant disease incidence and severity that would have impacted yield
- Complacency
- Managing diseases in cotton requires an integrated approach
- If we placed an economic value on management practices, would they be better adopted?

### 1.3 **Design and develop Geospatial database** (Prepared by Aphrika Gregson)

The disease survey team have utilised Fulcrum mobile-applications since 2017 to facilitate geospatial data capture. Fulcrum is a web-based application, offering customisable forms for mobile data collection in a geospatial context, such as plant disease surveys. A user creates a form from a template, with fields customised to capture information of interest. For example: date of collection, GPS location, geotagged pictures and annotations, checklists, text, and more. This form, once completed, can be shared with other users and used as a template for data collection.

Pre-requisites for a data collection system were as follows: intuitive interface, restricted data sharing in regards to anonymity of industry collaborators, ability to capture accurate GPS coordinates, compatibility

with a larger data-management system, affordability for a large seasonal user base (approximately 15 people), and overall simplicity for troubleshooting, training.

*In-Field Data Capture*

To date, the project field staff have retained the existing physical-field form for raw data capture. Within Fulcrum, a photograph of the completed field form during a survey creates a digital copy, linked to the geotagged record to be synced and stored in the cloud. This significantly improves data handling where loss or alteration of the original occurs, and in the case of field surveys where large volumes of data are generated by multiple users.

The mobile app also facilitates capture of geotagged pictures with annotations in an organised way; images may depict useful information regarding plant stand, disease symptoms, or unusual or suspect pathologies. In extremely poor reception, geotagging may be unavailable.

A limitation of fieldwork in remote cotton production regions is lack of reception and this can impair location tracking. To address this limitation rugged, portable and Bluetooth-enabled GPS units suitable for field use were trialled in combination with the mobile Fulcrum app. The units pair with a user's mobile device via Bluetooth, and improve satellite-GPS readings in-app to an accuracy of 1 metres, or 5-10 metres in poor coverage areas. The units also serve as a back-up to log GPS coordinates on the field form, should a mobile go flat or break during a field survey. The particular model used is: Bad Elf GPS Pro, and retails for $199.99 USD.

*Data Management*

Configurable role options mean access to project data is restricted; all users have access to the desktop version of Fulcrum, however only the data collected by the individual is visible. In the case of the administrator or project leader, permissions are set to make all data collected by all users available. This protects any potentially sensitive information collected.

The Fulcrum desktop app is a powerful visual tool in monitoring field activities across the cotton production regions of NSW and Qld (Figure 52). Filters allow records to be visualised specifically to region, farm, user or year (Figure 53).

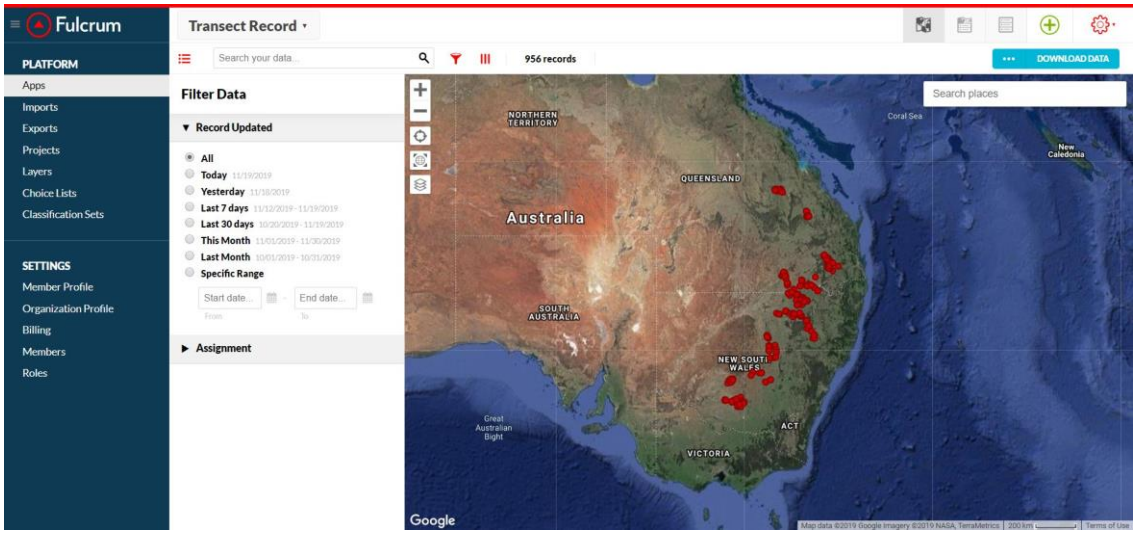



**Figure 52.** Desktop view of all records from all users, captured with the survey app 'Transect Record' distributed via starting GPS location, overlaid on base maps such as the satellite view above powered by Google.

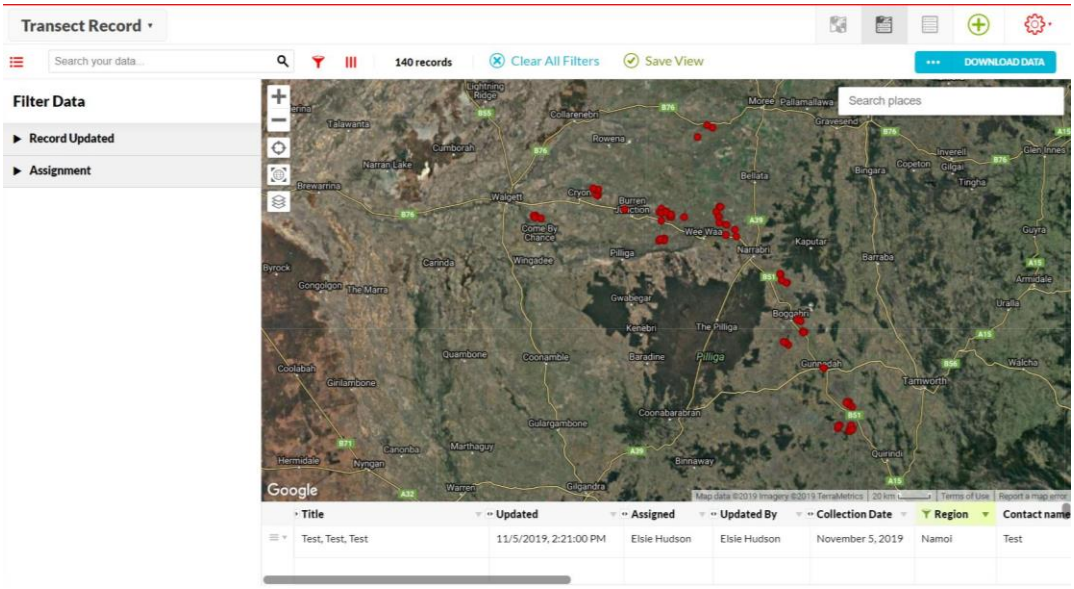



**Figure 53.** Desktop view of all records from all users, captured with the survey app 'Transect Record' distributed via starting GPS location, overlaid on base maps such as the satellite view above powered by Google.

Fulcrum provides cloud-based storage for records up to 100GB. This is invaluable for securing the large amount of valuable temporal and spatial data collected throughout seasons by multiple users. This feature relies on users 'syncing' from mobiles to the cloud: only in rare circumstances have users experienced any difficulty in this step.

Data can be easily exported to Excel or CSV format for manipulation and analysis. In RDE986, survey staff collate field data by transcribing from physical field forms into an excel database. There is potential in future to reduce this time-consuming process by direct input of raw data into a more complex Fulcrum digital form; when downloaded into a CSV/ excel file format, raw data such as incidence, plant stand and date, time and GPS would be pre-filled from field records.

The Fulcrum mobile feature, 'PDF Report Generator' (Figure 54), is a succinct summary of a single record in PDF which can be shared from your device. These summaries present raw data from an app record as a polished, professional report with particular emphasis on photographs captured in-record. PDF Report Generator is of benefit when growers, consultants, or staff request additional and timely information shortly after a disease survey has been conducted.

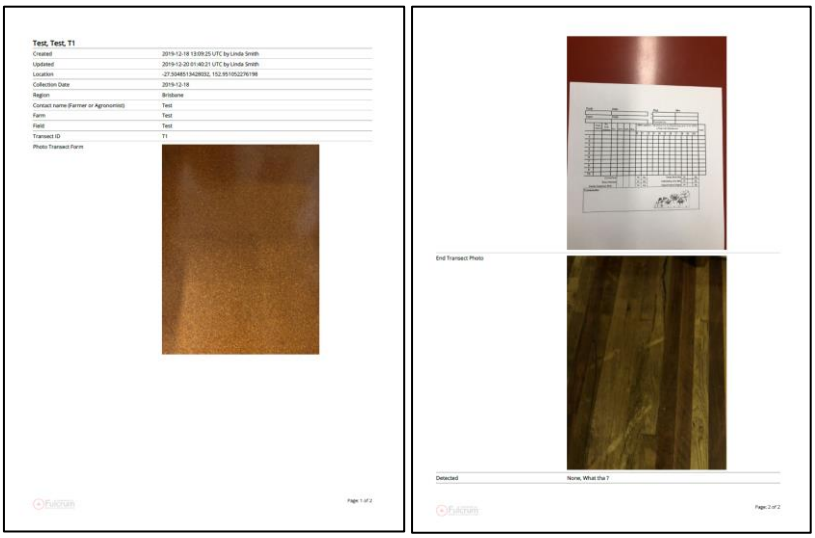


**Figure 54.** A test report demonstrating Fulcrum mobile's feature PDF Report Generator, including date, location data, user, and field data captured. Reports are sharable via text, email, or iCloud.

*Financial*

Fulcrum is subscription based, charged monthly and per user. A Professional plan valued at $38 USD per user, per month is currently administered by project staff of NSW Department of Industries. Two users, the project leader and a technical staff as administrator maintain a subscription throughout the year to support seasonal data collection during disease surveys, and retain access to previous datasets. For this reason, the monthly fee can fluctuate significantly month-month. This flexibility enables a degree of cost-saving; busy months result in higher monthly fees, and out-of-season months are minimal costs.

**1.4 Geospatial database developed and kept up to date, joint ownership of IP (refer IP schedule 2).**

An allocated administrator at NSW DPI manages the Fulcrum app. Administration moved from David Larson to Aphrika Gregson once the app was up and running effectively.

**1.5 Develop and implement disease survey template for growers to assist in collection of field data. This might include nutrition, previous crop history, irrigation schedules, significant events, yield.**

A grower information template was developed (Figure 55) and emailed to each grower whose field was surveyed, with the aim to capture additional information that could be considered in the data analyses to provide a better understanding of factors that influence disease incidence.

| | | |
|---|---|---|
| Surveyed by: | Survey date: | |
| Season: | Region: | Farm: |

GROWER INFORMATION

| | | |
|---|---|---|
| Field Surveyed | | |
| Variety | | |
| Field prep/tillage | | |
| Soil type | | |
| Configuration | | |
| Previous crop | | |
| Seed rate | | |
| Planting date | | |
| Pre-water/water up | | |
| Irrigation | | |
| Fertiliser application | | |
| Sprays | | |
| Yield | | |
| Comments | | |

**Figure 55.** Grower information template

**1.6 Determine the influence of environment, disease epidemiology, and cultural practices (e.g. row spacing, seed rate, sowing date, irrigation practices etc.) on disease through analysis of database supported by additional information and trials where required.**

Analyses of three seasons of surveys for each area in NSW and Qld

The analysis focused on interpreting the graphs of data showing diseases by area in each state and the amount of trash in each field by each area in each state and on the effect of previous crop on disease incidence, and lastly on the effect that trash had on disease incidence. Previous crops were binned into broad categories to help with small sample sizes and cotton was used as the baseline previous crop. All graphs shown here represent three seasons of surveys for each area.

*How to interpret plots and boxplots*

The following plots, boxplots, show the spread of the data for each area. Each point represents a field transect in the following plots. The boxplot shows the minimum value, max and quartiles with the middle of the box being the median, or 50 %. Dots indicate outliers in the data. The whiskers are each 25 % of the data values. A longer boxplot indicates more variation in the area's observations.

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Incidence of diseases

*General observations*

The following boxplots (Figures 56 – 73) summarise the incidence (%) of diseases observed early and late season across areas of NSW and Qld.

In NSW, the areas of Walgett, Whitton and Winton frequently displayed low levels of disease for all diseases observed. No major pattern stood out for any area having frequently high levels of disease. Pythium and TSV were the diseases with the lowest observed levels followed by Tight-lock and Seed rot. Rhizoctonia, Alternaria and Black root rot were frequently observed in most areas and at levels approaching or reaching 100% incidence in some fields. Verticillium wilt was observed in most areas at low levels with the highest in Merah North but Wyadrigah having the highest overall for the whole area.

Diseases in Queensland appeared to be less frequent and occurred in fewer areas at the levels observed in NSW. Black root rot and Fusarium wilt (Fov late season) were the only two diseases that approached 100% incidence in any fields surveyed in Queensland. Seed rot was widespread across the areas surveyed but levels did not exceed 10 %.

*Early season*

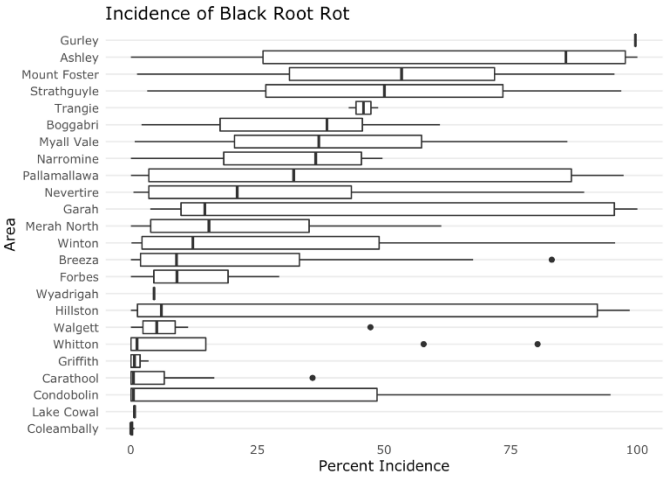



**Figure 56.** The incidence of Black Root Rot early season in NSW

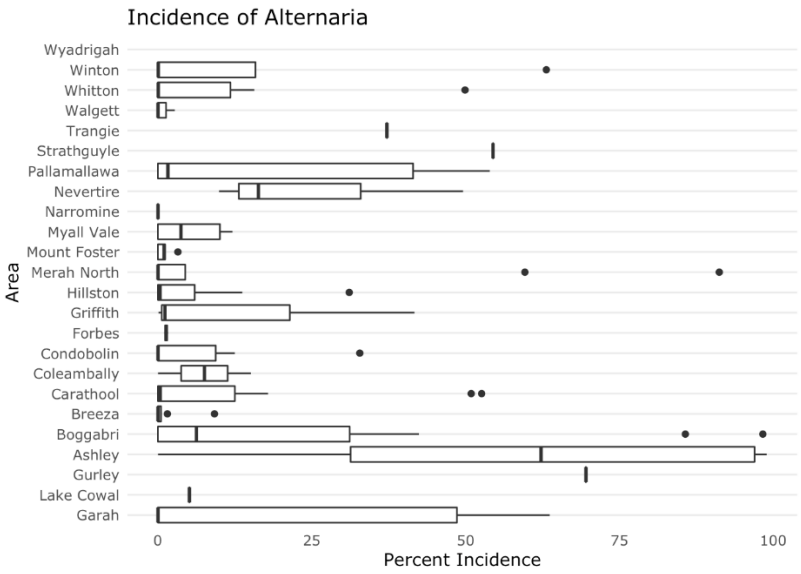



Figure 57. The incidence of Alternaria leaf spot early season in NSW

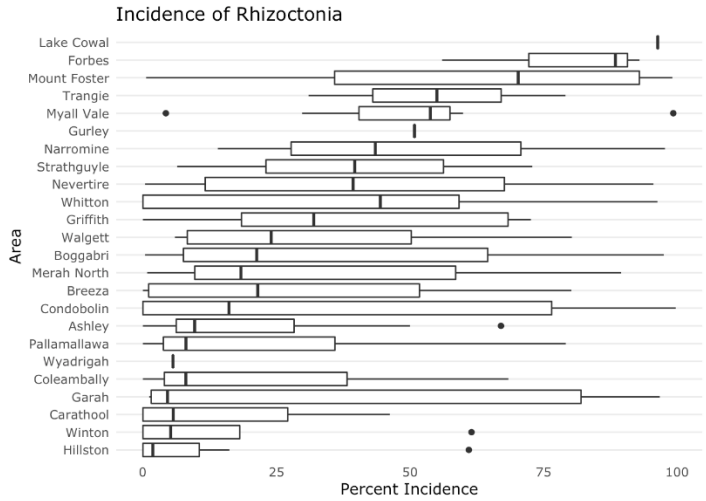



Figure 58. The incidence of Rhizoctonia early season in NSW

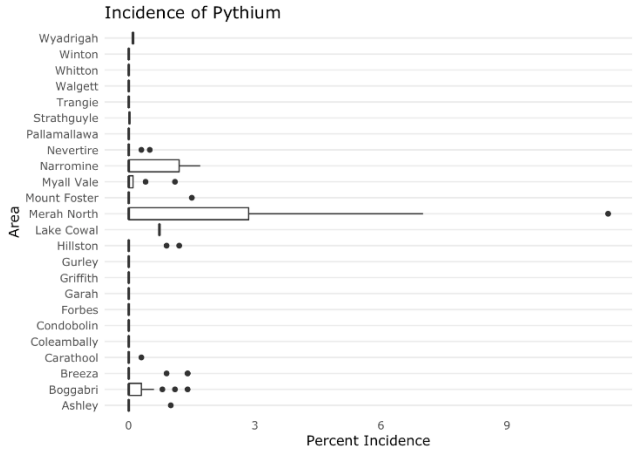



**Figure 59.** The incidence of Pythium early season in NSW

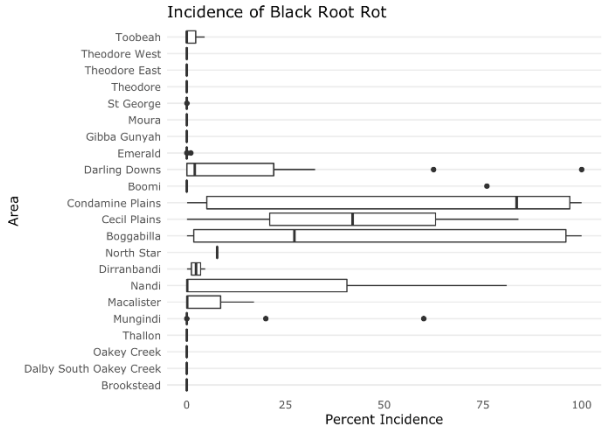



**Figure 60.** The incidence of Black Root Rot early season in Qld surveys

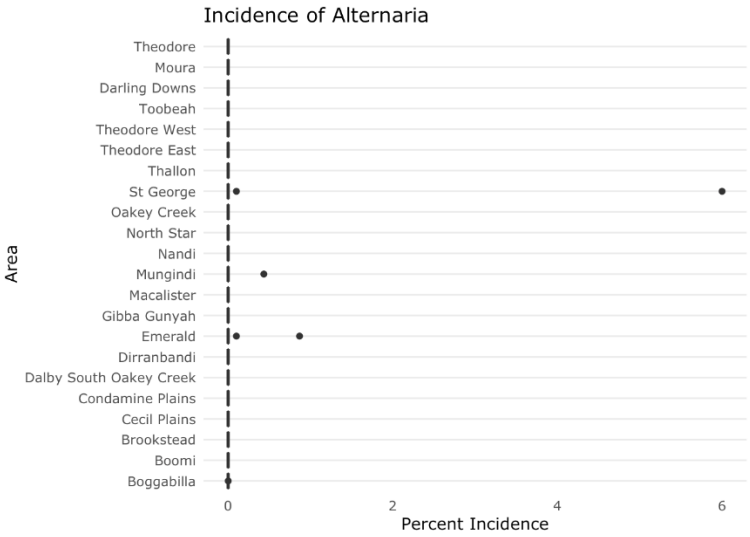



**Figure 61.** The incidence of Alternaria leaf spot early season in Qld surveys

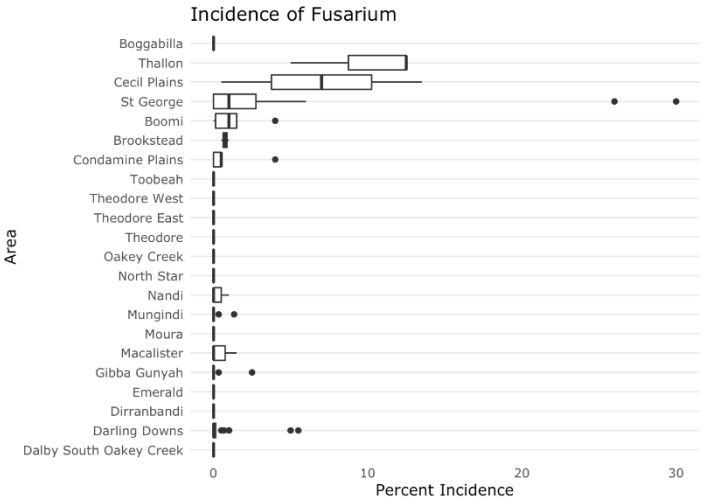



**Figure 62.** The incidence Fusarium wilt early season in Qld surveys

*Late season*

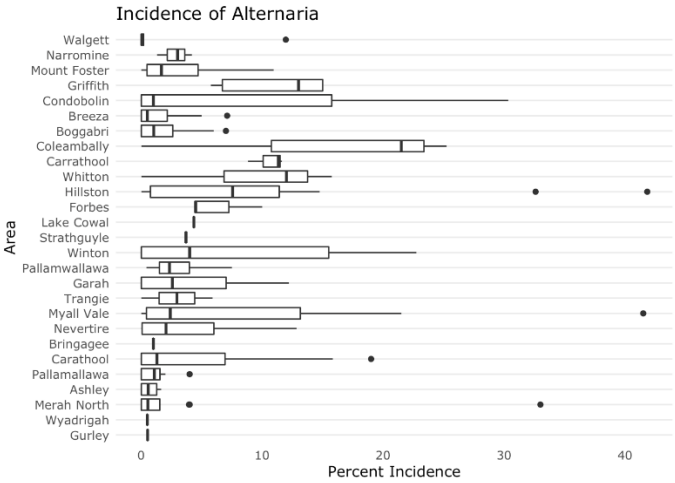



**Figure 63.** The incidence of Alternaria leaf spot late season in NSW

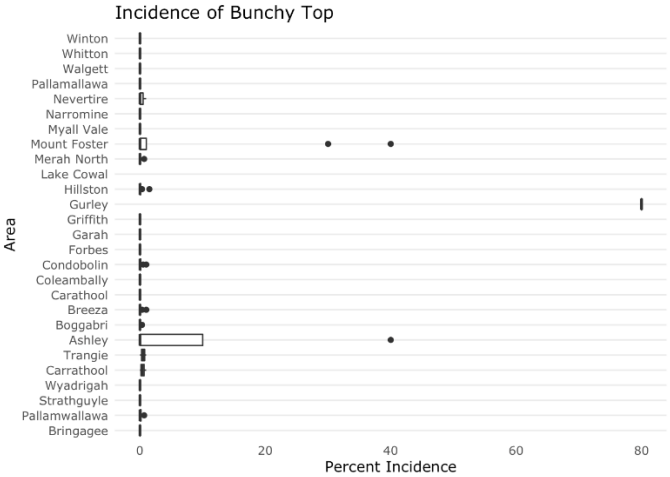



**Figure 64.** The incidence of Bunchy top late season in NSW

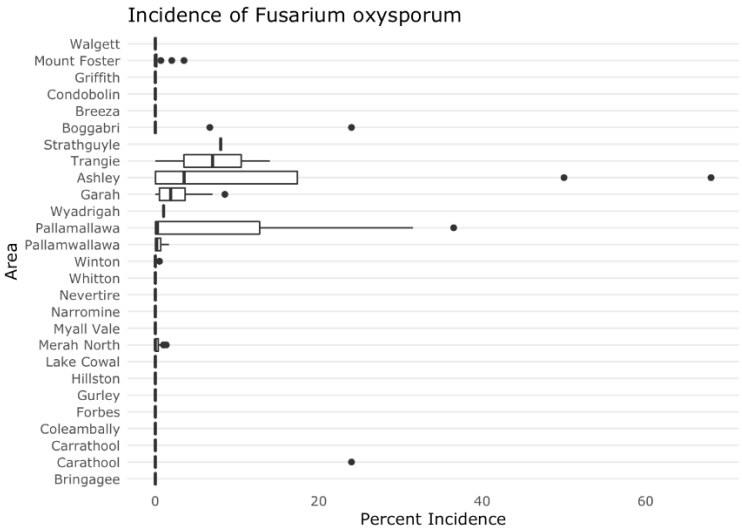



**Figure 65.** The incidence of Fusarium wilt late season in NSW

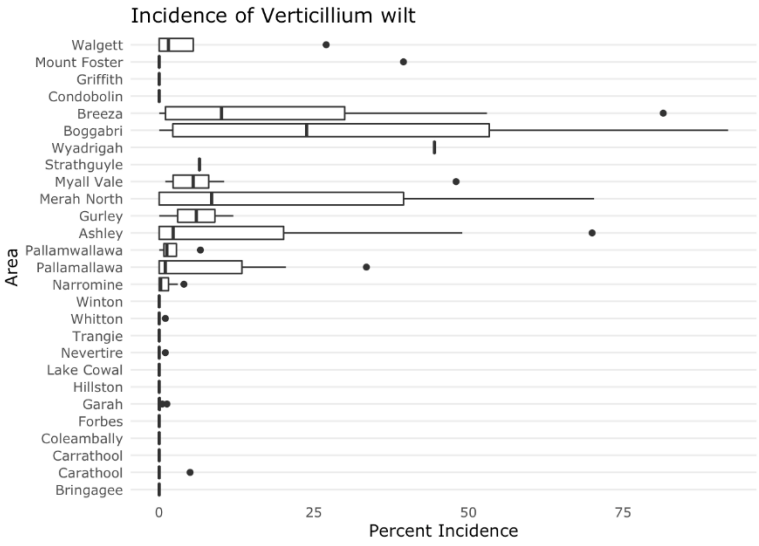



**Figure 66.** The incidence of Verticillium wilt late season in NSW

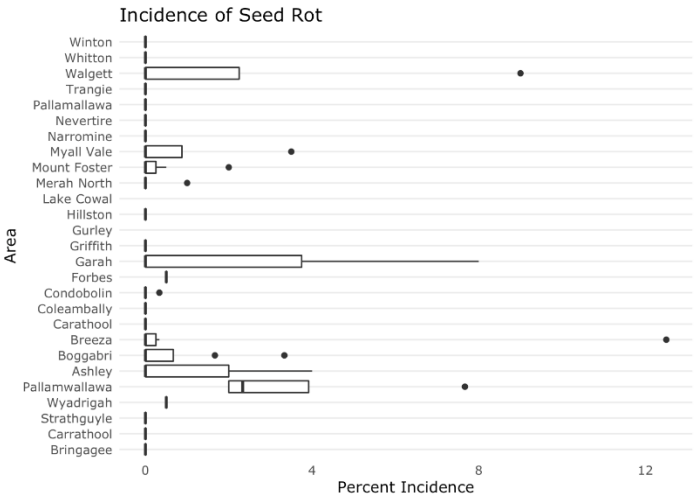



**Figure 67.** The incidence of Seed rot late season in NSW

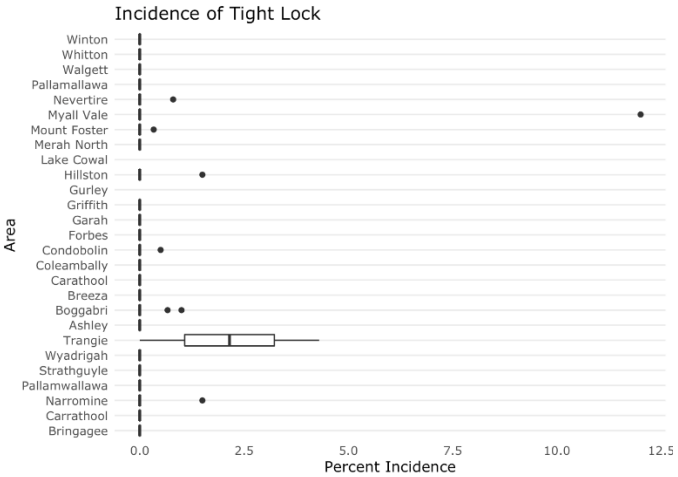



**Figure 68.** The incidence of Tight-lock late season in NSW

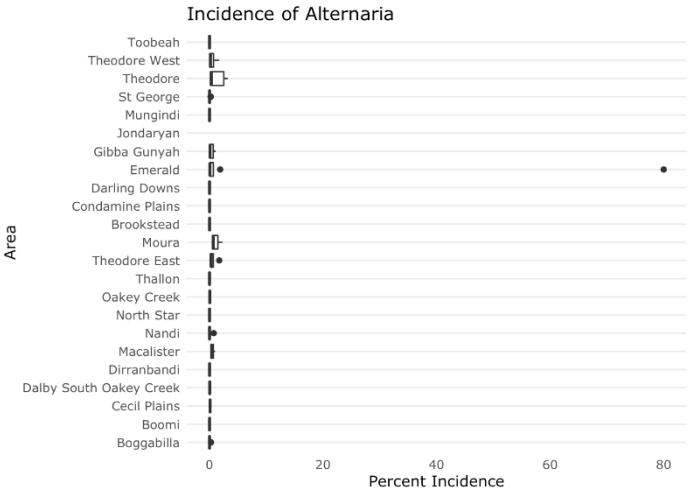



**Figure 69.** The incidence of Alternaria leaf spot late season in Qld surveys

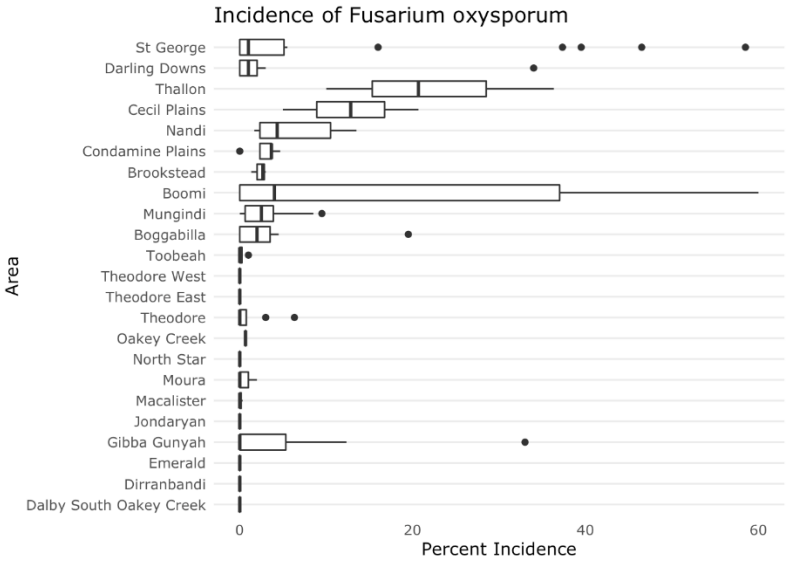



**Figure 70.** The incidence of Fusarium wilt late season in Qld surveys

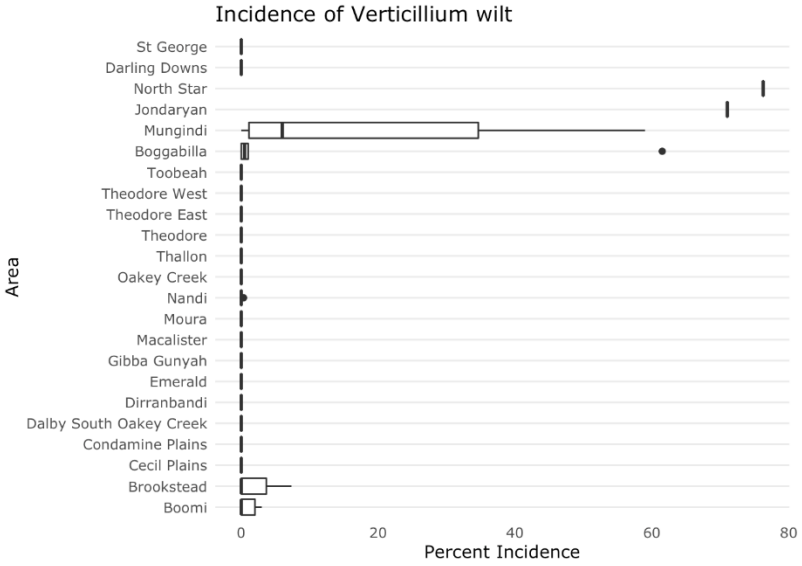



**Figure 71.** The incidence of Verticillium wilt late season in Qld surveys

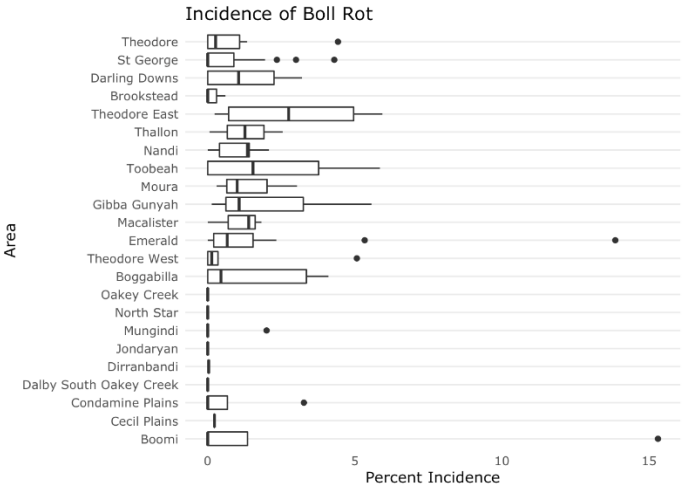



**Figure 72.** The incidence of Boll rot late season in Qld surveys

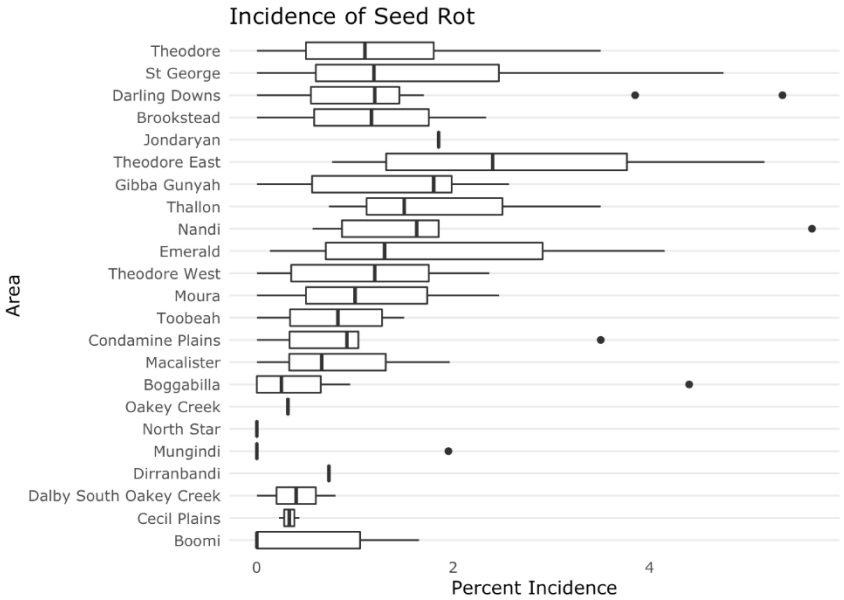



**Figure 73.** The incidence of Seed rot late season in Qld surveys

What Clusters Exist in the Whole Data?

Using a network plot we can identify clusters of variables and see relationships between diseases and yield. The red or blue lines are the paths that represent the correlation between variables. A red line means there is a negative correlation, e.g. Tight-lock (TL) is most strongly negatively correlated with yield. While a blue line indicates a positive relationship. The wider and less transparent the line, the stronger the correlation.

There are several very weak correlations in this data set. Fusarium (early season in Qld) and TSV were dropped from this analysis due to missing values. Here again we see that early Alternaria clusters with Black root rot (BRR) and bunchy top (BT). However, we need to be cautious interpreting relationships with Bunchy Top as the majority of potential Bunchy top samples yielded a negative result after testing for virus. Incidences recorded therefore do not reflect true incidence and this needs to be amended in the database. Most of the other diseases are weakly correlated with each other but Rhizoctonia appears to have mostly positive relationships with most other diseases in these data (Figure 74).

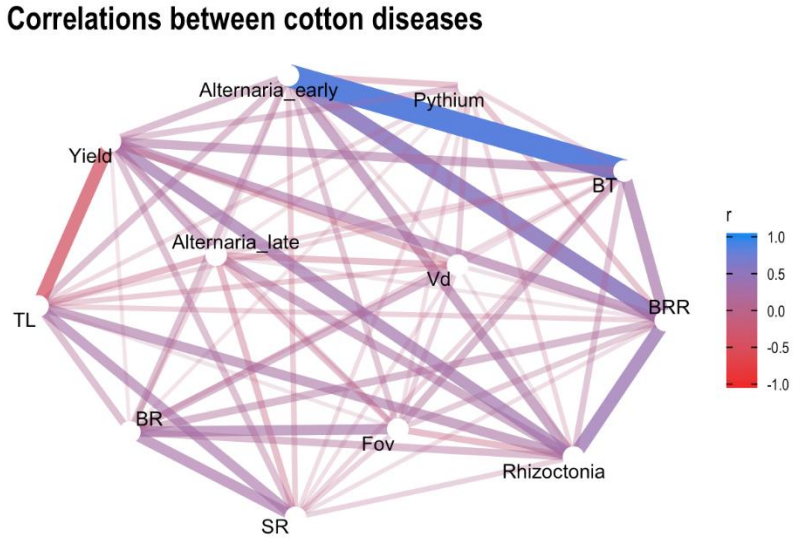



**Figure 74.** Network plot identifying correlations between cotton diseases and yield

What Clusters Exist in the State Data?

*Queensland*
It was necessary to drop Yield and Late season Alternaria observations due to missing values in Qld data. However, we can see that early season Fusarium (Fusarium) and late season Fusarium (Fov) are clustered and positively correlated. Seed rot, boll rot and tight-lock are clustered and positively correlated and have weakly negative relationships with Verticillium wilt (Vd), Bunchy top (BT) and Black root rot (Figure 75). None of the correlations are particularly strong.

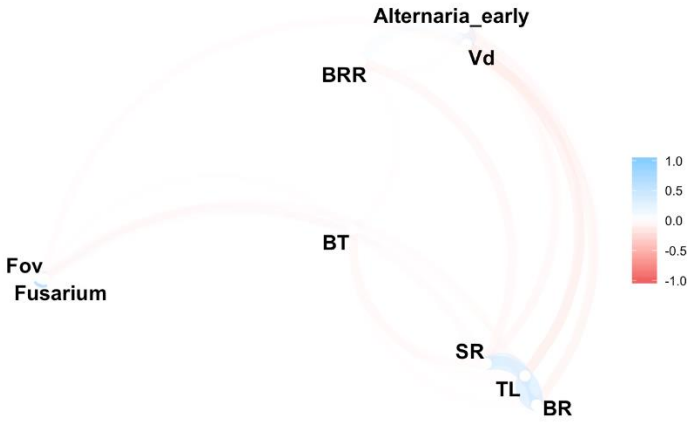



**Figure 75.** Qld survey network cluster for diseases

Environmental conditions are the key factors in determining infection rates of boll rots. The dry, hot conditions that generally prevailed in the 2018/19 season were not conducive to boll rot development. However, there was one exception on the Darling Downs. At time of surveying, 11% of bolls were tight-locked and 2% with seed rot and boll rot (Figure 76). At harvest at least 0.5b/ha yield loss was attributed to boll rot on lower lodged branches due to rain. This demonstrates that under disease conducive conditions, boll rots can have a significant impact on yield in Qld.

**Figure 76.** Images of tight-lock, boll rots and seed rot observed in one field on the Darling Downs

*New South Wales*

In the NSW data we see more positive correlations between diseases and a cluster of early Alternaria with black root rot (BRR) (Figure 77). However, as mentioned previously, the correct Bunchy top data needs to added to the database and be reanalysed.

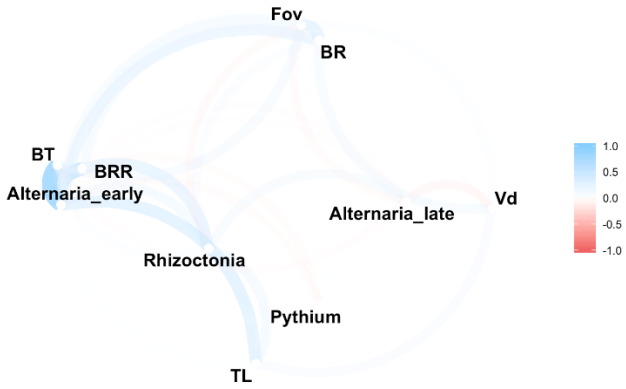



**Figure 77.** NSW network cluster for diseases

Area Networks

Due to data constraints, a network analysis on the area data was not able to be performed.

Summary of varieties grown

The varieties grown in fields surveyed in NSW and Qld are shown in Figures 78 and 79 respectively. Varieties “746”, “748” and “714” were the most common grown across NSW and Qld. How varieties were recorded in the database needs to be standardised for accurate interpretation of results. For example, in NSW and Qld, varieties recorded as 746, 748 etc. need to include the Bollgard technology that provides in-seed protection. In Qld, some varieties recorded as BG3 following conversation with grower on-farm in the 2018/19 season, may have been B3F. These issues will be addressed for the current database and future entries.

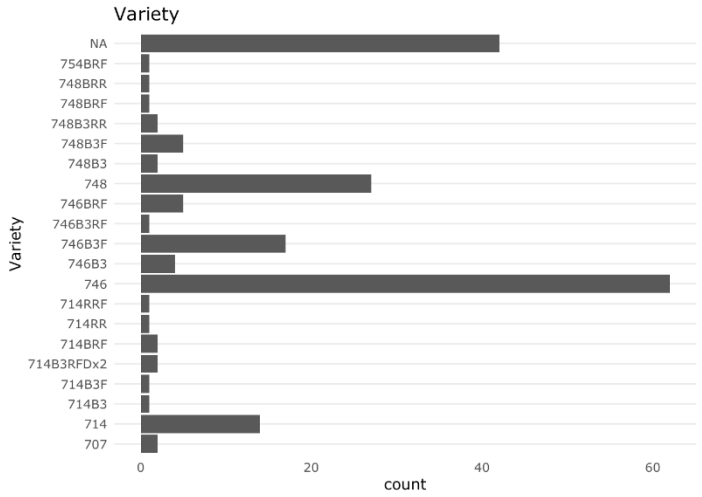



**Figure 78**.Varieties of cotton grown in fields surveyed in NSW

Qld survey

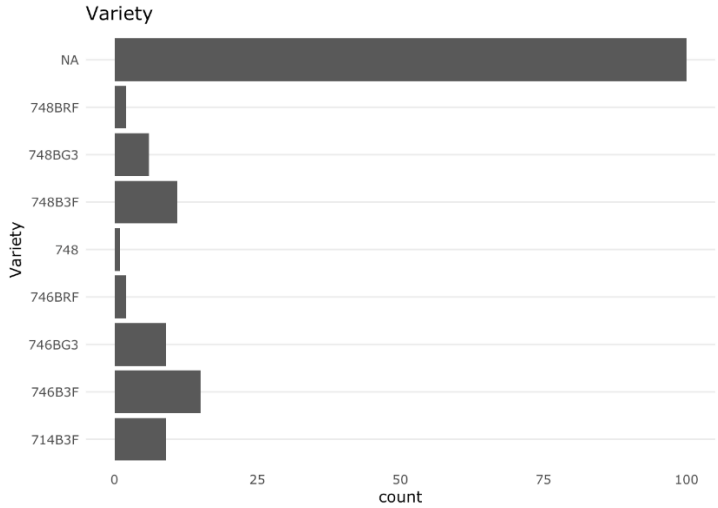



**Figure 79.** Varieties of cotton grown in fields surveyed in Qld

<u>Cotton trash and its influence on disease</u>

Cotton trash levels in NSW and Qld did not appear to vary greatly between the two states. In both, some fields recorded up to or more than 200 g/sq metre of cotton trash in the field (Figures 80 and 81).

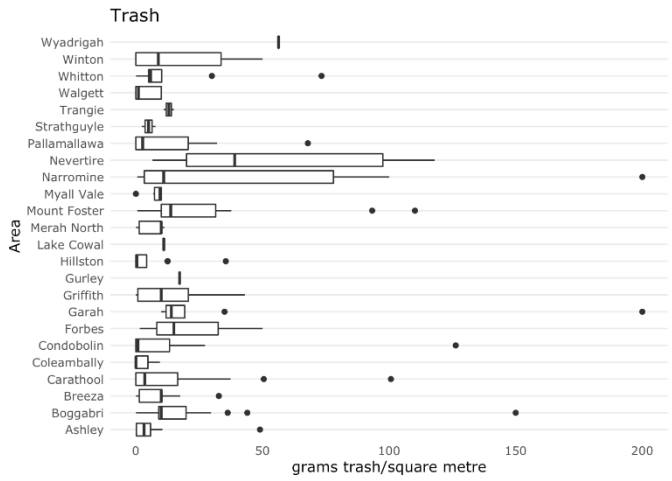


**Figure 80.** Cotton trash estimated early season on the soil surface in NSW surveys

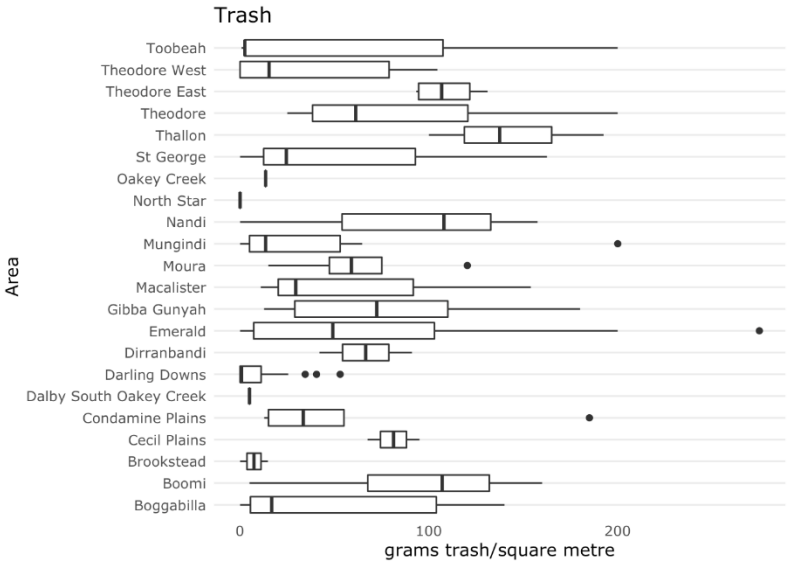



**Figure 81.** Cotton trash estimated early season on the soil surface in Qld surveys

Did the amount of cotton trash present early season affect diseases observed?

Management of cotton trash/residues plays an important part in an integrated approach to management of soil-borne diseases such as Verticillium wilt and Fusarium wilt. Hence, it was of interest to determine if the amount of cotton trash estimated on the soil surface early season had any effect on disease observed early and late season.

Analyses were performed using a generalised linear model (GLM) with a binomial family to handle the data that have clusters at zero. There may be better ways to handle these data, and this is being investigated.

The output displays the significance or non-significance of the quantity of cotton trash on the incidence of disease. To interpret analyses, if a star appears next to a variable in the summary table, it can be considered to have had a significant effect on disease.

The analyses performed on these data determined that the amount of cotton trash present in the field early season at time of surveys, did not have any significantly detectable effect on disease (Tables 11 – 21).

The following plots (Figures 82 – 92) show the relationship between cotton trash (g/sq m) at time of early season survey and incidence of disease determined early and late season. Each point represents a field transect in the following plots.

*Early Season*

**Fusarium**

**Table 11.** Summary table of analyses for effect of cotton trash (g/sq m) on the incidence (%) of Fusarium wilt

```
# Fusarium
glm_Fusarium <-
  glm(Fusarium ~ Trash,
      family = binomial,
```

```
      data = trash_Fusarium)

summary(glm_Fusarium)
##
## Call:
## glm(formula = Fusarium ~ Trash, family = binomial, data = trash_Fusarium)
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -0.2295  -0.1439  -0.1293  -0.1258   1.2575
##
## Coefficients:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept) -4.835971   1.168083  -4.140 3.47e-05 ***
## Trash        0.004409   0.011400   0.387    0.699
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 7.1480  on 153  degrees of freedom
## Residual deviance: 7.0086  on 152  degrees of freedom
## AIC: 7.2522
##
## Number of Fisher Scoring iterations: 7
ggplot(trash_Fusarium, aes(x = Trash, y = Fusarium)) +
  geom_point() +
  xlab("Trash (g/sq m)") +
  ylab("Incidence (%)") +
  ggtitle("Early Season Fusarium")
```

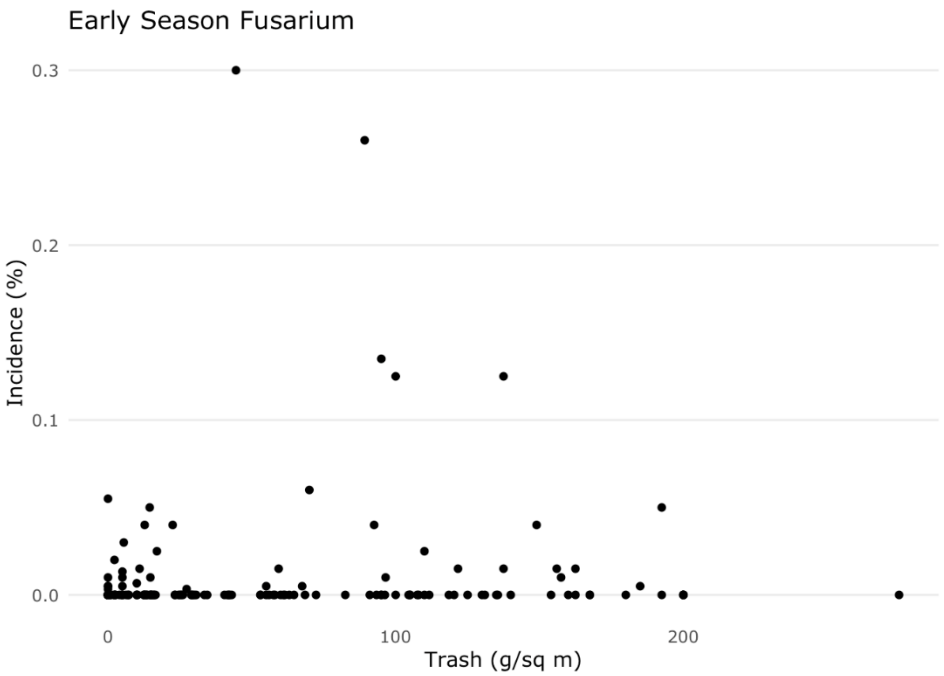



**Figure 82.** The relationship between cotton trash estimated early season and incidence of Fusarium wilt early season. Each point represents a field transect.

### Black Root Rot

**Table 12.** Summary table of analyses for effect of cotton trash (g/sq m) on the incidence (%) of Black root rot

```
# BRR
glm_BRR <-
  glm(BRR ~ Trash,
      family = binomial,
      data = trash_BRR)
```

```
summary(glm_BRR)
##
## Call:
## glm(formula = BRR ~ Trash, family = binomial, data = trash_BRR)
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -0.7144  -0.6822  -0.5723   0.3156   1.8797
##
## Coefficients:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept) -1.235508   0.164608  -7.506 6.11e-14 ***
## Trash       -0.002455   0.002950  -0.832    0.405
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 192.69  on 314  degrees of freedom
## Residual deviance: 191.96  on 313  degrees of freedom
## AIC: 298.29
##
## Number of Fisher Scoring iterations: 4
ggplot(trash_BRR, aes(x = Trash, y = BRR)) +
  geom_point() +
  xlab("Trash (g/sq m)") +
  ylab("Incidence (%)") +
  ggtitle("Early Season BRR")
```

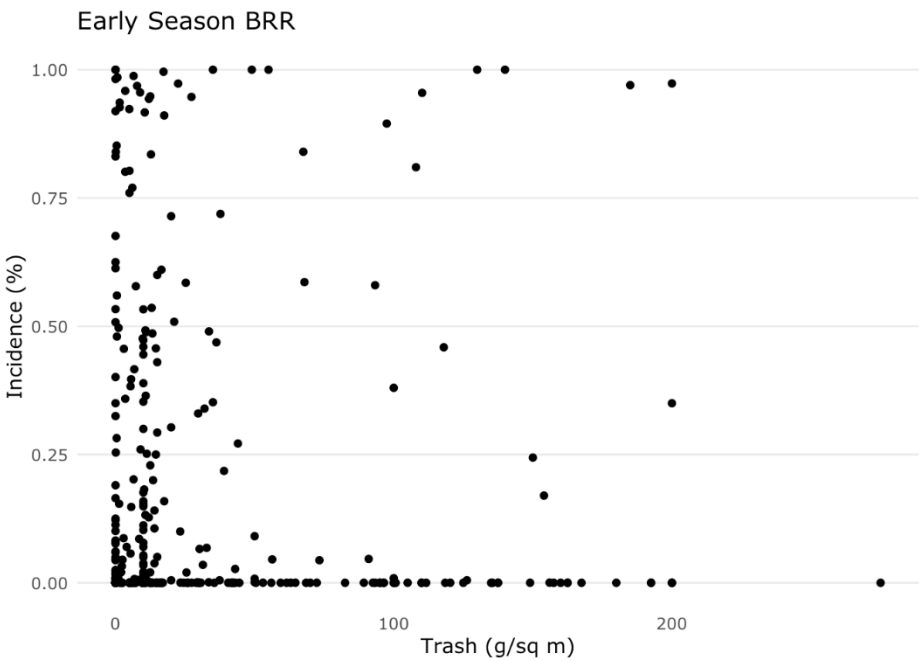



**Figure 83.** The relationship between cotton trash estimated early season and incidence of Black root rot. Each point represents a field transect.

### Pythium

**Table 13.** Summary table of analyses for effect of cotton trash (g/sq m) on the incidence (%) of Pythium

```
# Pythium
glm_Pythium <-
  glm(Pythium ~ Trash,
      family = binomial,
      data = trash_Pythium)

summary(glm_Pythium)
```

```
##
## Call:
## glm(formula = Pythium ~ Trash, family = binomial, data = trash_Pythium)
##
## Deviance Residuals:
##      Min        1Q    Median        3Q       Max
## -0.06820  -0.06819  -0.06789  -0.06714   0.82425
##
## Coefficients:
##               Estimate Std. Error z value Pr(>|z|)
## (Intercept) -6.0625158  1.7589186  -3.447 0.000567 ***
## Trash       -0.0009303  0.0505388  -0.018 0.985314
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 2.2744  on 187  degrees of freedom
## Residual deviance: 2.2740  on 186  degrees of freedom
## AIC: 4.8602
##
## Number of Fisher Scoring iterations: 9
ggplot(trash_Pythium, aes(x = Trash, y = Pythium)) +
  geom_point() +
  xlab("Trash (g/sq m)") +
  ylab("Incidence (%)") +
  ggtitle("Early Season Pythium")
```

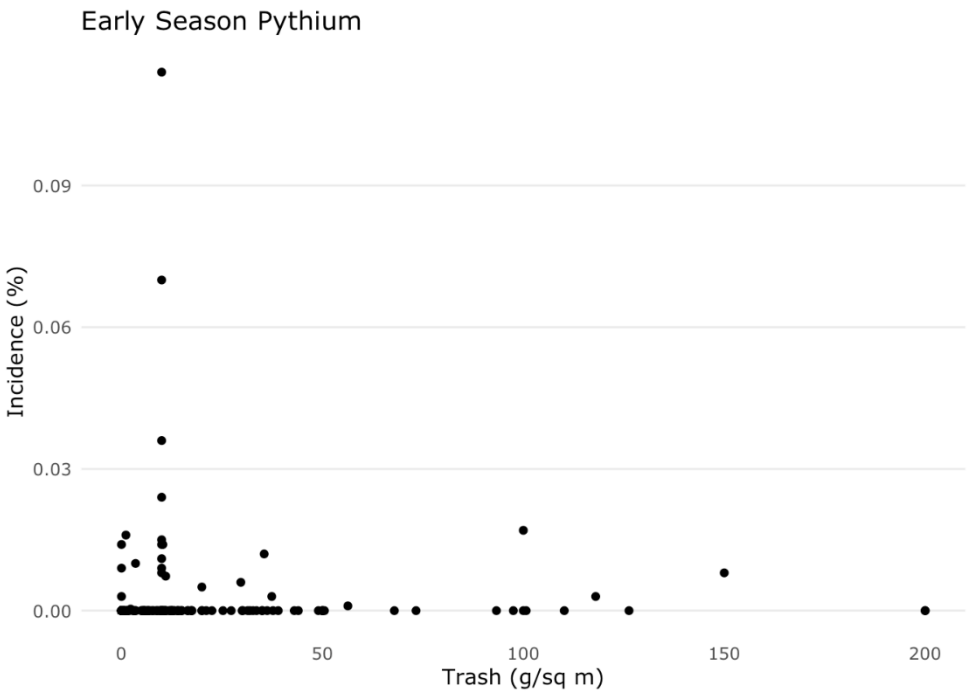



**Figure 84.** The relationship between cotton trash estimated early season and incidence of Pythium. Each point represents a field transect.

### Rhizoctonia

**Table 14.** Summary table of analyses for effect of cotton trash (g/sq m) on the incidence (%) of Rhizoctonia

```
# Rhizoc
glm_Rhizoctonia <-
  glm(Rhizoctonia ~ Trash,
      family = binomial,
      data = trash_Rhizoctonia)

summary(glm_Rhizoctonia)
##
## Call:
## glm(formula = Rhizoctonia ~ Trash, family = binomial, data = trash_Rhizoctonia)
```

```
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -1.0858  -0.7383  -0.2587   0.5040   1.4851
##
## Coefficients:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept) -0.821661   0.180376  -4.555 5.23e-06 ***
## Trash        0.007177   0.004738   1.515     0.13
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 107.06  on 187  degrees of freedom
## Residual deviance: 104.73  on 186  degrees of freedom
## AIC: 238.14
##
## Number of Fisher Scoring iterations: 3
ggplot(trash_Rhizoctonia, aes(x = Trash, y = Rhizoctonia)) +
  geom_point() +
  xlab("Trash (g/sq m)") +
  ylab("Incidence (%)") +
  ggtitle("Early Season Rhizoctonia")
```

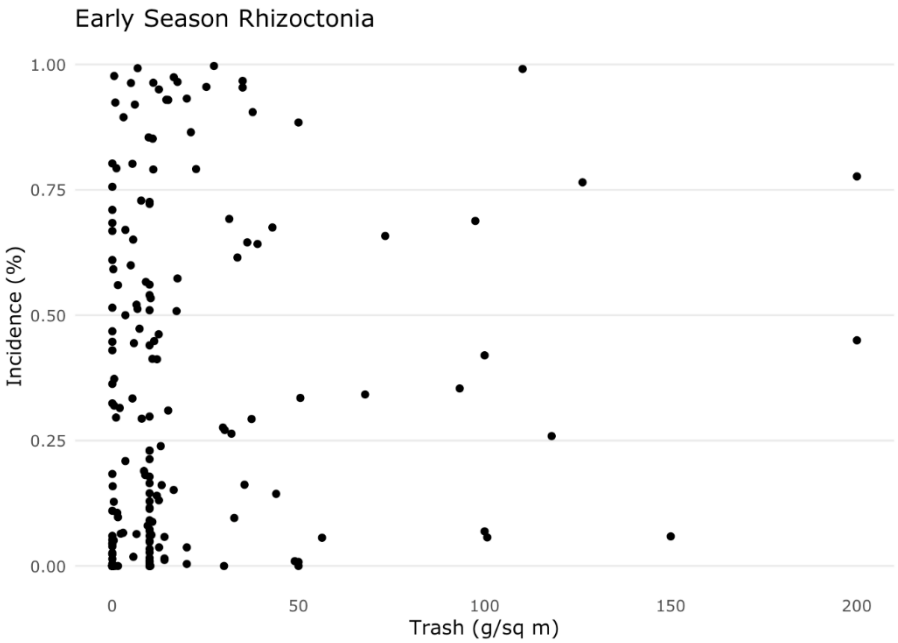



**Figure 85**. The relationship between cotton trash estimated early season and incidence of Rhizoctonia. Each point represents a field transect.

*Late Season*

**Alternaria**

**Table 15.** Summary table of analyses for effect of cotton trash (g/sq m) on the incidence (%) of Alternaria

**NOTE** Alternaria is recorded in both New South Wales and Queensland.

```
##
## Call:
## glm(formula = Alt ~ Trash, family = binomial, data = trash_Alternaria)
##
## Deviance Residuals:
##      Min        1Q    Median        3Q       Max
## -0.28103  -0.26706  -0.18114   0.00083   1.19790
```

```
##
## Coefficients:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept) -3.211961   0.441632  -7.273 3.52e-13 ***
## Trash       -0.006267   0.009391  -0.667    0.505
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 20.465  on 229  degrees of freedom
## Residual deviance: 19.930  on 228  degrees of freedom
## AIC: 19.055
##
## Number of Fisher Scoring iterations: 6
```

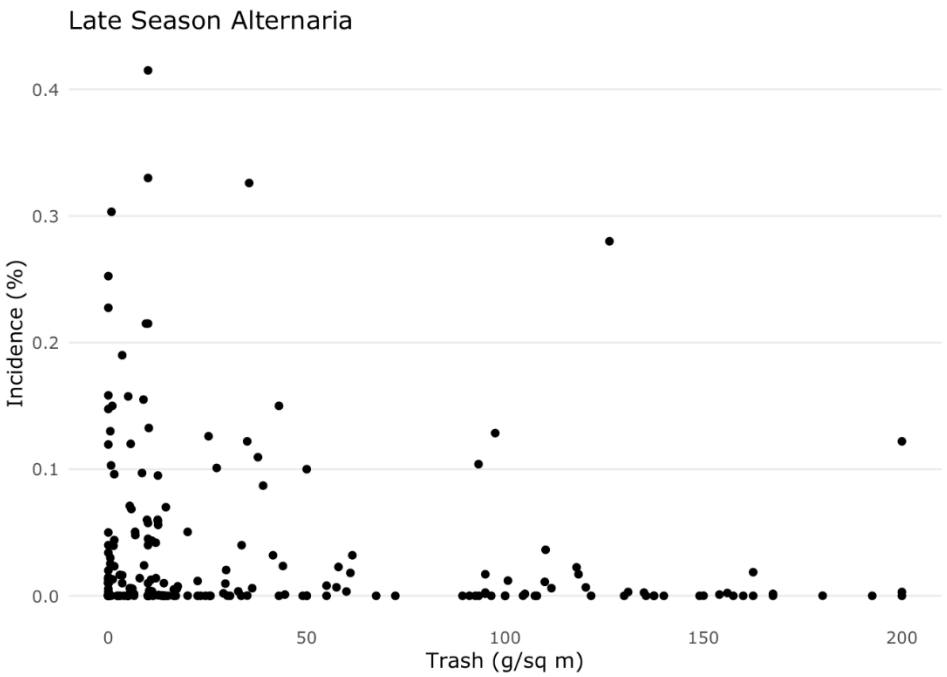



**Figure 86.** The relationship between cotton trash estimated early season and incidence of Alternaria leaf spot late season. Each point represents a field transect.

**Fusarium (Fov)**

**Table 16.** Summary table of analyses for effect of cotton trash (g/sq m) on the incidence (%) of Fusarium wilt

```
##
## Call:
## glm(formula = Fov ~ Trash, family = binomial, data = trash_Fov)
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -0.3827  -0.2461  -0.2374  -0.1247   1.8987
##
## Coefficients:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept) -3.562253   0.443209  -8.037 9.18e-16 ***
## Trash        0.004926   0.005618   0.877    0.381
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 38.185  on 265  degrees of freedom
## Residual deviance: 37.489  on 264  degrees of freedom
```

```
## AIC: 42.362
##
## Number of Fisher Scoring iterations: 6
```

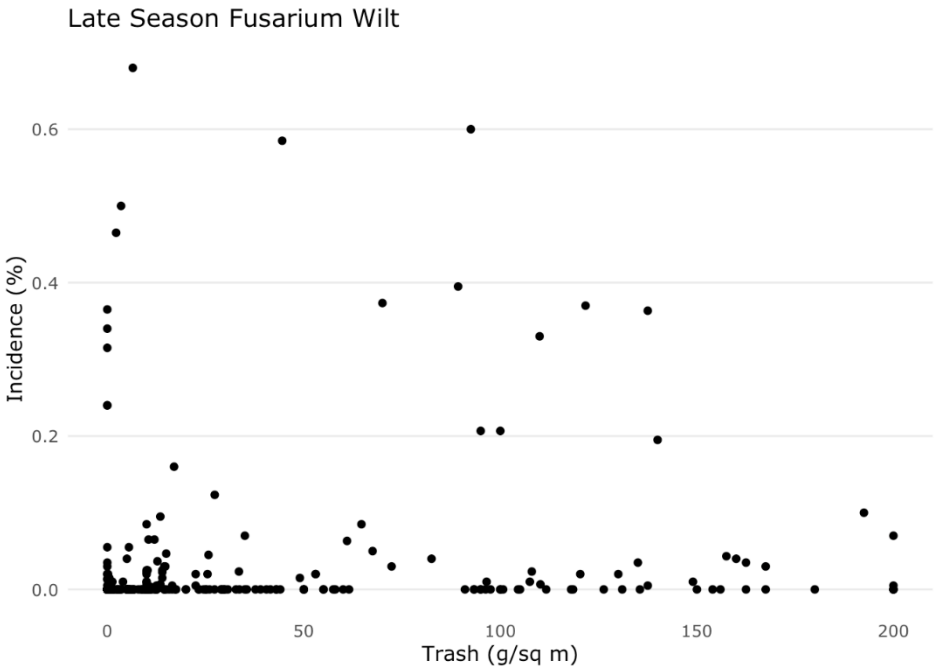



**Figure 87.** The relationship between cotton trash estimated early season and incidence of Fusarium wilt late season. Each point represents a field transect.

**Verticillium wilt**

**Table 17.** Summary table of analyses for effect of cotton trash (g/sq m) on the incidence (%) of Verticillium wilt

```
##
## Call:
## glm(formula = Vd ~ Trash, family = binomial, data = trash_Vd)
##
## Deviance Residuals:
##     Min       1Q   Median       3Q      Max
## -0.4138  -0.3908  -0.3246  -0.1626   2.2622
##
## Coefficients:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept) -2.414729   0.301430  -8.011 1.14e-15 ***
## Trash       -0.013726   0.009012  -1.523    0.128
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 71.997  on 266  degrees of freedom
## Residual deviance: 68.416  on 265  degrees of freedom
## AIC: 106.29
##
## Number of Fisher Scoring iterations: 6
```

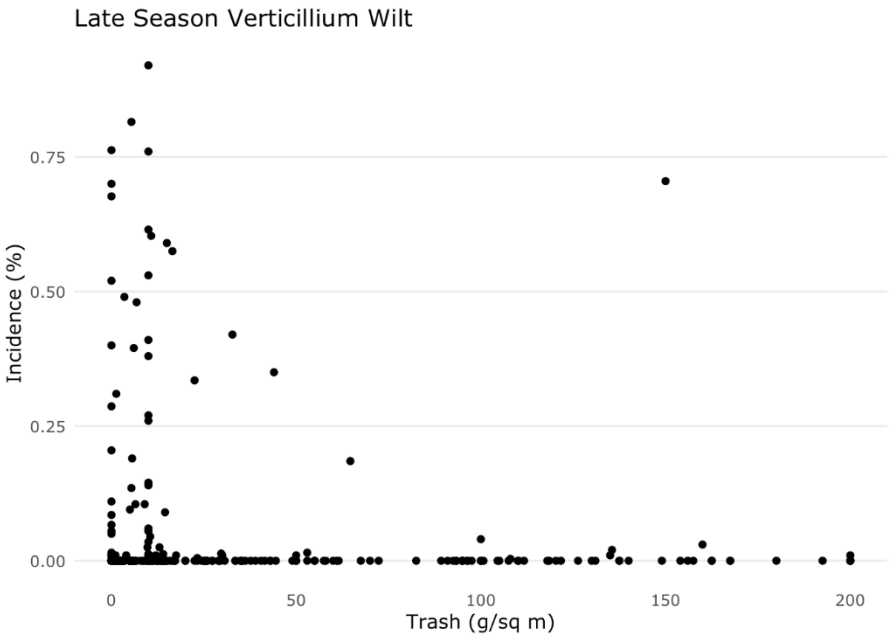



**Figure 88**. The relationship between cotton trash estimated early season and incidence of Verticillium wilt late season. Each point represents a field transect.

**Seed rot**

**Table 18.** Summary table of analyses for effect of cotton trash (g/sq m) on the incidence (%) of Seed rot

```
##
## Call:
## glm(formula = SR ~ Trash, family = binomial, data = trash_SR)
##
## Deviance Residuals:
##      Min        1Q    Median        3Q       Max
## -0.15140  -0.12150  -0.12010   0.02564   0.70001
##
## Coefficients:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept) -4.928329   1.007337  -4.892 9.96e-07 ***
## Trash        0.002326   0.013195   0.176     0.86
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 4.0234  on 211  degrees of freedom
## Residual deviance: 3.9938  on 210  degrees of freedom
## AIC: 7.3971
##
## Number of Fisher Scoring iterations: 7
```

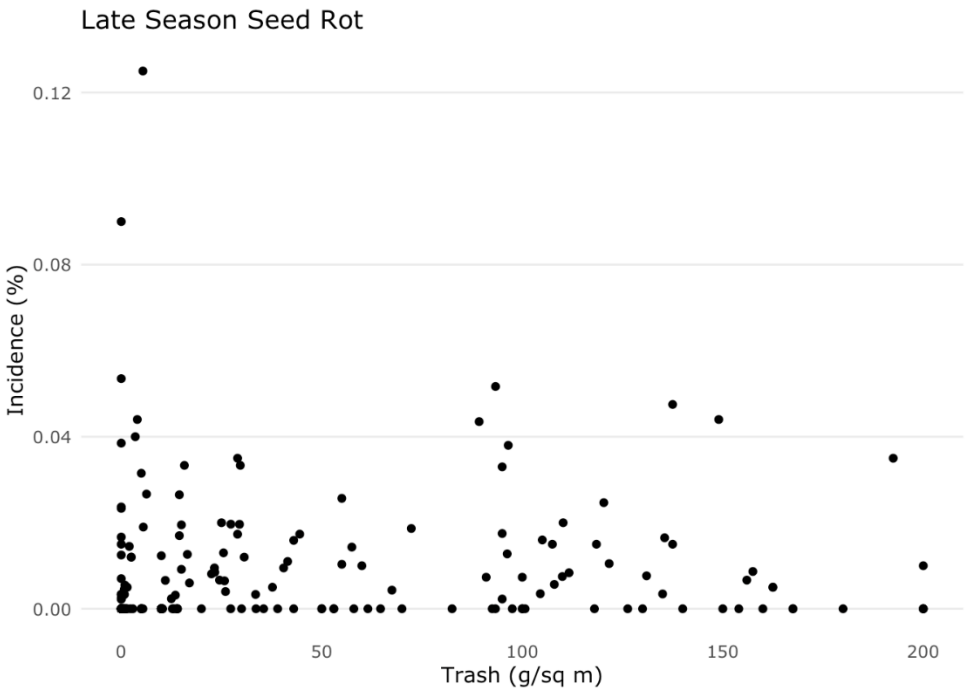



**Figure 89.** The relationship between cotton trash estimated early season and incidence of Seed rot late season. Each point represents a field transect.

**Boll rot**

**Table 19.** Summary table of analyses for effect of cotton trash (g/sq m) on the incidence (%) of Boll rot

**NOTE** In this Boll Rot variable, all boll rots recorded by Qld have been combined into a single value to align with the "Boll Rot" reported by NSW.

```
##
## Call:
## glm(formula = BR ~ Trash, family = binomial, data = trash_BR)
##
## Deviance Residuals:
##      Min        1Q    Median        3Q       Max
## -0.21154  -0.14748  -0.14470  -0.00582   1.29365
##
## Coefficients:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept) -4.554104   0.712979  -6.387 1.69e-10 ***
## Trash        0.003828   0.009445   0.405    0.685
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 9.6918  on 271  degrees of freedom
## Residual deviance: 9.5413  on 270  degrees of freedom
## AIC: 10.682
##
## Number of Fisher Scoring iterations: 7
```

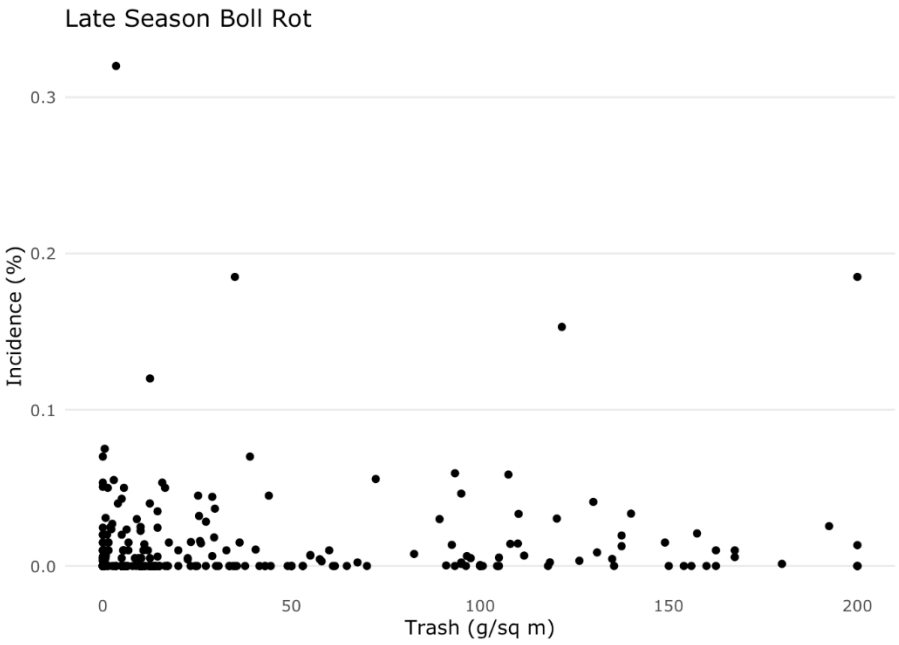



**Figure 90**. The relationship between cotton trash estimated early season and incidence of Boll rot late season. Each point represents a field transect.

**Tight-lock**

**Table 20.** Summary table of analyses for effect of cotton trash quantity on the incidence (%) of Tight-lock

```
##
## Call:
## glm(formula = TL ~ Trash, family = binomial, data = trash_TL)
##
## Deviance Residuals:
##      Min        1Q    Median        3Q       Max
## -0.20016  -0.15537  -0.15330   0.01713   0.59228
##
## Coefficients:
##              Estimate Std. Error z value Pr(>|z|)
## (Intercept) -4.437928   0.780160  -5.688 1.28e-08 ***
## Trash        0.002688   0.010148   0.265    0.791
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 7.1285  on 215  degrees of freedom
## Residual deviance: 7.0621  on 214  degrees of freedom
## AIC: 9.7295
##
## Number of Fisher Scoring iterations: 7
```

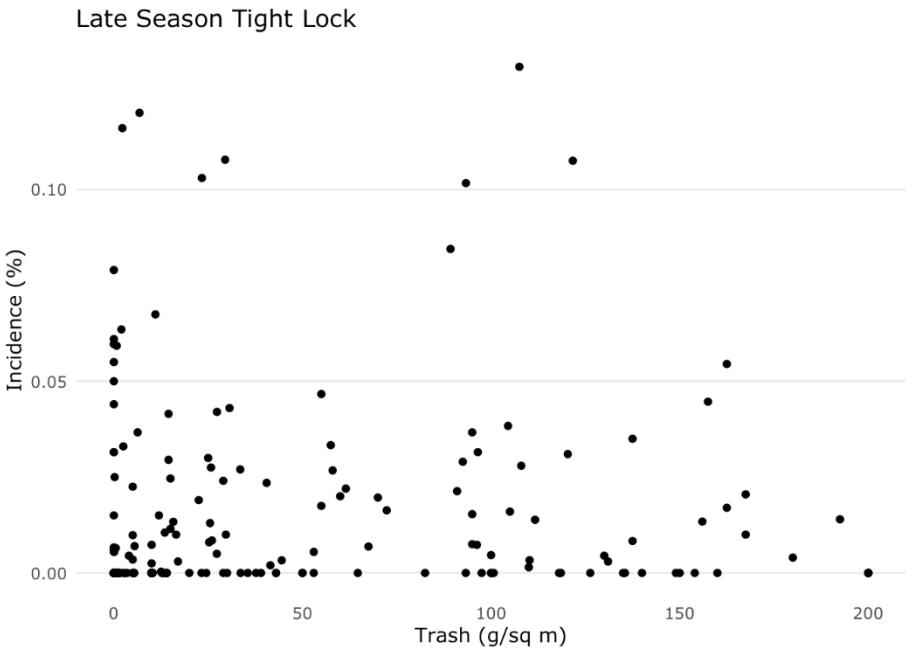



**Figure 91.** The relationship between cotton trash estimated early season and incidence of Tight-lock late season. Each point represents a field transect.

### Bunchy top

**Table 21.** Summary table of analyses for effect of cotton trash quantity on the incidence (%) of Bunchy top

**NOTE** Bunchy Top is only recorded in NSW.

```
##
## Call:
## glm(formula = BT ~ Trash, family = binomial, data = trash_BT)
##
## Deviance Residuals:
##      Min        1Q    Median        3Q       Max
## -0.15229  -0.15041  -0.13787  -0.07744   2.55610
##
## Coefficients:
##             Estimate Std. Error z value Pr(>|z|)
## (Intercept) -4.45129    0.88109  -5.052 4.37e-07 ***
## Trash       -0.01428    0.02632  -0.543    0.587
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## (Dispersion parameter for binomial family taken to be 1)
##
##     Null deviance: 14.565  on 214  degrees of freedom
## Residual deviance: 14.096  on 213  degrees of freedom
## AIC: 16.843
##
## Number of Fisher Scoring iterations: 9
```

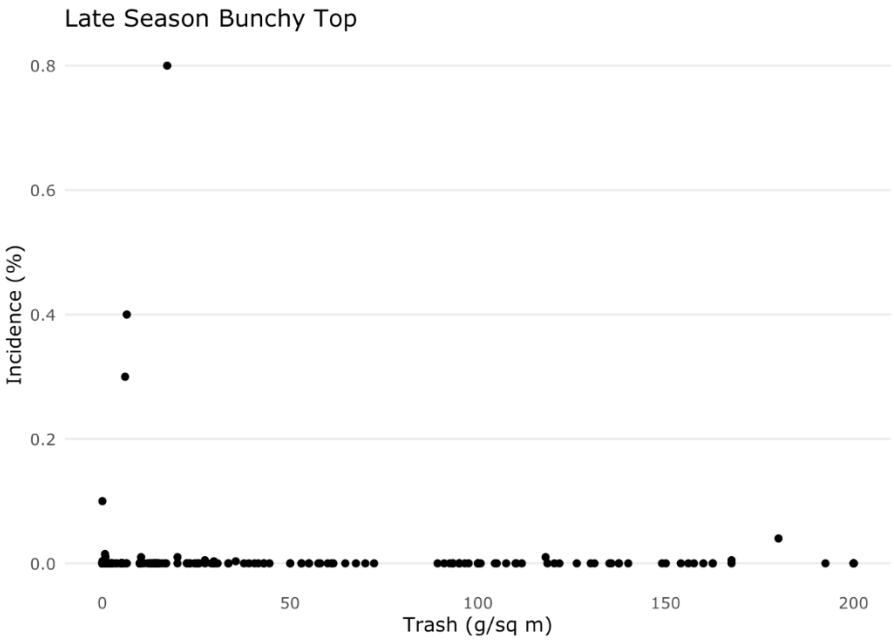



**Figure 92.** The relationship between cotton trash estimated early season and incidence of Bunchy top late season. Each point represents a field transect.

Previous crop

In NSW the most frequently recorded previous crop was wheat, followed by cotton (Figure 93). In Qld it was cotton followed by fallow (Figure 94). However, in both states the number of values for previous crop not recorded, NA, exceed the max values of the most frequently recorded previous crop, hence further follow-up is required with growers to complete dataset.

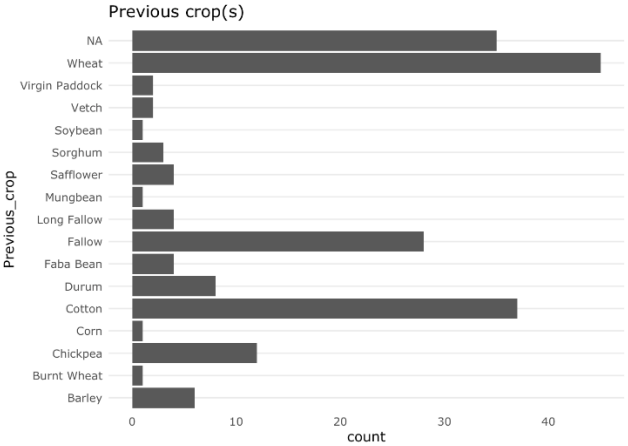



**Figure 93.** Previous crops grown in fields surveyed in NSW

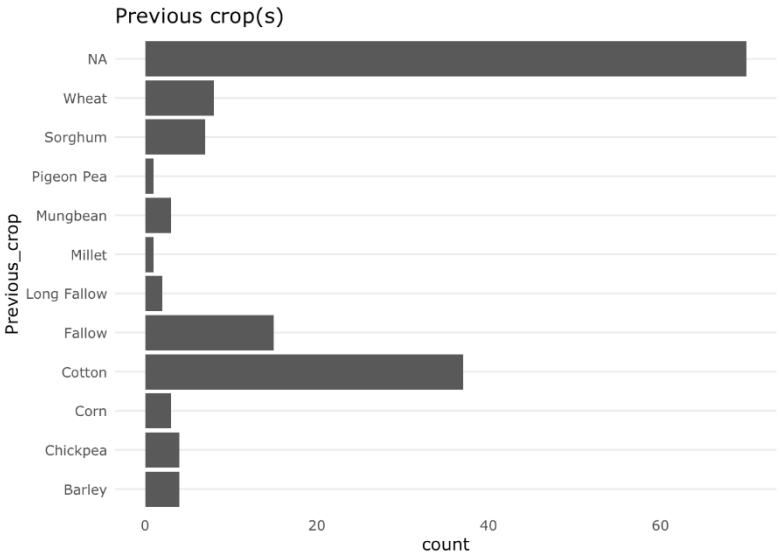



**Figure 94.** Previous crops grown in fields surveyed in Qld

Analysis of Previous Crop

Many diseases build up in the soil when a host crop is grown in the same field year after year. Rotation to a non-susceptible crop can help break this cycle by reducing pathogen levels. Therefore, analyses was conducted to determine if the previous crop had an effect on diseases in the following cotton crop.

Due to some crops or rotations having few observations, these were placed into groups to compare against cotton as the previous crop baseline. These analyses were performed using MCMCglmm, a Bayesian Generalised Linear Mixed Model with Previous_crop as a fixed effect and Code as a random effect was used. This will isolate the effects of the previous crop from the field effect.

The output displays the significance or non-significance of the previous crop on the disease. To interpret analyses, if a star appears next to a variable in the summary table, it can be considered significantly different from cotton as a previous crop.

The density plot figure shows the difference between the previous crops tested in the model. When the density plots mostly overlap, there is no significance. In cases where a density line is shifted to the right, then the effect is more disease. When shifted to the left, the effect is less disease. If the density plot mostly overlaps 0, then it is not significantly different than the cotton baseline being compared against.

Boxplots can also be viewed to determine if variable is higher or lower than cotton as a previous crop. The boxplots show the raw data for each field in an area that was tested. The boxplot shows the minimum value, max and quartiles with the middle of the box being the median, or 50 %. Dots indicate outliers in the data. The whiskers are each 25 % of the data values. A longer boxplot indicates more variation in the area's observations. For boxes where the centreline is to the left of cotton, the previous crop appears to have reduced the disease level. In cases where it is to the right, it has likely increased the amount of disease observed. There are other factors involved in the analysis, but this is an easy way to interpret these boxplots.

A pairwise comparison of all the previous crops with each other, not just cotton. When the horizontal solid black line of each comparison overlaps the 0 value of the x-axis, that pairwise comparison is not significantly different. This allows us to compare all the previous crops easily and visually.

*Early Season Diseases*

**Alternaria**

There was no significant effects of previous crop on the incidence (%) of Alternaria (Table 22).

**Table 22.** Summary table of analyses for effect of previous crop on the incidence (%) of Alternaria

**NOTE** Alternaria is recorded in both New South Wales and Queensland.

```
##  Iterations = 3001:12991
##  Thinning interval  = 10
##  Sample size  = 1000
##
##  DIC: -241.8114
##
##  G-structure:  ~uid
##
##     post.mean l-95% CI u-95% CI eff.samp
## uid     409.5    332.5      492     1000
##
##  R-structure:  ~units
##
##       post.mean l-95% CI u-95% CI eff.samp
## units   0.04701 0.001435   0.1807    44.61
##
##  Location effects: Alt ~ Previous_crop
##
##                             post.mean l-95% CI u-95% CI eff.samp pMCMC
## (Intercept)                    6.8439   1.3889  11.9856     1115 0.006 **
## Previous_cropFallow            3.1097  -4.7390  11.0924     1000 0.438
## Previous_cropPulse/Legume     -3.3101 -13.6763   5.8366     1000 0.500
## Previous_cropSummer Grain      1.0732 -11.3616  11.0998     1000 0.880
## Previous_cropWinter Cereal     0.3579  -7.4139   7.8588     1000 0.950
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

### Rhizoctonia

There were no significant effects of previous crop on the incidence of Rhizoctonia (Table 23).

**Table 23.** Summary table of analyses for effect of previous crop on the incidence (%) of Rhizoctonia

```
NOTE Rhizoctonia in New South Wales used only.
##  Iterations = 3001:12991
##  Thinning interval  = 10
##  Sample size  = 1000
##
##  DIC: 591.3852
##
##  G-structure:  ~uid
##
##     post.mean l-95% CI u-95% CI eff.samp
## uid      1109    852.1     1471    68.07
##
##  R-structure:  ~units
##
##       post.mean l-95% CI u-95% CI eff.samp
## units     33.46   0.2097    123.8    26.57
##
##  Location effects: Rhizoctonia ~ Previous_crop
##
##                             post.mean l-95% CI u-95% CI eff.samp  pMCMC
## (Intercept)                    36.885   27.255   47.953     1000 <0.001 ***
## Previous_cropFallow             4.855  -10.340   20.016     1000  0.524
## Previous_cropPulse/Legume     -12.693  -31.435    4.061     1120  0.160
## Previous_cropSummer Grain     -13.663  -39.769   12.199     1000  0.294
## Previous_cropWinter Cereal     -8.640  -21.491    4.940     1000  0.190
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

### Black Root Rot

There were no significant effects of previous crop on the incidence of Black root rot (Table 24).

**Table 24.** Summary table of analyses for effect of previous crop on the incidence (%) of Black root rot

```
NOTE Black root rot is recorded in both New South Wales and Queensland.

##  Iterations = 3001:12991
##  Thinning interval  = 10
##  Sample size  = 1000
##
##  DIC: 1550.167
##
##  G-structure:  ~uid
##
##     post.mean l-95% CI u-95% CI eff.samp
## uid     840.3 0.003576     1119    9.949
##
##  R-structure:  ~units
##
##       post.mean l-95% CI u-95% CI eff.samp
## units     167.1    4.114    938.1     25.8
##
##  Location effects: BRR ~ Previous_crop
##
##                             post.mean l-95% CI u-95% CI eff.samp  pMCMC
## (Intercept)                   21.1569  13.8192  28.7568     1000 <0.001 ***
## Previous_cropFallow            1.1789 -10.1163  12.9014     1000  0.844
## Previous_cropPulse/Legume      4.5701  -9.4760  20.2872     1000  0.536
## Previous_cropSummer Grain      4.5991 -12.2398  20.9611     1000  0.582
## Previous_cropWinter Cereal    -0.6477 -11.8896   9.3283     1115  0.874
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

### Pythium

There were no significant effects of previous crop on the incidence of Pythium (Table 25).

**Table 25.** Summary table of analyses for effect of previous crop on the incidence (%) of Pythium

**NOTE** Pythium in New South Wales is analysed only.

```
Location effects: Pythium ~ Previous_crop
##
##                             post.mean  l-95% CI  u-95% CI eff.samp pMCMC
## (Intercept)                  0.116251 -0.251749  0.480956   1000.0 0.544
## Previous_cropFallow         -0.091828 -0.628512  0.414257   1000.0 0.754
## Previous_cropPulse/Legume   -0.039244 -0.617387  0.589355   1000.0 0.924
## Previous_cropSummer Grain    0.297928 -0.519316  1.205765   1000.0 0.524
## Previous_cropWinter Cereal   0.446421 -0.005344  0.915896    897.5 0.060
##
## (Intercept)
## Previous_cropFallow
## Previous_cropPulse/Legume
## Previous_cropSummer Grain
## Previous_cropWinter Cereal .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

### Fusarium

There was no significant effects of previous crop on the incidence (%) of Fusarium wilt (Table 26).

**Table 26.** Summary table of analyses for effect of previous crop on the incidence (%) of Fusarium wilt

```
NOTE Fusarium is only measured in Queensland.
##
##  Iterations = 3001:12991
```

```
##  Thinning interval  = 10
##  Sample size  = 1000
##
##  DIC: 176.0878
##
##  G-structure:  ~uid
##
##     post.mean  l-95% CI u-95% CI eff.samp
## uid     10.09 0.0007429    14.73    54.01
##
##  R-structure:  ~units
##
##       post.mean l-95% CI u-95% CI eff.samp
## units     2.023  0.01386    10.55    40.11
##
##  Location effects: Fusarium ~ Previous_crop
##
##                             post.mean  l-95% CI  u-95% CI eff.samp pMCMC
## (Intercept)                  1.121595 -0.093510  2.077416   1000.0 0.052 .
## Previous_cropFallow         -0.872502 -2.801979  1.138285   1000.0 0.400
## Previous_cropPulse/Legume   -0.843492 -3.579684  1.780259   1000.0 0.534
## Previous_cropSummer Grain    0.005012 -2.174881  2.329581   1000.0 0.996
## Previous_cropWinter Cereal -0.983629 -3.304650  1.410652    874.3 0.406
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

***Late Season Diseases***

**Alternaria**

There were no significant effects of previous crop on the incidence of Alternaria (Table 27).

**Table 27.** Summary table of analyses for effect of previous crop on the incidence (%) of Alternaria

```
##
##  Iterations = 3001:12991
##  Thinning interval  = 10
##  Sample size  = 1000
##
##  DIC: -370.7481
##
##  G-structure:  ~uid
##
##     post.mean l-95% CI u-95% CI eff.samp
## uid     52.71    39.69    63.93    273.7
##
##  R-structure:  ~units
##
##       post.mean l-95% CI u-95% CI eff.samp
## units    0.5297 0.001788   0.7698     43.9
##
##  Location effects: Alt ~ Previous_crop
##
##                             post.mean l-95% CI u-95% CI eff.samp  pMCMC
## (Intercept)                    4.2420   2.4595   6.2504      646 <0.001 ***
## Previous_cropFallow           -0.5719  -3.7811   2.2958     1000  0.696
## Previous_cropPulse/Legume     -3.2443  -6.7396   0.4557     1000  0.090 .
## Previous_cropSummer Grain      3.9108  -1.1604   8.4927     1000  0.126
## Previous_cropWinter Cereal    -0.3603  -3.1615   2.3931     1000  0.822
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

**Fov (Fusarium wilt)**

There were no significant effects of previous crop on the incidence of Fusarium wilt (Table 28).

**Table 28.** Summary table of analyses for effect of previous crop on the incidence (%) of Fusarium wilt

```
##
##  Iterations = 3001:12991
```

```
##  Thinning interval  = 10
##  Sample size  = 1000
##
##  DIC: -19.19002
##
##  G-structure:  ~uid
##
##     post.mean l-95% CI u-95% CI eff.samp
## uid     94.28    75.24    114.3     1000
##
##  R-structure:  ~units
##
##       post.mean l-95% CI u-95% CI eff.samp
## units    0.1353 0.002471   0.5591    42.16
##
##  Location effects: Fov ~ Previous_crop
##
##                             post.mean l-95% CI u-95% CI eff.samp  pMCMC
## (Intercept)                    4.3183   1.7196   6.5728     1000 <0.001 ***
## Previous_cropFallow           -3.1919  -7.1977   0.4884     1000  0.128
## Previous_cropPulse/Legume     -3.8836  -8.7301   0.5697     1000  0.094 .
## Previous_cropSummer Grain      2.0553  -3.2677   7.4086     1000  0.506
## Previous_cropWinter Cereal    -2.1898  -5.8939   1.3263     1109  0.200
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

**Verticilium Wilt**

There was a significant effect of winter cereals as a previous crop on the incidence (%) of Verticillium wilt (Table 29). Verticillium wilt was significantly higher following winter cereal crops than cotton but no different following other previous crops (Figures 95 and 96). The most common winter cereal grown was wheat.

**Table 29.** Summary table of analyses for effect of previous crop on the incidence (%) of Verticillium wilt

```
##
##  Iterations = 3001:12991
##  Thinning interval  = 10
##  Sample size  = 1000
##
##  DIC: -123.6154
##
##  G-structure:  ~uid
##
##     post.mean l-95% CI u-95% CI eff.samp
## uid     286.7      232    347.8     1000
##
##  R-structure:  ~units
##
##       post.mean l-95% CI u-95% CI eff.samp
## units     1.589 0.001982     8.63    31.28
##
##  Location effects: Vd ~ Previous_crop
##
##                             post.mean l-95% CI u-95% CI eff.samp pMCMC
## (Intercept)                    4.6483   0.4591   9.3021   1000.0 0.030 *
## Previous_cropFallow            0.2293  -7.1408   6.6452    935.6 0.940
## Previous_cropPulse/Legume     -4.0367 -11.8827   4.2027   1000.0 0.330
## Previous_cropSummer Grain      2.9602  -6.8604  12.1158   1000.0 0.552
## Previous_cropWinter Cereal     8.4193   2.3770  14.9982   1000.0 0.016 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Figure 95 shows the difference between the previous crops tested in the model above for Verticillium wilt. When the density plots mostly overlap, there is no significance. In cases where a density line is shifted to the right, such as with winter cereals and summer grain crops, then the effect is more disease. When shifted to the left, such as for pulse.legume crops, the effect is less disease. If the density plot mostly overlaps 0, as for fallow, then it is not significantly different than the cotton baseline being compared against.

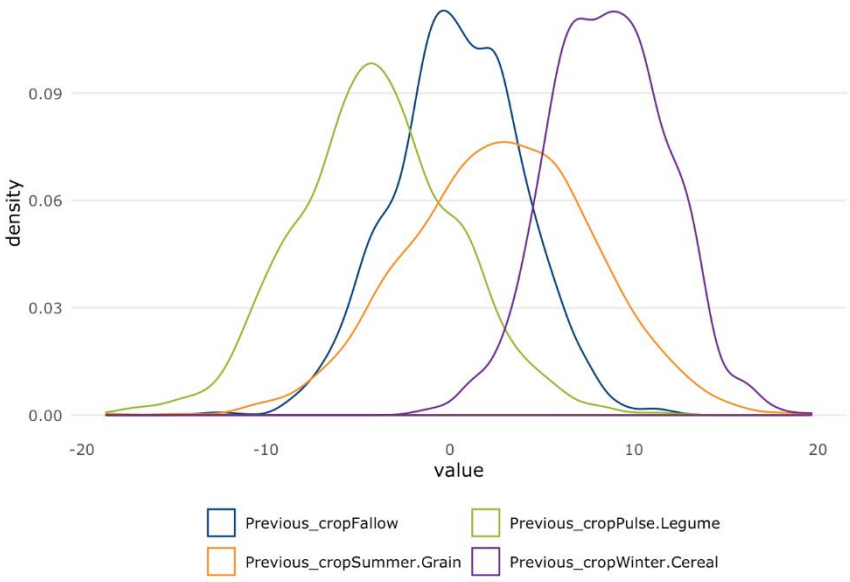



**Figure 95.** Density plots showing the difference between the previous crops tested in the model for Verticillium wilt

The boxplots show the raw data for each field in an area that was tested. For boxes where the centreline is to the left of cotton, the previous crop appears to have reduced the disease level. In cases where it is to the right, it has likely increased the amount of disease observed. There are other factors involved in the analysis, but this is an easy way to interpret these boxplots. Figure 96 shows that both winter cereals and summer grains increase the incidence of Verticillium wilt, with the increase being significantly higher than cotton when winter cereal was the previous crop.

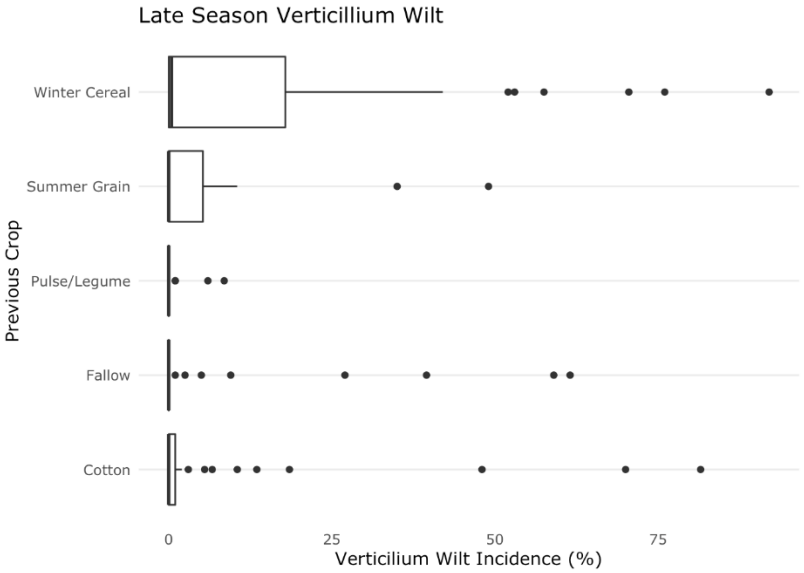



**Figure 96.** Boxplots showing the raw data for Verticillium wilt incidence for each field grouped by previous crop Note: Lower = to the left, higher = to the right, of 0.

The plot below shows a pairwise comparison of all the previous crops with each other, not just cotton. When the horizontal solid black line of each comparison overlaps the 0 value of the x-axis, that pairwise comparison is not significantly different. This allows us to compare all the previous crops easily and visually. Hence, Figure 97 shows that the only pairwise comparison of previous crops that had a significant effect on Verticillium wilt incidence was cotton and winter cereals. This is an important finding

because a common crop rotation implemented to manage Verticillium wilt has been a fallow, wheat cotton rotation. Anecdotally in the Macintyre valley, this strategy has not managed Verticillium wilt and disease incidence has continued to rise.

Analyses of disease incidence data collected in these surveys provides statistical evidence that winter cereals have the potential to increase the incidence of Verticillium wilt compared to a host crop such as cotton. Further research is required through field trials to confirm this finding and to determine the mechanism(s) involved. A better understanding of relationships among cereal crops and *V. dahliae* may allow us to use crop rotations more effectively in efforts to reduce soil inoculum levels as an efficient management practice.

There are reports in the literature of non-hosts, including wheat, confirmed as asymptomatic hosts of *V. dahliae* (Davis et al., 1997). Research conducted by Linda Scheikowski and reported in this report (Milestone 2.1) provides support that wheat when artificially inoculated can become infected by *V. dahliae*. Whether infection of wheat occurs naturally in the field, causing an increase in soil inoculum, requires investigation to confirm.

Davis, J. R., Huisman, O. C., Sorensen, L. H., and Schneider, A. T. 1997. Field studies comparing the susceptibility of various crops to colonization by Verticillium dahliae. Page 29 in: Proc. 7th Int. Verticillium Symp. Athens, Greece.

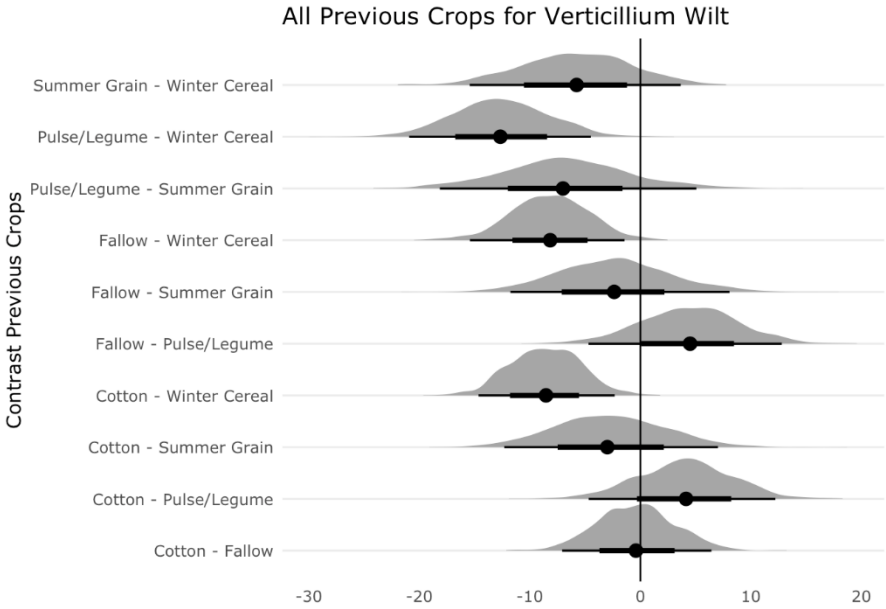



**Figure 97.** Pairwise comparison of previous crops for effect on Verticillium wilt incidence

**Seed Rot**

There was a significant effect of winter cereals and fallow as a previous crop on the incidence (%) of Seed rot (Table 30). Seed rot was significantly lower in cotton crop following a fallow and winter cereals than cotton, but no different in other previous crops (Figure 98).

**Table 30**. Summary table of analyses for effect of previous crop on the incidence (%) of Seed rot

```
##
##  Iterations = 3001:12991
##  Thinning interval  = 10
##  Sample size  = 1000
##
##  DIC: -45.68525
##
##  G-structure:  ~uid
##
##     post.mean l-95% CI u-95% CI eff.samp
```

```
## uid     1.871 8.38e-08    2.614    29.73
##
##  R-structure:  ~units
##
##       post.mean l-95% CI u-95% CI eff.samp
## units    0.3732 0.002273    1.952    16.63
##
##  Location effects: SR ~ Previous_crop
##
##                            post.mean l-95% CI u-95% CI eff.samp  pMCMC
## (Intercept)                  1.10887  0.68394  1.49570   1000.0 <0.001 ***
## Previous_cropFallow         -0.79373 -1.45004 -0.11270    784.4  0.026 *
## Previous_cropPulse/Legume   -0.08656 -0.89480  0.69489   1000.0  0.832
## Previous_cropSummer Grain   -0.48601 -1.48312  0.48716   1000.0  0.324
## Previous_cropWinter Cereal  -0.88683 -1.48782 -0.26440   1000.0  0.002 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

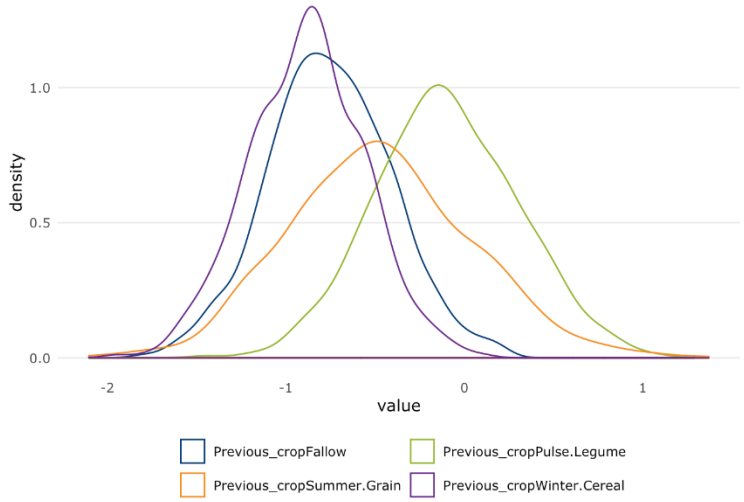



**Figure 98.** Density plots showing the difference between the previous crops tested in the model for Seed rot

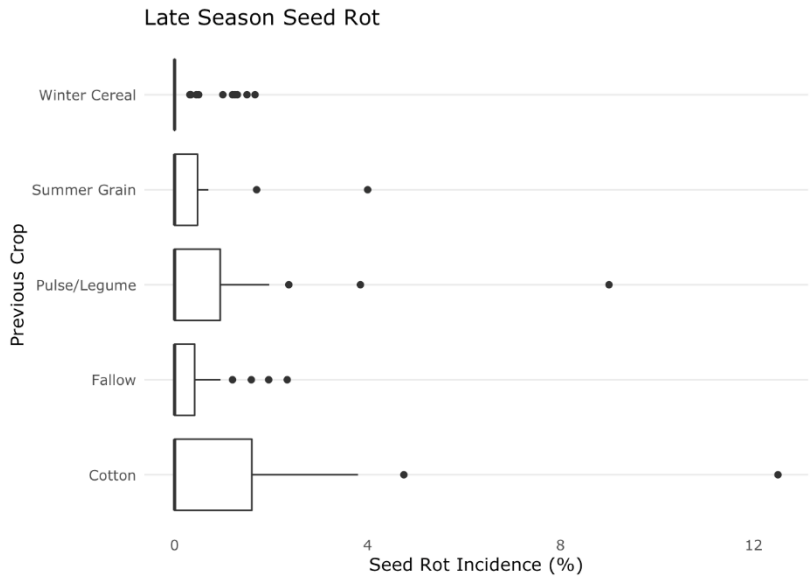



**Figure 99.** Boxplots showing the raw data for Seed rot incidence for each field grouped by previous crop Note: Lower = to the left, higher = to the right, of 0.

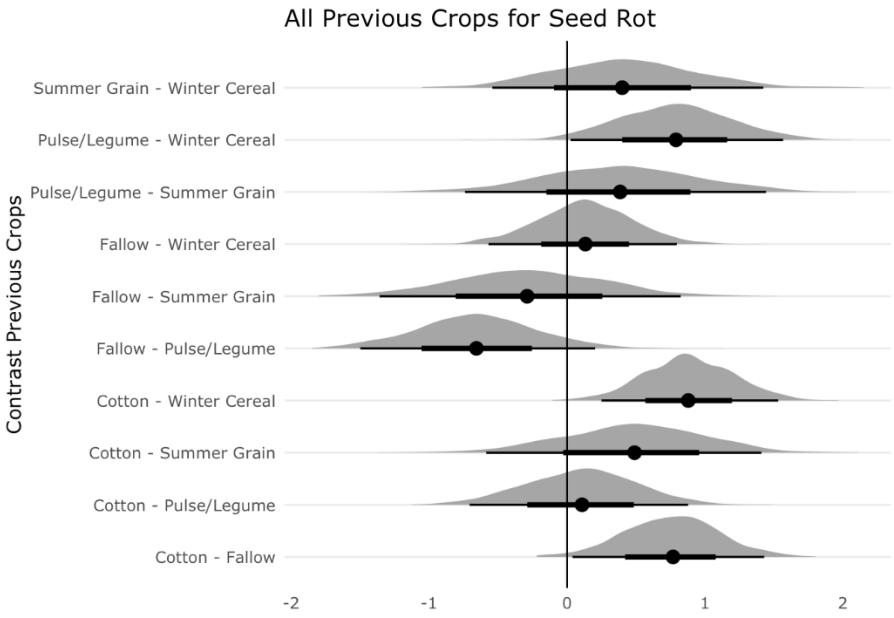



**Figure 100.** Pairwise comparison of previous crops for effect on Seed rot incidence

**Boll Rot**

There was a significant effect of summer grains as a previous crop on the incidence of Boll rot (Table 31).

Boll rot was significantly higher in summer grains than cotton but no different in other crops (Figures 101 - 103). However it is important to note that there was an outlier from sorghum that is likely to be causing this effect, and therefore it may be spurious.

Boll Rot is known to be caused by several fungi and bacteria including Fusarium, Diplodia, and Alternaria species. Head blight and molds found infecting sorghum are caused by a variety of fungal pathogens. Head molds generally refer to fungi that mold the grains as they mature on the seed head. *Fusarium moniliforme*, *Fusarium semitectum*, *Curvularia lunata*, *Phoma sorghina*, *Helminthosporium* spp. and *Alternaria* spp. are generally considered to be head molds. Therefore, sorghum may not be an outlier in the analyses and may be building up fungi in the soil that cause head mould of sorghum as well as boll rots of cotton. This effect of sorghum grown prior to cotton, on subsequent incidence of boll rots requires further investigation.

**Table 31.** Summary table of analyses for effect of previous crop on the incidence (%) of Boll rot

**NOTE** In this Boll Rot variable, all boll rots recorded by Qld have been combined into a single value to align with the “Boll Rot” reported by NSW.

```
#
##  Iterations = 3001:12991
##  Thinning interval  = 10
##  Sample size  = 1000
##
##  DIC: 82.72583
##
##  G-structure:  ~uid
##
##     post.mean l-95% CI u-95% CI eff.samp
## uid      5.79 0.007328    7.976    28.59
##
##  R-structure:  ~units
##
##       post.mean l-95% CI u-95% CI eff.samp
## units     1.233 0.001371    6.272    26.95
##
```

```
##  Location effects: BR ~ Previous_crop
##
##                            post.mean l-95% CI u-95% CI eff.samp  pMCMC
## (Intercept)                   1.1213   0.3988   1.7237     1137 <0.001 ***
## Previous_cropFallow          -0.8801  -1.9283   0.1390     1000  0.092 .
## Previous_cropPulse/Legume    -0.2659  -1.4721   1.0112     1000  0.676
## Previous_cropSummer Grain     1.5682   0.1866   3.0206     1000  0.036 *
## Previous_cropWinter Cereal   -0.2715  -1.2094   0.7201     1000  0.580
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

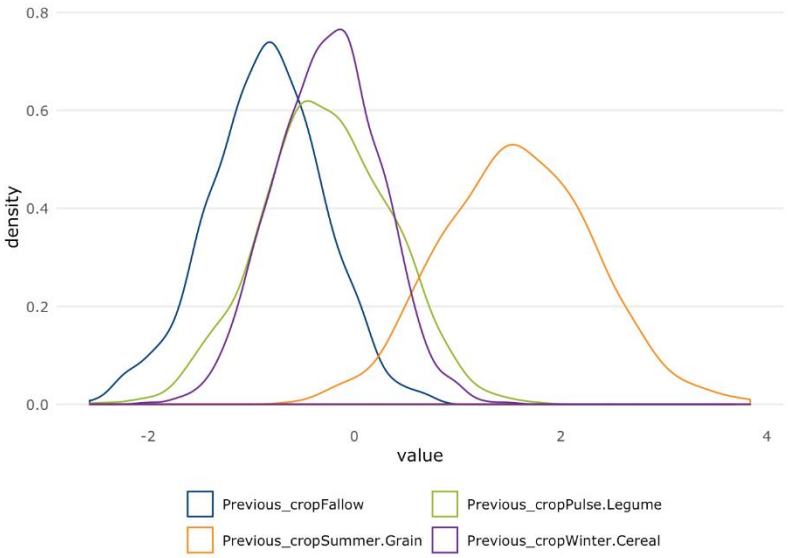



**Figure 101.** Density plots showing the difference between the previous crops tested in the model for Boll rot

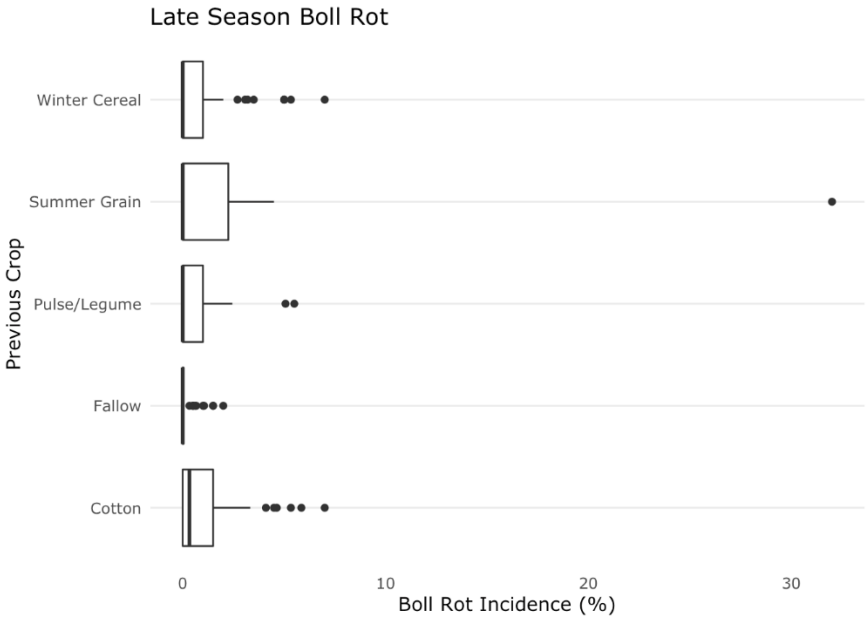



**Figure 102.** Boxplots showing the raw data for Boll rot incidence for each field grouped by previous crop Note: Lower = to the left, higher = to the right, of 0.

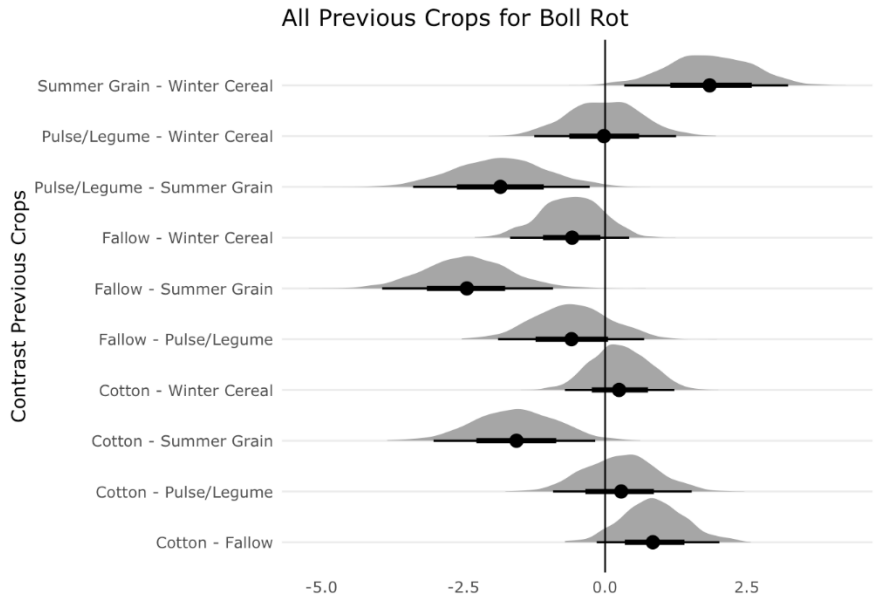



**Figure 103.** Pairwise comparison of previous crops for effect on Boll rot incidence

**Tight Lock**

There was a significant effect of winter cereals and fallow as a previous crop on the incidence (%) of Tight-lock (Table 32). Tight-lock was significantly lower following fallow and winter cereals than cotton but no different with other previous crops (Figures 104 - 106). Tight-lock was identified in this project as having the strongest negative relationship with yield (Figure 74). The principal economic effect is that the boll's lint is unharvestable by mechanical cotton pickers. This disease is endemic to the Southeastern US and can cause severe yield losses up to 70% in some fields (Srivastava et al., 2010). When conditions are conducive for tight-lock development, cotton crops in CQ can be significantly affected with potential yield loss. The relationship between previous crop and tight-lock in cotton requires further investigation as a potential management strategy.

Srivastava, P., Mailhot, D.J., Leite, B. et al. Curr Microbiol (2010) 61: 79. https://doi.org/10.1007/s00284-009-9578-5

**Table 32.** Summary table of analyses for effect of previous crop on the incidence (%) of Tight-lock

```
##
##  Iterations = 3001:12991
##  Thinning interval  = 10
##  Sample size  = 1000
##
##  DIC: -106.8498
##
##  G-structure:  ~uid
##
##     post.mean l-95% CI u-95% CI eff.samp
## uid     4.777    3.613    6.078    195.3
##
##  R-structure:  ~units
##
##       post.mean l-95% CI u-95% CI eff.samp
## units    0.1344 0.001984   0.6339    60.98
##
##  Location effects: TL ~ Previous_crop
##
##                             post.mean l-95% CI u-95% CI eff.samp  pMCMC
## (Intercept)                   1.63940  1.05097  2.28696     1000 <0.001 ***
## Previous_cropFallow          -1.17978 -2.13881 -0.16354     1000  0.022 *
## Previous_cropPulse/Legume     0.09701 -1.10514  1.19349     1000  0.872
## Previous_cropSummer Grain    -0.36621 -1.86773  1.12087     1000  0.662
## Previous_cropWinter Cereal   -1.07030 -1.95816 -0.18040     1000  0.020 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

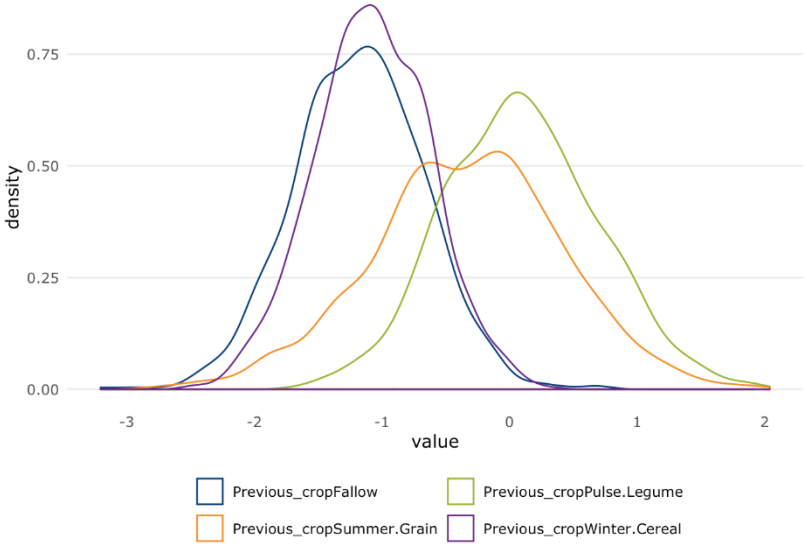



**Figure 104.** Density plots showing the difference between the previous crops tested in the model for Tight-lock

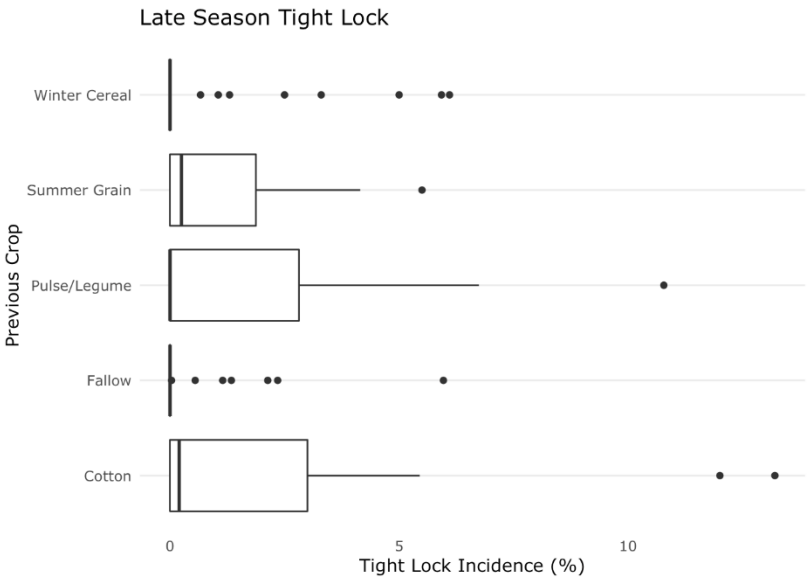



**Figure 105.** Boxplots showing the raw data for Tight-lock incidence for each field grouped by previous crop Note: Lower = to the left, higher = to the right, of 0.

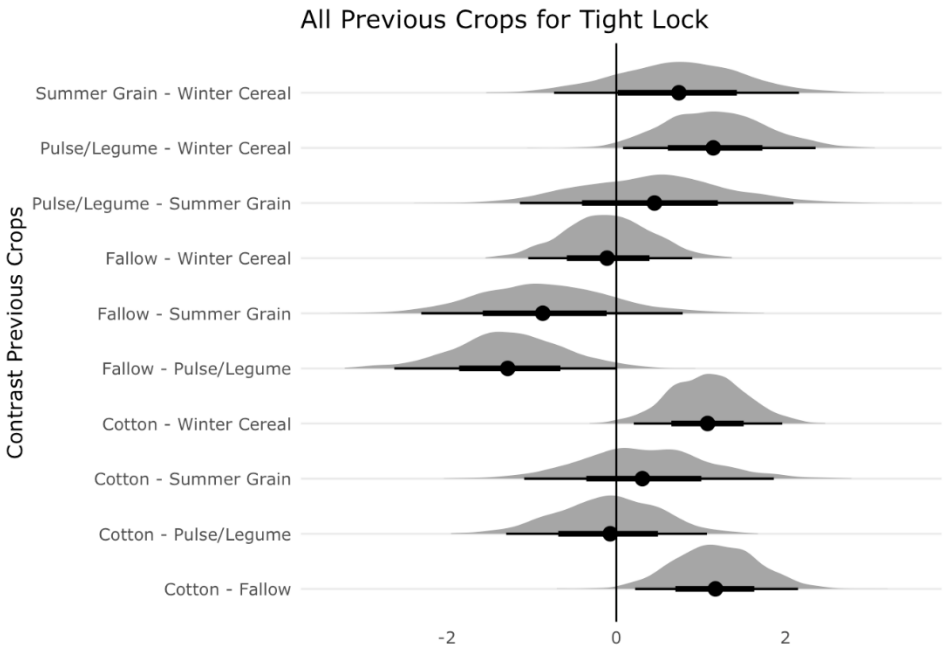



**Figure 106.** Pairwise comparison of previous crops for effect on Tight-lock incidence

**Bunchy Top**

There was no significant effects of previous crop on the incidence (%) of Bunchy top (Table 33).

**Table 33.** Summary table of analyses for effect of previous crop on the incidence (%) of Bunchy top

```
##
##  Iterations = 3001:12991
##  Thinning interval  = 10
##  Sample size  = 1000
##
##  DIC: -132.8193
##
##  G-structure:  ~uid
##
##     post.mean l-95% CI u-95% CI eff.samp
## uid     57.55     45.5    72.74    786.3
##
##  R-structure:  ~units
##
##       post.mean l-95% CI u-95% CI eff.samp
## units    0.4214 0.001573    2.433    41.95
##
##  Location effects: BT ~ Previous_crop
##
##                             post.mean l-95% CI u-95% CI eff.samp pMCMC
## (Intercept)                    0.9046  -1.4386   3.0229   1008.4 0.448
## Previous_cropFallow            2.4184  -0.7200   6.1776   1000.0 0.162
## Previous_cropPulse/Legume     -0.8384  -5.0751   3.2776   1000.0 0.690
## Previous_cropSummer Grain      0.1252  -5.2778   5.0955    909.1 0.980
## Previous_cropWinter Cereal    -0.8467  -4.1376   2.2568   1000.0 0.568
```

**TSV**

There was no significant effects of previous crop on the incidence (%) of TSV (Table 34).

**Table 34.** Summary table of analyses for effect of previous crop on the incidence (%) of TSV

```
NOTE TSV is only recorded in NSW.

##
##  Iterations = 3001:12991
##  Thinning interval  = 10
##  Sample size  = 1000
##
##  DIC: -573.3028
##
##  G-structure:  ~uid
##
##     post.mean l-95% CI  u-95% CI eff.samp
## uid 3.137e-06 5.62e-13 1.192e-05     1000
##
##  R-structure:  ~units
##
##       post.mean  l-95% CI u-95% CI eff.samp
## units  0.000246 0.0001742 0.000327    778.4
##
##  Location effects: TSV ~ Previous_crop
##
##                              post.mean   l-95% CI   u-95% CI eff.samp pMCMC
## (Intercept)                 -0.0002372 -0.0067543  0.0066652     1000 0.952
## Previous_cropFallow          0.0001259 -0.0108416  0.0100185     1000 0.972
## Previous_cropPulse/Legume    0.0001166 -0.0103336  0.0114027     1166 0.956
## Previous_cropSummer Grain    0.0004114 -0.0142417  0.0161446     1271 0.986
## Previous_cropWinter Cereal   0.0001910 -0.0081789  0.0087545     1101 0.970
```

Yield

Yields were sparsely reported, more from NSW (Figure 107) than Qld (Figure 108) with higher yields being reported for NSW and the lowest yields per area overall in Brookstead and Condamine Plains in Queensland. Low yields in these areas were due to drought forcing cotton to be grown as a dryland or part dryland crop.

Despite yields being sparsely reported, as mentioned earlier, network analyses determined that Tight-lock had the strongest negative relationship with yield in the data set (Figure 74).

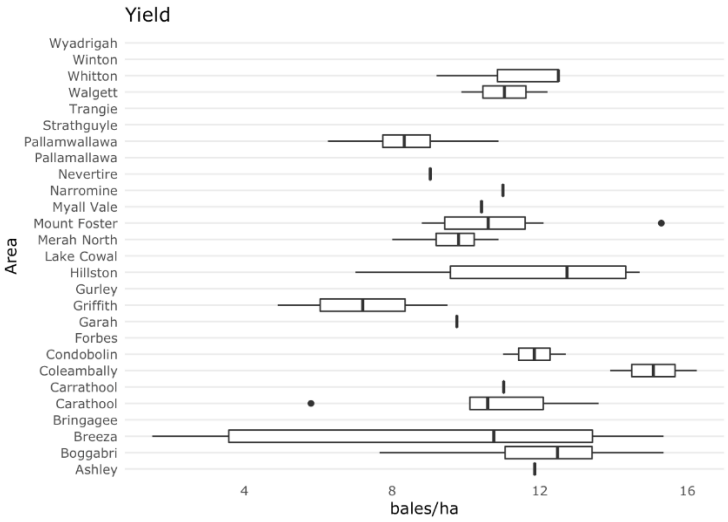



**Figure 107.** Cotton yields recorded for fields in NSW

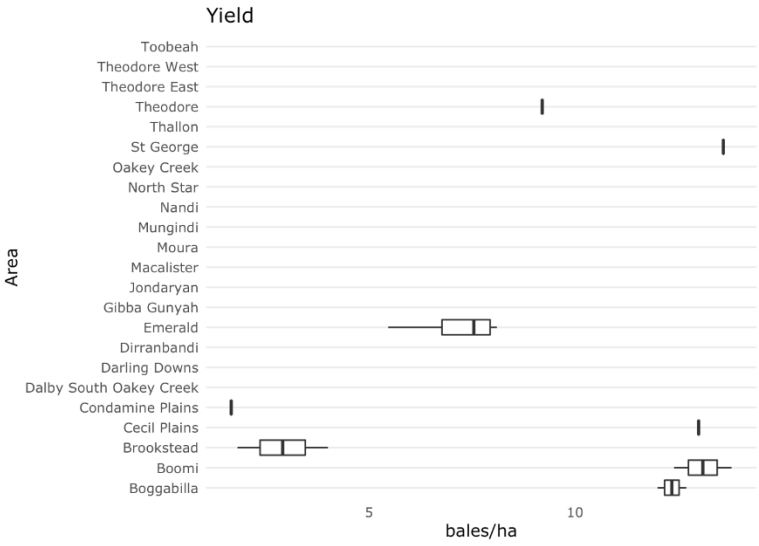



**Figure 108.** Cotton yields recorded for fields surveyed in Qld and Border Rivers

**Summary of Findings**

Analyses were performed to test for any effect of previous crop and cotton trash present on early and late season diseases. Previous crops were binned into broad categories to help with small sample sizes and cotton was used as the baseline previous crop.

Further correlation network analyses were performed on the data to identify relationships between diseases and yield and diseases themselves in the whole data set and in each state.

**Significant Findings**

- Verticillium wilt was significantly higher in winter cereal crops than cotton but no different in other previous crops.
- Seed rot was significantly lower in fallow and winter cereals than cotton but no different in other previous crops.
- Boll rot[1] was significantly higher in summer grains[2] than cotton but no different in other crops.
- Tight-lock was significantly lower in fallow and winter cereals than cotton but no different in other previous crops.
- Tight-lock had the strongest negative relationship with yield in the data set.
- Early season Alternaria[3] had a strong correlation with early season Black root rot.
- Clusters of positively associated diseases in the entire data set included early Alternaria and Black root rot
- Clusters in the Queensland only data showed weaker relationships
    - Early season Fusarium wilt and late season Fusarium wilt being clustered and positively correlated.
    - Tight-lock, Boll rot and Seed rot were clustered with weakly positive correlations
    - Early season Alternaria and late season Verticillium wilt clustered with negative correlations to tight-lock and boll rot
- Clusters in New South Wales showed some stronger correlations than Queensland
    - Early Alternaria clustered more closely with Black root rot than in Queensland.

**Non-significant Findings**

The amount of cotton trash present in the field did not have any significantly detectable effect on disease in the analyses performed on these data.

1. This includes all individual types of boll rot from Queensland to align with the category reported by NSW↩
2. Note that there is an outlier from sorghum that likely is causing this effect, it may be spurious↩

3. NSW only↩

**Recommendations for future analyses (Adam Sparks)**

The data are well structured and fairly easy to use but some changes should be made going forward.

- Every effort should be made to collect complete data. For example, there are many missing values for previous crop, which hinders the analysis due to missing data.
- Unique identifiers for each field should be created and assigned to allow for linking fields between seasons and early or late observations. These identifiers do not have to enable the identification of the field per se, but rather just be unique in the data set and allow for easy identification.
- Care should be taken to ensure that the data are entered in a standardised format. Using a Fulcrum form rather than handwritten notes is one way to ensure data integrity and formatting.
    - Examples include values entered in columns that should be only numeric, *e.g.* "Black Root Rot", which was to be a percentage value that contained values such as "Moderate" or "High" rather than actual numeric values. These values had to be re-encoded as numeric or in some cases dropped altogether due to lack of information about the actual value.
    - The yield column was mainly bales per hectare, but also contained some that were encoded as bales per acre (including the text). This should be reported only as one value in numeric format only.
    - Seed rate information was recorded in different rates, some had to be completely removed due to differences in reporting and text in the column.
- Standardisation of data collected should be undertaken. This leads to incompatibility weakening the analyses that could be performed.
- Information on fertilisation practices, configuration, volunteers, irrigation, row spacing, etc. should be collected in a standardised form to allow for analysis. Currently the irrigation and configuration are somewhat standardised, but still free-form and fertilisation practices are free-form. Some basic set of standard values should be agreed upon for use. The row spacing is sometimes referred to as 1m other times 40" or even 40'' in the data.
- Previous crop values should be standardised rather than free-form.
- Any text entry values should not only standardise the values, but capitalisation, spaces and spelling, *e.g.* cotton variety. Some varieties are reported with spaces, others without. Previous crops are reported some capitalised, others not. In some cases, the area names were even misspelled.
- The data were formatted in several spreadsheets, some with separate tabs. This is understandable but for analysis, two sheets would be preferable. One for early season and one for late season with data from both states (collected in a standardised format) would be preferable. I created two workbooks with two sheets each and combined the data in R for the analysis.
- Missing values should be reported as NA not left blank, it is unclear if the intention is to have a missing value that was not recorded, lost, could not be recorded or simply a "0" value that wasn't entered as "0" in the data.

The above-mentioned recommendations can easily be addressed to improve workability and accuracy of future analyses.

**Future Analyses**

Much further work can be done with these data including but not limited to:

- Analysing the effects of weather provided by BOM from the nearest station for each field
- Cluster analysis to determine clusters of areas acting in a similar or dissimilar fashion
- Analyse effects of the following on disease:
    - Variety
    - Seed rate
    - Configuration
    - Irrigation
    - Fertilisation regime

- Soil type

In summary, these findings provide direction for research to investigate cropping rotations that potentially will decrease/increase disease incidence of important diseases of cotton. The groundwork achieved in this project has provided the foundation knowledge and critical directions to improve the collection and storage of data, and to build on the analyses already conducted.

### 1.7 Survey team (QDAF, NSW DPI, and CottonInfo) meet quarterly.

A Cotton Pathology Survey Project Meeting was held on the 23rd January 2017 via tele-conference to discuss how early season surveys progressed, and to address any concerns that participants may have regarding the process. A summary of comments from CottonInfo Regional Officers was provided by Annabel Twine.

An Informal fortnightly disease catch up meeting was initiated on the 1 September 2019 at the start of the new CRDC funded project. The aim of this meeting is to 1. Keep all team members informed of what is happening across the regions in regards to disease, 2. Provide an opportunity for each of us to briefly update the team on what we are currently working on, and 3. What we are planning to work on next. This fortnightly meet provides a forum to not only discuss project work, but also discuss any issues we are having, to seek advice, and to learn from each other as well as flag emerging industry issues and provide a forum for collaboration. Gupta Vadakattu organised videoconferencing for these meetings, which was much more engaging than just talking on the phone. Participants include Susan Maas/Elle Storier, Gupta Vadakattu, Duy Le, Aphrika Gregson, Tim Green, Alison Young, Linda Scheikowski, Dinesh Kafle and Linda Smith.

### 1.8 Develop a sample diagnostic flow chart/chain of custody procedures for all diseases and virus samples that are either collected during surveys or submitted for diagnostics.

**General principles for enquiries and diagnostics**
Detailed process (Who, where, how, when, and data/sample storage/management)

Enquiry
1. Point of call - CottonInfo or state pathologist;
2. Take a photo and send to pathologist, and fill form during enquiry;
3. CottonInfo has bags for samples with clear procedure for Cottoninfo on how to send samples

Diagnostic results reported back to pathologist for state where sample came from, who will then inform whoever sent the sample (CottonInfo if they sent the sample, or direct to grower/consultant). Relevant state Pathologist to enter on database.

Process for sample collection and diagnosis
The process initially developed for sample collection and where to send for pathogen confirmation is detailed in Table 35. All samples from NSW are to be sent to EMAI for processing. All samples from QLD to be sent to ESP. However, after Dr Duy Le was employed by NSW DPI diagnostics for all pathogens, except VCG of the Fusarium wilt pathogen, was undertaken at ACRI in NSW.

**Table 35.** Process initially developed for sample collection and where to send for pathogen confirmation

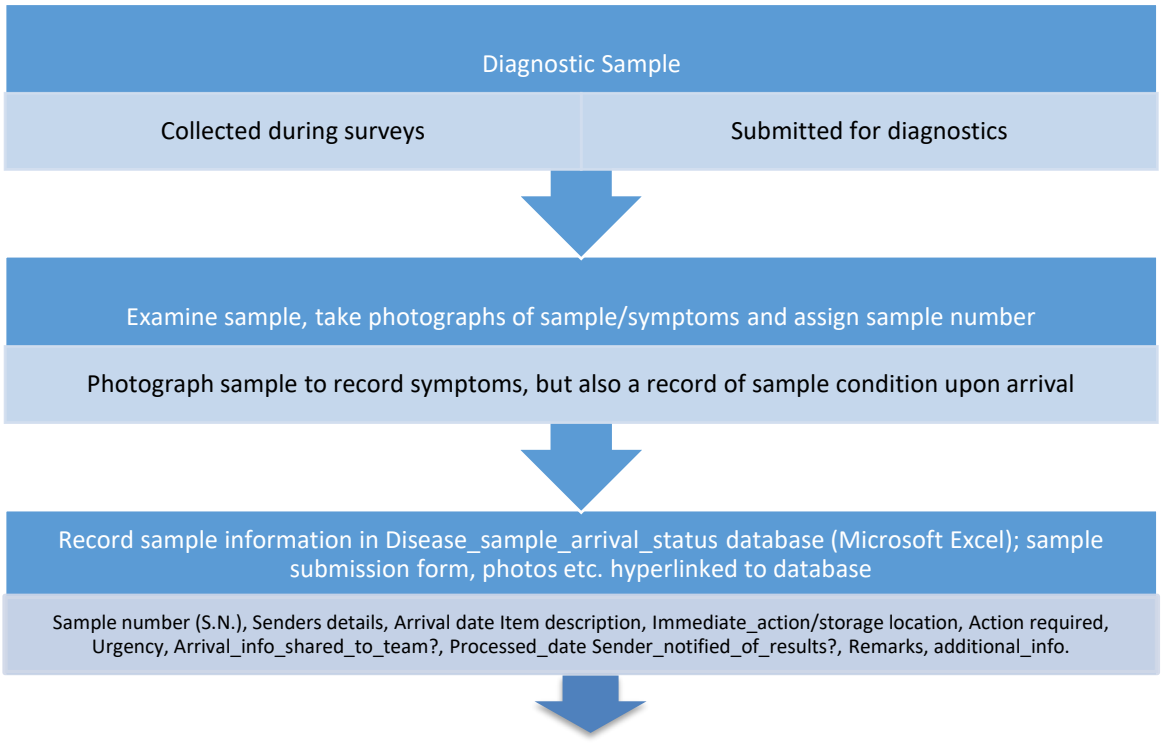
| Disease/issue | How to sample | How to send | Where to send first | Where next? | Storage of sample |
|---|---|---|---|---|---|
| Verticillium | Close to ground; Stem only Stored in fridge prior to sending | In paper NOT PLASTIC Send express | NSW – send to EMAI Qld – send to ESP | | NSW – using in project QLD – Provide isolates to NSW |
| Fusarium | Close to ground; Stem only Stored in fridge prior to sending | In paper NOT PLASTIC Send express | NSW – send to EMAI Qld – send to ESP | NSW - Isolated on PDA Novo and sent to ESP | Qld - Extract DNA and keep representative samples |

| | | | | | |
|---|---|---|---|---|---|
| Black Root rot | Dig sample out – DON'T PULL IT OUT<br>Stored in fridge prior to sending | In paper<br>NOT PLASTIC<br>Send express | NSW – EMAI<br>Qld – pathologist | | No sample kept |
| Seedling diseases/ Unknowns seedling vigour | Dig sample out – DON'T ULL IT OUT<br>Stored in fridge prior to sending | In paper<br>NOT PLASTIC<br>Send express | NSW – EMAI<br>Qld – pathologist | | |
| Virus | Plant tissue with symptoms<br>Stored in fridge prior to sending | Zip lock back;<br>Send express | NSW – EMAI then send to Murray Sharman<br>Qld - Send to Murray | | |
| Bacterial blight | Plant tissue | In paper<br>NOT PLASTIC<br>Send express | NSW – EMAI<br>QLD – ESP | All samples sent to Toni Chapman | |
| Other - pathology | Sent as per discussion with state pathologist | As per discussion | NSW – send to EMAI<br>Qld – send to ESP | | |
| Other – not pathology | Sent as per discussion with state pathologist | As per discussion | NSW – send to EMAI<br>Qld – send to ESP | | |
| Exotic | **CALL 1800 084 881** | | | | |

Chain of custody

- NSW DPI have automatic chain of custody process
- DAF have a lab book where details of samples are documented and database recording system detailed in Figure 109.

A diagnostic flowchart was developed for fungal diseases and viruses collected during surveys or submitted for diagnostics at ESP (Figure 109).



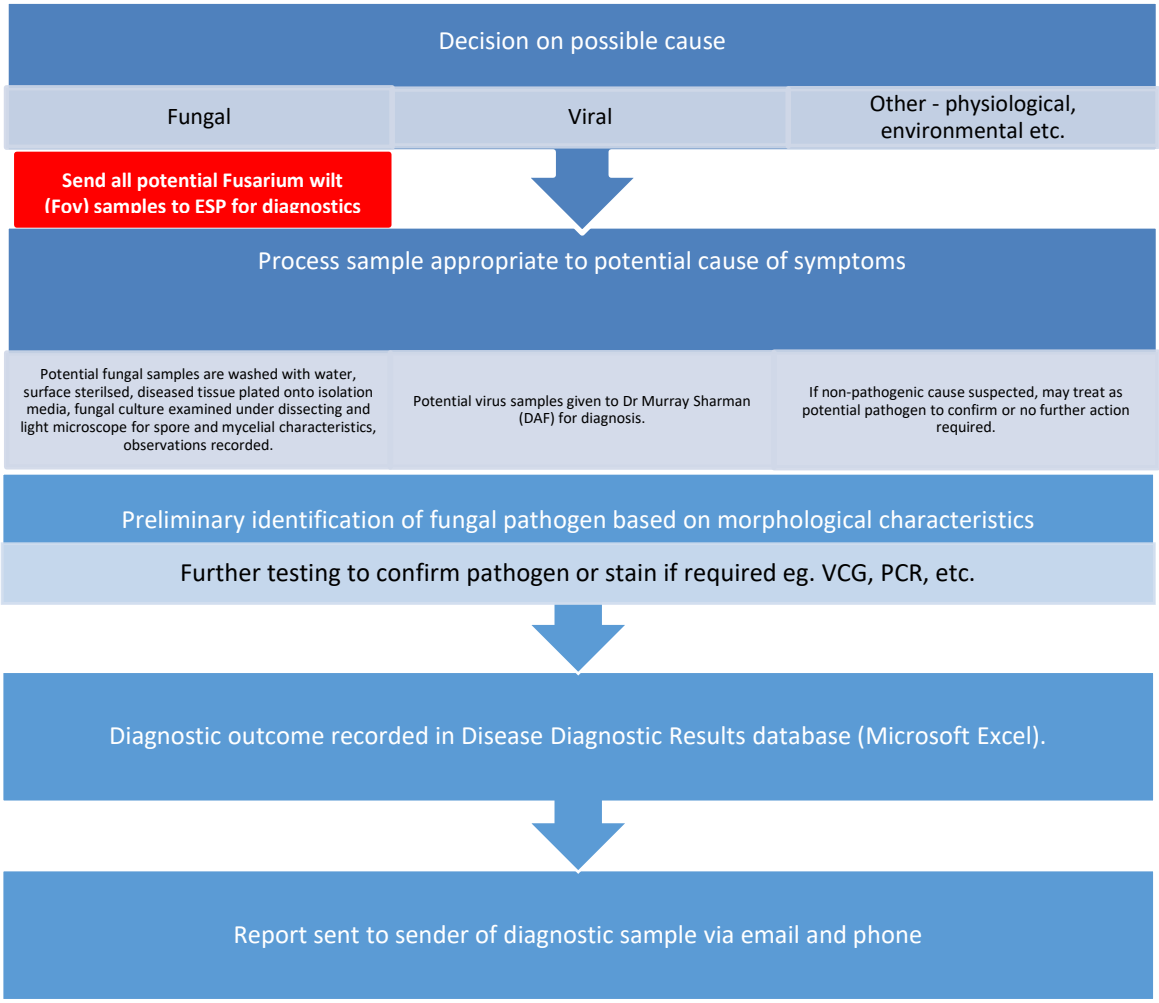



**Figure 109.** Diagnostic flowchart for fungal diseases and viruses collected during surveys or submitted for diagnostics at ESP

## 1.9 Process diagnostic cotton samples (including samples collected during surveys), isolate pathogen and conduct VCG and/or PCR to characterise pathogen.

**Summary of disease diagnostics at ACRI (Duy Le)**

In the 2017/18 season, a total 79 cotyledon and seedling samples and 56 mature cotton samples were collected by NSW DPI or received at the Australian Cotton Research Institute (ACRI) for diagnostics. A total of 361 and 359 fungal isolates were recovered during early and late season disease surveillance across NSW, respectively (Table 36).

In the 2018/19 season, a total of 82 seedling samples and 44 mature cotton samples were processed, and in total 185 and 452 fungal isolates were recovered during early and late season disease surveillance across NSW, respectively (Table 36).

**Table 36.** Number of samples processed and pathogens recovered during season 2017/18 and 2018/19

| Seasons | Samples | | Pathogens[1] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Collected (NSW DPI) | Received | BR | Rhi | Py | Alt | Fu | Vert |
| Early 17/18 | 65 | 14 | 84 | 46 | 22 | 86 | 123 | - |
| Late 17/18 | 38 | 18 | - | - | - | 126 | 53 | 180 |
| Early 18/19 | 64 | 18 | 36 | 9 | - | 62 | 78 | - |
| Late 18/19 | 40 | 4 | - | - | - | 234 | 106 | 112 |

[1] No. of isolates recovered of the following pathogens:
BR *Berkeleyomyces rouxiae* Rhi *Rhizoctonia* spp. Py *Pythium* spp; Alt *Alternaria* spp.; Fu *Fusarium* spp.; Vert *Vertcillium dahliae*

A total of 120 *Berkeleyomyces rouxiae* (=*Thielaviopsis basicola*) isolates, causal agent of BRR, were collected from early season surveys across cotton farms in NSW in the last two seasons indicating this pathogen remains pandemic.

Alternaria leaf spot was present across the entirety of the cotton-growing season allowing collection of isolates (Alt) at both sampling times (Table 36). Molecular sequence data indicated that *Alternaria alternata* accounted for around 85% of the pathogen population. No *A. macrospora* was detected within this population. A number of *Alternaria* isolates could represent a novel species for Australia and the world; and this characterization work is still under way.

A total of 188 putative *Fusarium* isolates were recovered from cotton seedlings exhibiting atypical symptoms of either Fusarium damping-off or Fusarium wilt, which were sampled across NSW during the 2017/18 and 2018/19 seasons. Based on sequence analysis of the translation elongation factor 1-alpha (TEF1), eight *Fusarium* species were identified, that being *Fusarium oxysporum*, *F. equesiti* and *F. falciforme* species complex, *F. nygamai, F. brachygibbosum*, *F. acuminatum, F. chlamydosporum*, and *F. redolens*. Of these, *F. oxysporum* species complex (FOSC) accounted for over 80% of the total number of isolates recovered. The predominant number of isolates (78.5%) were clustered with non-Australian Fov race 1-8. The remaining 21.5% of FOSC isolates in our study was clustered with Australian Fov biotypes. Further characterization of these FOSC isolates is required.

Most of the *Fusarium* isolates recovered during the late season survey in 2017/18 and 2018/19 seasons clustered well with Australian Fov biotypes based on the sequence data retrieved from the TEF1 region. Most of the isolates from 2017/18 season were further identified as VCG 01111, except for some that did not pair with VCG testers. VCG determination for 2018/19 collection is still under way.

*Verticillium dahliae* isolates were solely recovered from mature cotton samples, which were further molecularly characterized into 27 defoliating (17.4%) and 153 non-defoliating pathotypes during the 2017/18 season. A lower number of *V. dahliae* isolates were recovered in the 2018/19 season (Table 36), but the percentage of defoliating pathotype isolates increased and accounted for 34% of the recovered population.

**DAF Vert patho-typing (2019)**
Late 2019 NSW DPI requested *V. dahliae* isolates from Qld surveys for further research and therefore assisted in characterising isolates detailed below.
A total of 115 isolates were grown for mycelia and DNA extraction. A total of 115 DNA extracts were subjected to duplex PCR amplification using specific primers. Six DNA extracts failed to amplify.
2019 isolates: total 65 = 39 defoliating + 26 non-defoliating
2018 isolates: total 52 = 6 defoliating + 38 non-defoliating

**DAF Diagnostics**
Samples were processed, and identification and distribution of results was in accordance with agreed procedures (2.1). Some Fusarium and Verticillium isolates are still pending. The documentation of samples arriving at ESP has been improved to ensure accurate documentation and processing in a timely manner.

**2. Research question: What is the host range of Vd, is Vd seed-borne in cotton, and what is the temperature sensitivity of strains?** (Prepared by Linda Scheikowski)

**2.1 DAF Qld to conduct glasshouse trials (Tor Street and ESP) to determine pathogenicity of different strains of Vd under different environmental conditions on commercial cultivars of selected rotation crops e.g. Chickpea, cereals, corn.**

**Field Trial "Getta Getta"**
Trial 1 – Cotton following one year of rotation crop treatments

Development of foliar symptoms and climatic data

The progress of external disease symptoms, in relation to climatic conditions, was followed in the 2016/17 season when cotton was planted following one year of the different rotation treatments. Disease expression was largely suppressed by the hot summer temperatures until mid-February (Figure 110). Minimal disease expression was noted on February 17th (4% symptomatic plants). Slightly more disease was evident on March 3rd (10% symptomatic plants) and then increased greatly by the end of March (68% of assessed plants expressed foliar symptoms). This increase in disease coincided with decreasing soil and air temperatures and a week of rainy, overcast weather mid-March. Verticillium is favoured by cooler and wetter conditions.

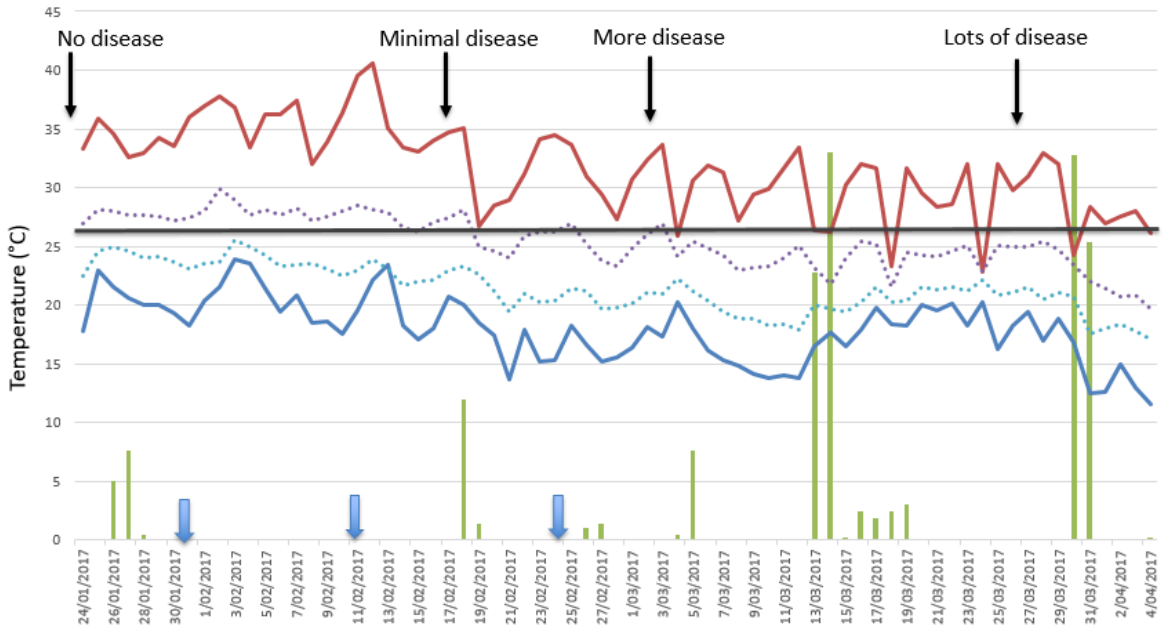



**Figure 110.** Climatic conditions (soil & air temperature and rainfall), and indications of foliar symptom development in Getta Getta trial 2016/17 season. (-max air temperature, -min air temperature, --max soil temperature, --min soil temperature, green bars are rainfall, blue arrows indicate irrigation event. Temperature and rainfall data was obtained from the CSD FastStart™ Fund Soil Temperature Network)

The average disease intensity graph (Figure 111) highlights that the incidence and severity of foliar symptoms was greatest in cotton following cotton compared to cotton following sorghum, corn or fallow when assessed late March.

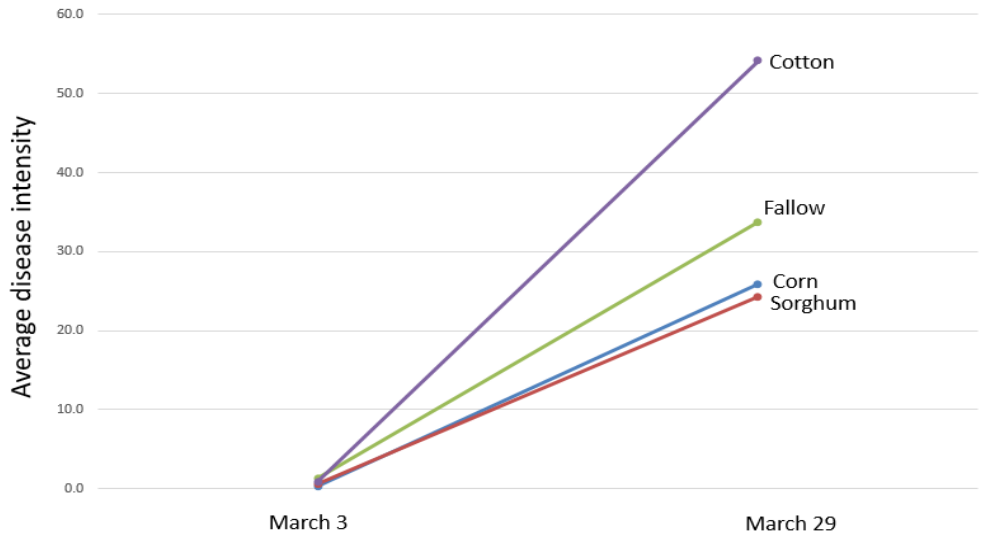



**Figure 111.** Average disease intensity between March 3 and March 29, 2017 in cotton following one year of rotation to corn, sorghum, fallow or cotton (expressed as a % of the maximum possible disease)

This highlights that previous rotation crop was having an effect on the expression and severity of disease. By the end of March, more plants growing in the cotton following cotton had developed Verticillium foliar symptoms and the leaf symptoms were more severe compared to cotton following fallow, corn or sorghum. This could be of greater significance in a season where cooler conditions, more conducive to disease development, begin earlier.

End of season disease incidence, measured through vascular brown discolouration of stems indicated that 81% of plants were infected in cotton following cotton, compared to 77% in cotton following fallow and 74% in cotton following both corn and sorghum. These were not significantly different (Figure 112) and suggest greater than one season of rotation is needed to lower disease levels when the soil population of *V. dahliae* is high. Pathogen isolation from assessed stems showed that VCG 2A was the prevalent strain (90% of all plants) in the trial area with a small (1%) of plants yielding both the 1A and 2A strain.

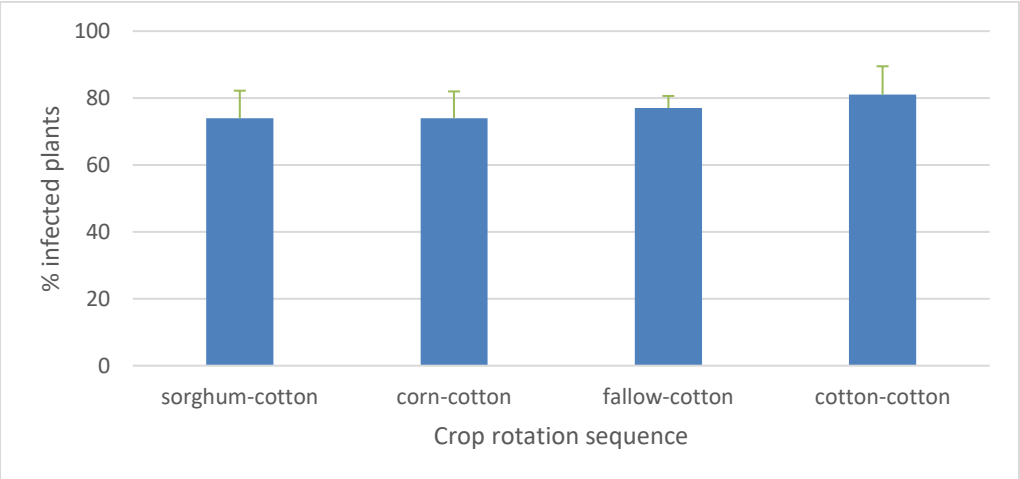



**Figure 112.** Trial 1- disease incidence in cotton following one year of rotation with corn, sorghum, cotton or bare fallow. (NB: not significantly different at $p<0.05$)

Being so hot for so long and only cooling down late in the season when Verticillium started to develop meant yield loss was also minimised during this season. Yield losses tend to be greatest when foliar symptom development occurs early, especially before opening of first flowers. Whilst individual

treatment yield was not obtained the entire trial area averaged 11.2b/ha which was quite good considering the end of season disease levels in the field. The level of inoculum in the large number of infected plants, however would be present to carry over into the following season.

There was a significantly greater number of average bolls per plant on diseased plants compared to non-diseased plants in all treatments except for where cotton followed fallow (Table 37). This is probably in relation to greater boll numbers adding additional stress to plants, increasing susceptibility to infection. There appeared to be no difference in the number of bolls/plant between the two strains (data not shown).

**Table 37.** Average boll numbers per plant in cotton following one year of rotation in plants with and without vascular browning, April 2017

| | Average number of bolls/plants (Diseased plants) | Average number of bolls/plants (Clean plants) |
|---|---|---|
| Sorghum-cotton | 14.8 a | 10.2 b |
| Corn-cotton | 14.0 a | 10.0 b |
| Cotton-cotton | 16.4 a | 10.6 b |
| Fallow-cotton | 12.9 a | 10.9 a |

The level of *V. dahliae* measured using the PreDicta-Pt test is shown in Figure 12 for the duration of the trial. The only significant difference occurred in the first year when the levels of *V. dahliae* measured post first cropping sequence were significantly higher where cotton was grown.

2017-2018 (Two years of crop rotation followed by cotton)
Cotton was assessed in the final year of this trial following two seasons of crop rotation treatments. The disease incidence in each treatment sequence as the season progressed is shown in Figure 114.

Disease was first noted in the continuous cotton plots on December 14th 2017 (Figure 113). These infected plants were largely stunted and expressing foliar symptoms, so disease did come in quite early with the continuous cotton treatment and likely is reflective of the increased inoculum in the continuous cotton.

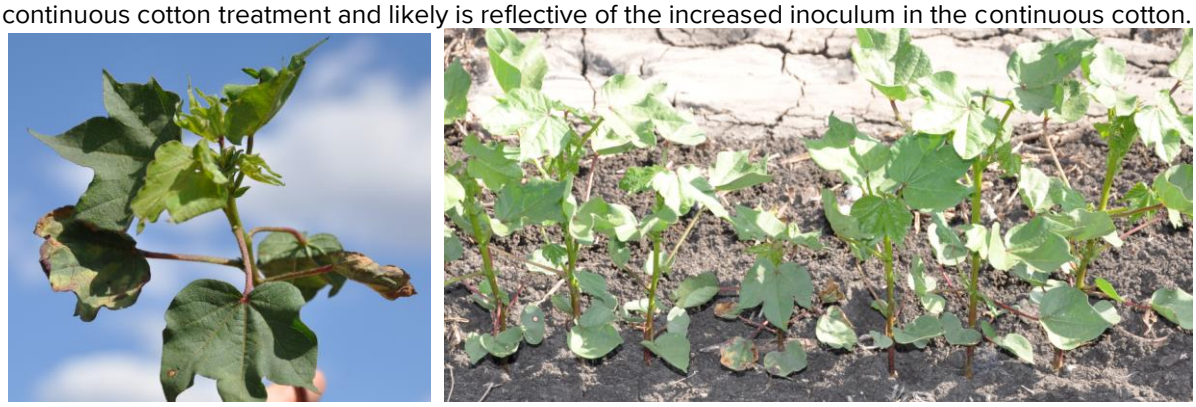


**Figure 113.** Stunted diseased cotton plants in the continuous cotton treatments on December 14 2017

At the first intensive assessment made on January 17th 2018 the continuous cotton treatment had at least twice as many diseased plants as the other treatments, and minimal (0.8%) infected plants were observed in the cotton following two years of fallow (Figure 114). As the season progressed this trend continued. There were significantly more diseased plants in assessments made from February until the end of the season (Figure 114). By the end of the season disease incidence was significantly higher at 96% in the continuous cotton compared to the other treatments. This study showed that the lower inoculum levels in the bare fallow and where there had been two years of non-host crops delayed disease development and resulted in less diseased plants at the end of the season.

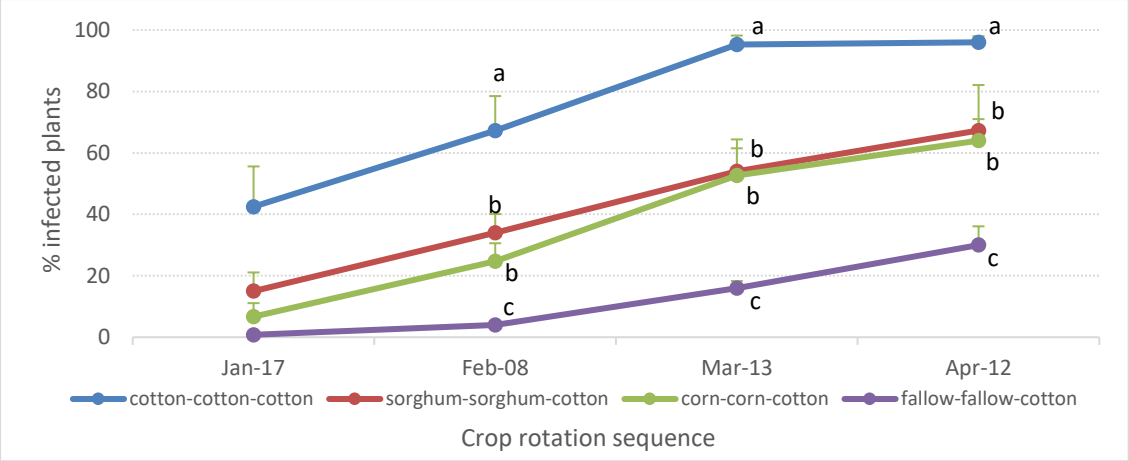



**Figure 114.** Trial 2- disease progress in cotton following two years of rotation with non-hosts, cotton or bare fallow during the latter part of the 2017/18 season (half the trial only from the head ditch end was assessed for all sampling dates for this study) (NB. Due to a planting error, replications of the sorghum-sorghum rotation in the 2015/16 -2016/17 season were actually corn-sorghum, fallow-sorghum or sorghum-sorghum: one replication of the corn-corn rotation was actually sorghum-corn) Treatments within the same sampling time followed by different letters are significantly different, $p<0.05$.

Disease assessments across the entire plot area at the end of the season showed three years of continuous cotton had significantly more disease than the other treatments (Table 38). Additional data where the severity of stem vascular browning was estimated highlighted that cotton had the greatest proportion of plants (70%) with severe vascular browning (>51-100% of stem discoloured) compared to 16%, 24% and 25% of plants in the previous fallow, sorghum and corn treatments respectively (data not shown). Yield measured was significantly greater in the corn-corn-cotton and fallow-fallow-cotton treatment compared to sorghum-sorghum-cotton and continuous cotton (Table 38).

**Table 38.** Effect of two years of crop rotation on yield and disease incidence of Verticillium wilt in the subsequent cotton crop at the end of the 2017-18 season

| Rotation sequence | Disease incidence (% plants with vascular browning)* | Yield (b/ha)[1] |
|---|---|---|
| Sorghum-sorghum[2] | 50 (0.7801) a | 9.87 a |
| Corn-corn[3] | 54 (0.8242) a | 11.53 b |
| Fallow-fallow | 27 (0.5436) a | 11.50 b |
| Cotton-cotton | 95 (1.3438) b | 9.63 a |

Values within a column followed by different letters are significantly different at $p<0.05$
*Values in brackets has been transformed for analysis using arcsine square root transformation
[1]Yield (b/ha) is based on 42% gin turnout
[2]1 rep of this treatment was fallow-sorghum & 1 rep was corn-sorghum
[3]1 rep of this treatment was sorghum-corn

While there was a similar trend to the previous season where average boll numbers were greater in diseased plants compared to clean plants, these differences in the 2017/18 season were not significantly different (Table 39). Targeted segmented picking and plant assessment was not performed which may have provided greater insights into disease effects on plant structure and individual boll weights. If infection occurs early in the season Verticillium wilt can reduce development of squares and bolls and fibre production. Fibre quality, such as length, strength and fineness of fibre produced by diseased plants may also be reduced, so these are additional production losses that could occur as well especially in early infected plants.

**Table 39.** Average boll numbers per plant in cotton following two years of rotation in diseased and non-diseased plants (as determined by vascular stem browning), April 2018 (results not significant, $p=0.05$**)**

| | Average number of bolls/plants (Diseased plants) | Average number of bolls/plants (Clean plants) |
|---|---|---|
| 2 x Sorghum-cotton | 16.28 | 13.54 |
| 2 x Corn-cotton | 13.41 | 9.79 |
| 2 x Cotton-cotton | 9.46 | 7.70 |
| 2 x Fallow-cotton | 10.27 | 9.98 |

Climatic data from late November for the 2017/18 season is shown in Figure 115. As noted in the previous season disease tends to increase the most in the later part of the season when plants are developing with fruit loads and soil temperatures in particular are decreasing.

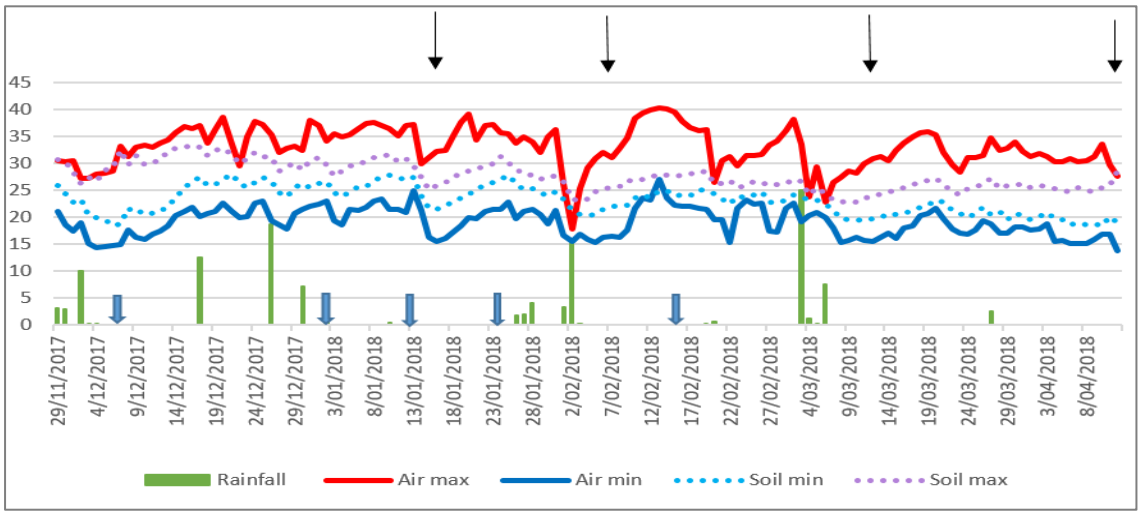
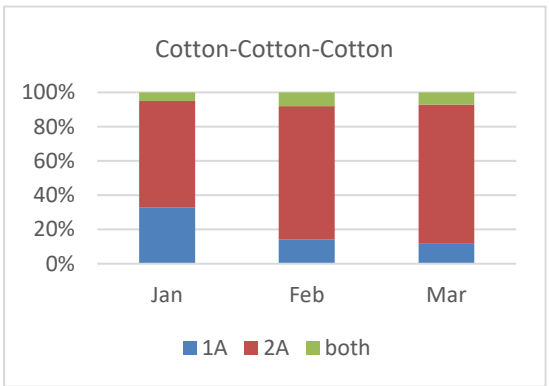
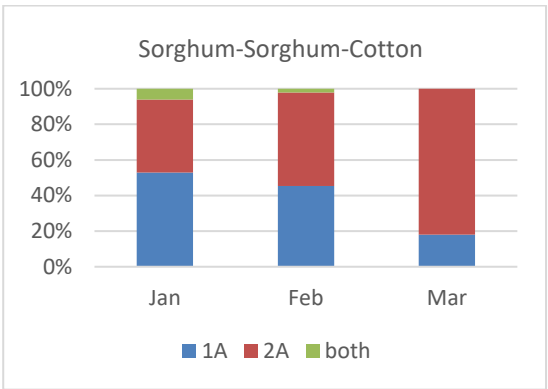



**Figure 115.** Climatic data for later part of 2017/18 season. Min and max soil temperature measured in furrow, air max and min and rainfall obtained from on-site farm weather station. Blue arrows are irrigation dates. Black arrows were plant assessment dates

Again during this season the extensive plating of plant stems to confirm pathogen infection, confirmed the majority of the Verticillium belonged to VCG 2A, with the presence of VCG 1A as well. Both VCG groups were isolated from a small number of plants. It was noted with the destructive sampling done in January, February and March 2018 that the proportion of isolates did change over time: the proportion of VCG 1A infected plants became less as the proportion of VCG 2A increased between January and March 2018. Also the proportion of VCG 1A was generally higher in the rotation treatments (corn, sorghum or fallow) compared to continuous cotton. Whether this is of significance, related to climatic conditions or the potential influence of different crops, is unclear but could warrant further monitoring if ever the occasion should arise.



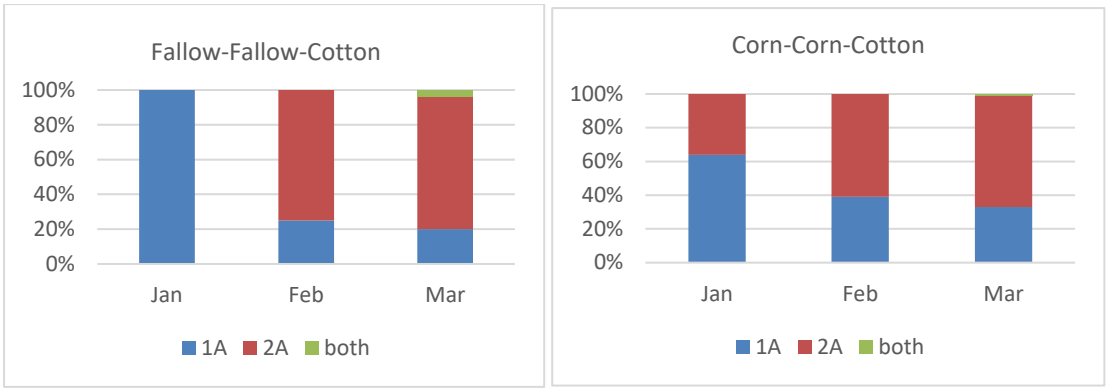



**Figure 116.** Proportion of VCG 1A, VCG2A or both strains isolated from cotton following two years of rotation to non-hosts or fallow when sampled mothly between January and March 2018 at "Getta Getta"

In Trial 2 (Figure 118), the higher quantity of *V. dahliae* DNA measured with the PreDicta-Pt test under continuous cotton correlated with the disease incidence observed in the field during the final season.

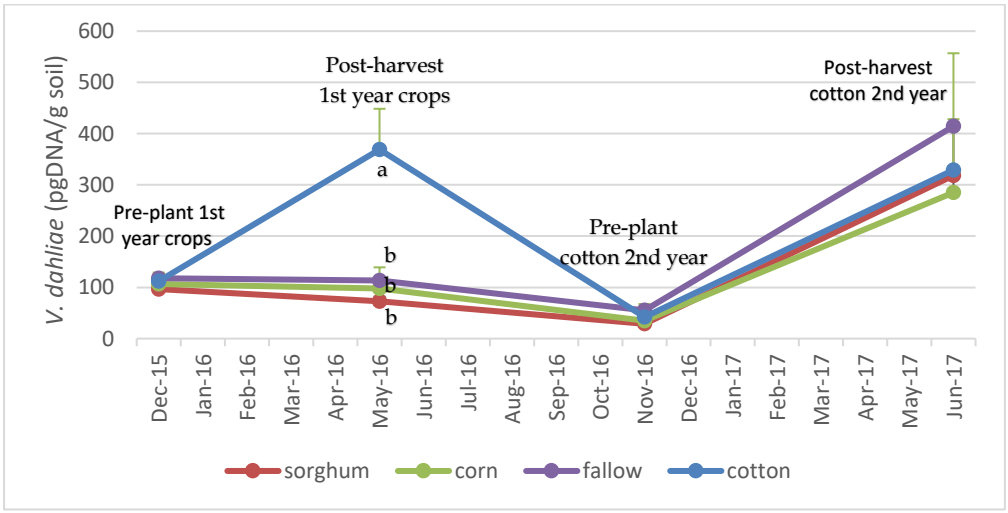



**Figure 117.** Trial 1 - quantity of *Verticillium dahliae* (pgDNA/g soil) measured during one year of crop sequences (2015-16) followed by cotton (2016/17) in a fully irrigated trial. Treatments within the same sampling time followed by different letters are significantly different, $p<0.05$.

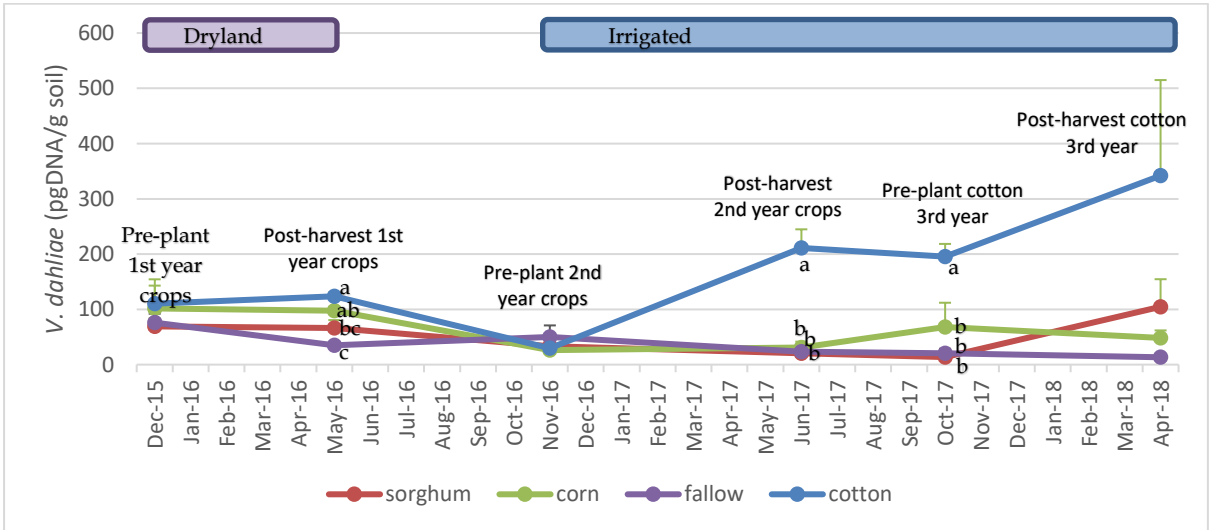



**Figure 118.** Trial 2 -quantity of *Verticillium dahliae* (pgDNA/g soil) measured during two seasons of rotation sequences (2015-17) followed by cotton (2017-18). December 2015 to May 2016 was dryland cropping and November 2016 to April 2018 was irrigated cropping. (NB. Due to a planting error,

replications of the sorghum-sorghum rotation in the 2015/16 -2016/17 season were actually corn-sorghum, fallow-sorghum or sorghum-sorghum: one replication of the corn-corn rotation was actually sorghum-corn) Treatments within the same sampling time followed by different letters are significantly different, $p<0.05$.

In the first year of the trial (2015/16) the quantity of measured *V. dahliae* DNA peaked after the first year of irrigated cotton (Figure 117) compared to where cotton was grown dryland (Figure 118) in the same season. This supported what was observed in the field (63% diseased plants in the dryland cotton compared to 71% in the irrigated trial) and concurs with findings from overseas studies which have shown that more frequently irrigations increase the incidence of disease. Drought stressed cotton typically suffers less infection by *Verticillium dahliae* than irrigated cotton and this is likely to be related to irrigation cooling the soil and enhancing pathogen survival and infection.

For research purposes the PreDicta-Pt test was a useful tool to monitor general trends in Verticillium levels following the different rotation management strategies, complementing the disease incidence data.

2018-2019 season
Due to drought conditions during the 2018/19 season the trial ran out of water and was not able to be completed. The forage sorghum treatment was unable to be incorporated. The cotton plants were very small and stunted and an estimation of vascular browning on February 28th 2019 was difficult due to the size and condition of the plants. An estimated 2.9% of plants examined along transects the length of the cotton plots appeared to have possible vascular browning but this was unable to be confirmed in the laboratory with isolations. Pre-plant and post-harvest soil levels of *V. dahliae* are shown in Table 40.

**Table 40.** *V. dahliae* (pgDNA/g soil) pre-plant and post-harvest during the drought season 2018/19

| | pre-plant Nov 12 18 | post-harvest May 22 19 |
|---|---|---|
| sorghum | 33 | 18 |
| forage sorghum | 42 | 15 |
| fallow | 11 | 9 |
| cotton | 48 | 31 |

**Soil Solarisation Trials**

**Soil solarisation, Mungindi (2017/18)**
Plastic on the hill tops had degraded by six weeks (Figure 119).

**Figure 119.** Degraded plastic in solarisation trial 11 January 2018

Figures 120 and 121 compare the maximum and minimum soil temperature at 10cm under two plastic replications compared to a control treatment. The slight differences in the maximum temperature between the two replicates shown is likely reflective of when the plastic would have blown off. Application of plastic did consistently increase maximum soil temperature for the duration of the experiment.

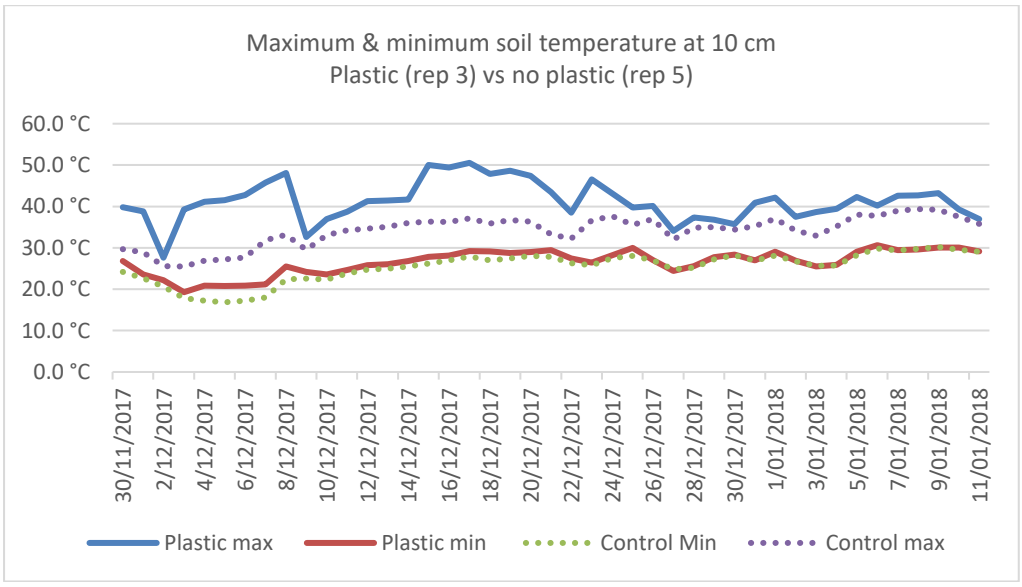



**Figure 120.** Maximum and minimum soil temperature at 10 cm under plastic (rep 3) and no plastic, November 30 2017 until January 11, 2018, Mungindi

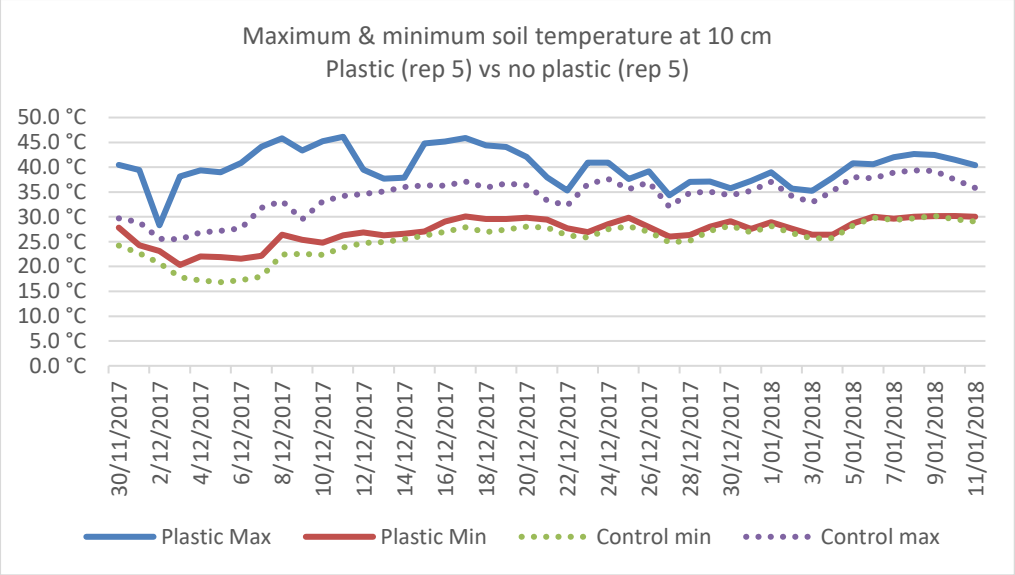



**Figure 121.** Maximum and minimum soil temperature at 10 cm under plastic (rep 5) and no plastic, November 30 2017 until January 11, 2018, Mungindi

Once the plastic was re-established on the hill the soil temperature was close to remaining above 35°C, the temperature believed to be required to have an effect on the soil population if consistently at that temperature for six weeks. Other overseas studies have also indicated that a constant soil temperature of 45°C for one to two hours killed more than 90% of the Verticillium population. This high temperature was achieved under plastic on several occasions during this study in the top 10 cm of soil as shown in Table 41 and 42. *V. dahliae* microsclerotia are multicellular and can produce many germ tubes upon germination. Soil heating at sublethal temperatures impairs the ability of *V. dahliae* to penetrate the plant and cause disease by weakening the microsclerotia through death of either individual cells or entire propagules. Additionally heating the soil may alter the population of surrounding soil organisms increasing the survival and activity of antagonistic microflora, which either inhibit the germination of microsclerotia or cause the death of *V. dahliae*. Death is also increased with soil moisture so being able to get adequate water into the profile before the plastic is applied is quite important. It has been found that beneficial mycorrhizal fungi have a higher thermal tolerance and survive the solarisation process.

**Table 41.** Time periods when soil temperature exceeded 45°C for at least I hour under plastic (rep 3)

| Date | Time | Duration | Av temp (°C) |
|---|---|---|---|
| 7-12-17 | 1:45-4:00 pm | 2 ¼ hrs | 45.5 |
| 8-12-17 | 12:30-3:00 pm | 2 ½ hrs | 47.0 |
| 15-12-17 | 12:00-5:30 pm | 5 ½ hrs | 48.0 |
| 16-12-17 | 12:15-5:30 pm | 5 ¼ hrs | 47.9 |
| 17-12-17 | 11:45-5:45 pm | 6 hrs | 48.6 |
| 18-12-17 | 12:45-5:00 pm | 4 ¼ hrs | 47.0 |
| 19-12-17 | 12:15-5:15 pm | 5 hrs | 47.5 |
| 20-12-17 | 12:30-4:45 pm | 4 ¼ hrs | 46.7 |
| 23-12-17 | 1:15-4:15 pm | 3 hrs | 46.0 |

**Table 42.** Time periods when soil temperature exceeded 45°C for at least I hour under plastic (rep 5)

| Date | Time | Duration | Av temp (°C) |
|---|---|---|---|
| 8-12-17 | 1:45-3:45 pm | 2 hrs | 45.5 |
| 10-12-17 | 3:00-4:00 pm | 1 hr | 45.7 |
| 11-12-17 | 2:00-4:45 pm | 2 ¾ hrs | 45.7 |
| 16-12-17 | 3:00-4:15 pm | 1 ¼ hrs | 45.1 |
| 17-12-17 | 2:00-4:45 pm | 2 ¾ hrs | 45.6 |

Glasshouse bioassay. At the completion of the glasshouse bioassay experiment all plants were essentially asymptomatic. No Verticillium was isolated. 0.85% *Fov* was isolated from the control treatment and 0.56% *Fov* from the plastic solarisation treatment. The amount of *V. dahliae* DNA measured pre and post solarisation was only small as well (Table 43). Given the low level of Verticillium present in the field and lack of Verticillium in the glasshouse bioassay it could not be concluded if solarisation helped reduce the population or not. The soil temperatures certainly indicated potential to kill or damage pathogen structures if present.

**Table 43.** Mean pgDNA *V. dahliae*/g soil from solarisation trial, Mungindi, measured before (28-11-19) and after (23-1-18) experiment (data not analysed due to differences in soil core depths measured)

| | Controls | Plastic |
|---|---|---|
| 0-10 cm pre- (28-11-17) | 6 | not assessed |
| 0-20 cm post- (23-1-18) | 3 | 2 |
| 20-40 cm post- (23-1-18) | 1 | 2 |

**Soil solarisation at "Getta Getta" (2018/19)**

In the "Getta Getta" trial, the plastic solarisation significantly reduced the quantity of *V. dahliae* measured using the Pre-Dicta Pt test compared to the control where there was no plastic (Table 44).

**Table 44.** *V. dahliae* pg DNA/g soil measured pre- and post- solarisation compared to the control

| Treatment | Vd pg DNA/g soil 29/11/2018 (pre-) | Vd pg DNA/g soil 19/3/2019 (post-) |
|---|---|---|
| Plastic solarisation | 63 a | 1 a |
| No plastic (control) | 66 a | 18 b |

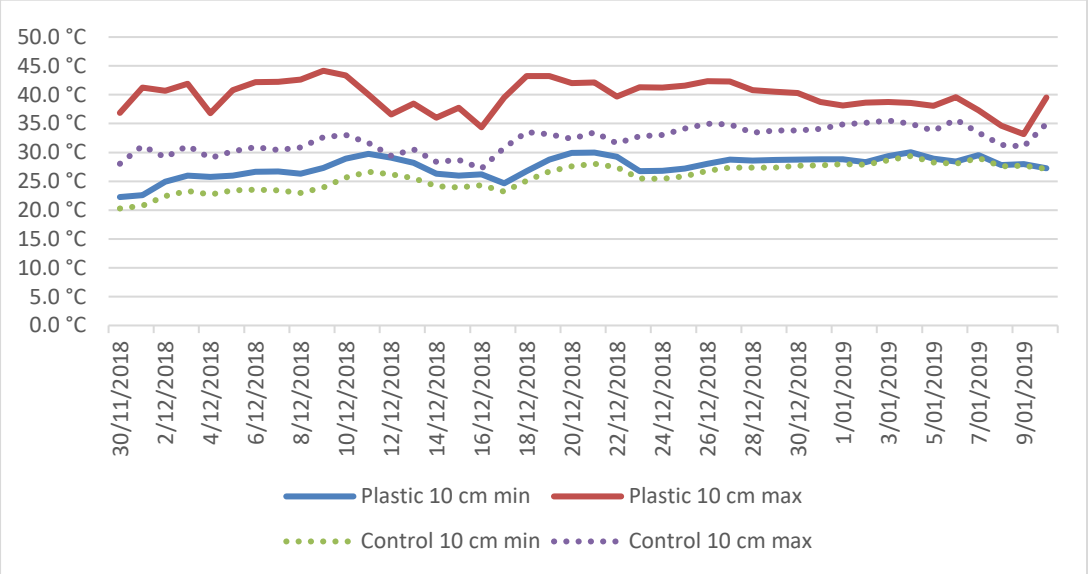



**Figure 122.** Maximum and minimum soil temperature at 10 cm under plastic and no plastic, November 30 2018 until January 10, 2018, "Getta Getta"

Solarisation did maintain maximum soil temperatures almost consistently above 35°C for the duration of the experiment. The average maximum temperature at 10 cm under solarisation was 39.8°C with a maximum of 44°C measured during the six week period (Figure 122). This compares to the average maximum temperature under the control being 32.2°C with an upper maximum of 35.7°C reached during the same period. This appears to have been adequate enough to cause a reduction in the amount of *V.dahliae* measured post solarisation (Table 44).

Heat was also increased at a depth of 20 cm under solarisation compared to the control with no plastic (Figure 123). The average maximum temperature at 20 cm under plastic was 34.1°C (and upper maximum of 37 °C) compared to an average maximum of 29.6°C (and upper maximum of 32.8°C) in the control. Whilst the soil was moist at the time the experiment was set up, it would be assumed that an even wetter

profile would enhance the likelihood of increased temperature at depth to aid in further death of the pathogen deeper in the profile.

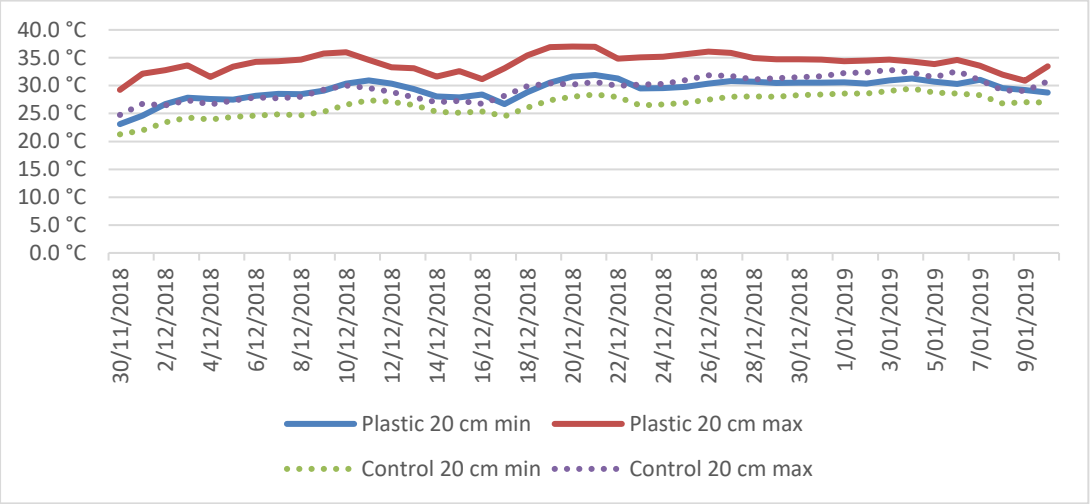



**Figure 123.** Maximum and minimum soil temperature at 20 cm under plastic and no plastic, November 30 2018 until January 10, 2018, "Getta Getta"

Glasshouse bioassay. The glasshouse pot bioassay was largely problematic with all plants exhibiting abnormally slow growth (perhaps due to some chemical applied across the fallow to control weeds in the field). When rating the stunted plants at twenty eight weeks no vascular browning was observed in any of the plants from the solarisation treatment. 7% of plants in the control treatment showed slight signs of vascular browning but *V. dahliae* was only isolated from one of these plants when plated onto isolation media.

Based on the field data the solarisation appears to have significantly reduced the measurable V. *dahliae* DNA in the soil which shows promise for treating small 'hot spots' of Verticillium if practical to do so. The effectiveness of soil solarisation can be influenced by numerous factors such as soil texture, soil temperature, soil moisture, rainfall and cloud cover. While solarising entire fields may not be practical there may be opportunities to target hot spots especially for early identified small areas. Services such as "Crown Analytical" who have the ability to map and identify the potentially worse Verticillium areas in a field could be used to identify such spot.

**Incorporation of forage sorghum and verticillium**

Disease incidence in the cotton plants assessed in the following cotton season showed no significant difference between where forage sorghum had been incorporated (93.5%) compared to where the millet had been grown and baled for hay (96%). Similarly the quantity of *V. dahliae* measured pre and post treatment (Table 141) was not significantly different between the two treatments.

**Table 41.** *V. dahliae* (pgDNA/g soil) measured pre-plant and post- treatment (growing millet vs incorporating forage sorghum (not significantly different, $p<0.05$)

| | Millet | Forage sorghum |
|---|---|---|
| Pre-treatment | 90 | 83 |
| Post-treatment | 90 | 75 |

This is only one field study and would be worthwhile repeating to see if incorporation of certain crops such as forage sorghum, as well as others, have the capacity to aid in managing Verticillium given some successful reports from overseas. A full microbial analysis of the soil would be needed to identify shifts in the soil microbial populations.

**Host susceptibility studies (glasshouse trials)**

Both strains (VCG 1A and VCG 2A) of *V. dahliae* caused symptoms and were able to be re-isolated from some plants of all inoculated test crops with no pathogen recovery from any non-inoculated control

plants. The greatest pathogen recovery of *V. dahliae* was from cotton followed by mungbean (green), barley, chickpea, mungbean (black gram) and then wheat. There were significant effects on growth parameters such as fresh shoot weight and plant height for all crops tested except wheat. Both strains (1A and 2A) significantly reduced fresh shoot weight in all cotton cultivars compared to the controls and caused severe and significant stunting (Table 42).

**Table 42.** Mean plant height, fresh shoot weight and percentage of pathogen recovery of cotton cultivars following root dip inoculation with isolates of *V.dahliae* (values followed by the same letter within a cultivar are not significantly different, $p < 0.05$)

| Cotton Cultivar | Isolate | Fresh shoot weight (g) | Plant Height (cm) | *V. dahliae* recovery (%) |
|---|---|---|---|---|
| Sicot 754B3F | Control | 9.10 a | 37.20 a | 0 |
| | 2A | 0.60 b | 9.7 b | 100 |
| | 1A | 0.40 b | 10 b | 100 |
| Sicot 746B3F | Control | 8.56 a | 34.35 a | 0 |
| | 2A | 1.22 b | 11.00 b | 95 |
| | 1A | 1.08 b | 11.13 b | 95 |
| Sicot 748B3F | Control | 9.03 a | 36.25 a | 0 |
| | 2A | 0.86 b | 11.38 b | 100 |
| | 1A | 0.68 b | 11.10 b | 100 |
| Sicot 707B3F | Control | 10.28 a | 40.80 a | 0 |
| | 2A | 2.43 b | 15.90 b | 100 |
| | 1A | 0.66 b | 11.30 c | 100 |
| Sicot 714B3F | Control | 9.55 a | 36.15 a | 0 |
| | 2A | 0.39 b | 8.63 b | 100 |
| | 1A | 0.58 b | 11.70 c | 100 |

There were also significant reductions in both fresh shoot weight and plant height in all barley cultivars inoculated with both strains compared to non-inoculated controls (Table 43). The fresh shoot weight of cv. Compass was significantly reduced from infection with VCG 1A compared to VCG 2A.

**Table 43.** Mean plant height, fresh shoot weight and percentage of pathogen recovery of barley cultivars following root dip inoculation with isolates of *V.dahliae* (values followed by the same letter within a cultivar are not significantly different, $p < 0.05$)

| Barley cultivar | Isolate | Fresh shoot weight (g) | Plant Height (cm) | *V. dahliae* recovery (%) |
|---|---|---|---|---|
| Compass | Control | 22.25 a | 60.44 a | 0 |
| | 2A | 14.75 b | 47.12 b | 88 |
| | 1A | 8.68 c | 47.15 b | 100 |
| Rosalind | Control | 24.09 a | 55.40 a | 0 |
| | 2A | 14.41 b | 46.9 b | 100 |
| | 1A | 15.03 b | 49.2 b | 90 |
| Flinders | Control | 23.66 a | 59.2 a | 0 |
| | 2A | 14.54 b | 50.7 b | 100 |
| | 1A | 13.73 b | 45.75 c | 100 |
| Commander | Control | 25.84 a | 64.85 a | 0 |
| | 2A | 14.32 b | 51.56 b | 100 |
| | 1A | 13.03 b | 50.35 b | 95 |

Inoculation with both strains significantly reduced fresh shoot weight in the three chickpea cultivars compared to the controls (Table 44). Average plant height was significantly reduced in the 1A inoculated chickpea cultivars compared to the controls, but only HatTrick inoculated with the 2A strain were significantly shorter than the control plants.

There was no significant effect caused by inoculating any wheat cultivar with either strain of *V. dahliae*. Greatest pathogen recovery occurred in cv. Dart (Table 45).

**Table 44.** Mean plant height, fresh shoot weight and percentage of pathogen recovery of chickpea cultivars following root dip inoculation with isolates of *V.dahliae* (values followed by the same letter within a cultivar are not significantly different, $p < 0.05$)

| Barley cultivar | Isolate | Fresh shoot weight (g) | Plant Height (cm) | *V. dahliae* recovery (%) |
|---|---|---|---|---|
| HatTrick (Desi) | Control | 17.73 a | 63.47 a | 0 |
| | 2A | 9.08 b | 52.87 b | 93 |
| | 1A | 7.83 b | 48.87 b | 87 |
| Drummond (Desi) | Control | 17.07 a | 56.00 a | 0 |
| | 2A | 10.4 b | 50.07 ab | 87 |
| | 1A | 9.6 b | 48.33 b | 87 |
| Monash (Kabuli) | Control | 21.07 a | 54.20 a | 0 |
| | 2A | 12.00 b | 42.73 ab | 93 |
| | 1A | 8.19 b | 38.00 b | 67 |

**Table 45.** Mean plant height, fresh shoot weight and percentage of pathogen recovery of wheat cultivars following root dip inoculation with isolates of *V.dahliae* (values followed by the same letter within a cultivar are not significantly different, $p < 0.05$)

| Barley cultivar | Isolate | Fresh shoot weight (g) | Plant Height (cm) | *V. dahliae* recovery (%) |
|---|---|---|---|---|
| Mitch | Control | 11.17 a | 52.16 a | 0 |
| | 2A | 12.88 a | 50.10 a | 60 |
| | 1A | 14.70 a | 50.00 a | 65 |
| Wallup | Control | 9.62 a | 54.1 a | 0 |
| | 2A | 10.86 a | 54.1 a | 50 |
| | 1A | 10.91 a | 51.4 a | 75 |
| Dart | Control | 6.30 a | 48.25 a | 0 |
| | 2A | 6.61 a | 48.40 a | 85 |
| | 1A | 8.15 a | 48.25 a | 85 |

There was more varietal differentiation and reaction to strain observed in the green mungbean cultivars when inoculated with the pathogen. The mean fresh shoot weight of plants inoculated with 1A strain was significantly less compared to both the controls and 2A inoculated plants (Table 46). The fresh shoot weight of Celera II-AU and Crystal cultivars inoculated with VCG 2A were not significantly different to the controls. The black gram cultivar (Onyx-AU) on the other hand was far more resistant to the 1A strain which was not significantly different to the control. Pathogen re-isolation was also less with the 1A inoculated cultivar Onyx-AU (75%) compared to all other mungbean cultivars where the pathogen was re-isolated from between 90 and 100% of plants.

With the exception of Crystal mungbean (control and VCG 2A not significantly different) plant height was significantly reduced in all inoculated plants compared to the non-inoculated controls (Table 46). In all green mungbean cultivars (Berken, Celera II-AU, Crystal, Jade-AU and Satin-II) the plants inoculated with the 1A strain were significantly more stunted than those inoculated with the 2A strain.

**Table 46.** Mean plant height, fresh shoot weight and percentage of pathogen recovery of mungbean cultivars following root dip inoculation with isolates of *V.dahliae* (values followed by the same letter within a cultivar are not significantly different, $p < 0.05$)

| Barley cultivar | Isolate | Fresh shoot weight (g) | Plant Height (cm) | *V. dahliae* recovery (%) |
|---|---|---|---|---|
| Onyx-AU (Black gram) | Control | 15.65 a | 51.40 a | 0 |
| | 2A | 8.49 b | 31.05 b | 95 |
| | 1A | 14.95 ab | 39.53 b | 75 |

| | | | | |
|---|---|---|---|---|
| Berken (green) | Control | 14.85 a | 53.75 a | 0 |
| | 2A | 4.00 b | 23.00 b | 100 |
| | 1A | 0.12 c | 11.90 c | 100 |
| Celera II-AU (green) | Control | 11.25 a | 49.30 a | 0 |
| | 2A | 9.05 a | 33.28 b | 95 |
| | 1A | 1.01 b | 14.26 c | 90 |
| Crystal (green) | Control | 10.95 a | 42.21 a | 0 |
| | 2A | 10.75 a | 37.19 a | 100 |
| | 1A | 1.64 b | 13.75 b | 100 |
| Jade-AU (green) | Control | 15.22 a | 45.17 a | 0 |
| | 2A | 8.35 b | 35.32 b | 95 |
| | 1A | 0.17 c | 13.00 c | 100 |
| Satin-II (green) | Control | 16.3 a | 55.25 a | 0 |
| | 2A | 9.3 b | 40.79 b | 100 |
| | 1A | 0.25 c | 13.00 c | 100 |

Typical Verticillium wilt symptoms occurred with premature leaf chlorosis, necrosis and leaf abscission observed, especially in mungbean (Figure 124). VCG 1A was especially aggressive on inoculated green mungbean cultivars with foliar symptom development noted soon after inoculation and plants largely dead and defoliated by five weeks.


**Figure 124.** Symptom development on mungbean a) and b) leaf chlorosis and necrosis c) severe stunting, leaf chlorosis and necrosis on cv Berken inoculated with VCG 1A

Six weeks following inoculation, the barley cultivars were more symptomatic than the wheat cultivars with more leaf chlorosis noted (Figure 125).


**Figure 125.** A) Commander barley control, B) Commander barley inoculated with VCG2A, C) Commander barley inoculated with VCG 1A, D) Dart wheat control, E) Dart wheat inoculated with VCG 2A, F) Dart wheat inoculated with VCG 1A

Despite the reduced symptom development and no significant reductions in measured plant biomass of inoculated wheat plants, *V. dahliae* had established itself systemically in both wheat and barley causing above ground symptoms (leaf chlorosis) and was able to be re-isolated from both stem and leaf tissue (Figure 126).

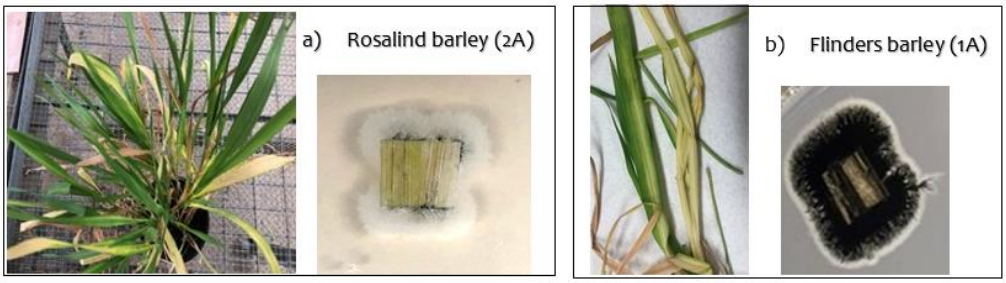



**Figure 126.** Leaf chlorosis on a) Rosalind barley inoculated with VCG 2A strain b) Flinders barley inoculated with VCG 1A strain and Verticillium observed readily growing from infected leaf pieces on isolation plates

Vascular stem browing was also observed in all test crops (Figure 127).

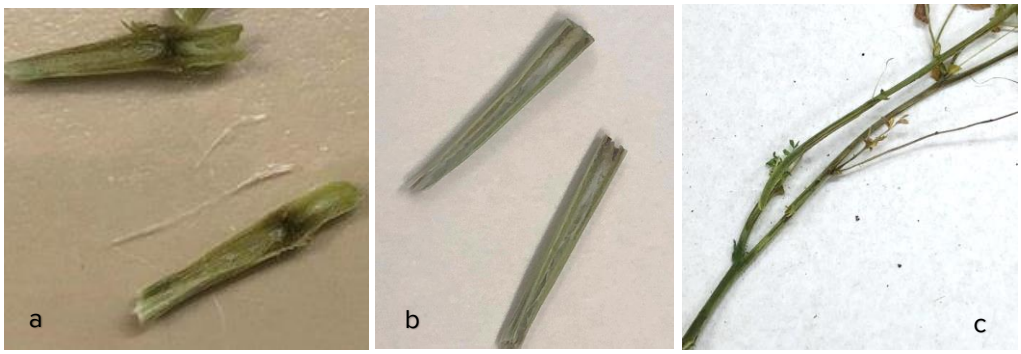



**Figure 127.** Stem vascular browning noted in a) barley b) wheat c) chickpea and d) mungbean

This data suggests that some rotation crops commonly rotated with cotton are potential hosts and have the ability to contribute to, and aid in maintaining soil inoculum levels in the field. Evidence of symptomatic infection of *V. dahliae* on rotation crops presented here will be useful in designing rotations for management of Verticillium wilt.

Commercial field monitoring: To date, Verticillium has not been isolated from any wheat samples collected from fields with a known history of Verticillium. Random asymptomatic wheat plants were collected from Verticillium infected plants during the 2015 and 2017 seasons and plated onto ¼ PDAS - *no V. dahliae* was recovered. Field infected plants of other barley, mungbean and chickpea have not been observed either to date. It is recognised that *V.dahliae* can persist in symptomless host and non-host roots, providing a means of multiplication and dissemination, so continued monitoring of these crops in the field is certainly recommended.

**2.2 Collect seed from Vd infected plants/receive seed from CSIRO trials at Narrabri nursery, surface sterilise and plate onto semi-selective medium. Incubate plates and observe for growth of Vd, isolate, single-spore and characterise using PCR and VCG analysis.**

**Testing cotton seed for *V. dahliae* infection**

*V. dahliae* (VCG1A) was only isolated from one seed of the 3976 plated (0.025%). The recovery of the pathogen following the disinfestation of the seed surface suggests that the fungus was inside the seed. Overseas reports confirm dissemination of the pathogen with cotton seed and microsclerotia associated with lint. The most common means of spread of this pathogen from field to field would include through irrigation and flood water and soil adhering to farm equipment, animals and humans. This study shows, however, there is also a small potential for the pathogen to be present in acid-delinted seed and transmitted into clean fields which could potentially initiate a disease in a new area. With the continued spread of Verticillium in recent years, it would be difficult to determine what has caused the appearance of disease in new fields. Certainly the disease can be spread on soil by infected machinery and humans, highlighting the importance of farm hygiene. Weeds harbour the pathogen and can be seed borne (e.g. Noogoora burr) and contribute to disease spread. Asymptomatic non-host rotation crops harbouring the pathogen endophytically or potentially susceptible new hosts (such as mungbean, chickpea and cereals as reported in this current report) may have the potential to spread the disease in infected plant material.

As with Fusarium wilt, perhaps thought should be given to increasing seed of new varieties on known disease free sites to try to minimise the potential of even slight seed transmission. Disease prevention is a far better option than trying to manage this disease, since once it is established microsclerotia enable it to survive long term in the soil.

**2.3 DAF Qld - Test different Vd strains on cotton at different temperatures under controlled environmental conditions to determine temperature sensitivity on virulence. Conduct growth tests of isolates at different temperatures to determine growth curves of each strain.**

**Temperature sensitivity of strains**

Mean growth rates for the twenty four isolates tested are recorded in Table 47. All of the isolates were able to grow at all temperatures from 5°C to 32°C. Temperature had an effect on growth and development of *V. dahliae* and differences were observed between isolates at the same temperature. The greatest variation among isolates occurred at the higher temperature range. 32°C had the greatest differences among isolates followed by 30° then 27° and 24°C. There was the least variation at the lowest temperature of 5°C where minimal growth still occurred. Growth continued in all isolates at temperatures

considered less optimal for *V. dahliae* (5°C and 32°C) suggesting *V. dahliae* has some tolerance to less optimal temperature conditions.

**Table 47.** Average radial mycelial growth rates (mm $d^{-1}$) of different isolates of *V. dahliae* on Czapek-Dox agar at different temperatures (mean of 5 reps +/- SE)

| Isolate No. | VCG | 5°C | 10°C | 15°C | 20°C | 24°C | 27°C | 30°C | 32°C |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1A | 0.65±.007 | 1.40±.021 | 2.59±.012 | 3.35±.016 | 3.54±.015 | 3.26±.049 | 1.80±.117 | 0.29±.047 |
| 2 | 1A | 0.67±.004 | 1.45±.009 | 2.55±.011 | 3.29±.005 | 3.59±.019 | 3.41±.030 | 1.81±.025 | 0.34±.024 |
| 3 | 1A | 0.64±.007 | 1.31±.011 | 2.65±.027 | 3.40±.016 | 3.35±.014 | 3.02±.079 | 2.04±.053 | 0.57±.018 |
| 4 | 1A | 0.63±.003 | 1.28±.023 | 2.97±.035 | 3.93±.031 | 2.84±.031 | 1.90±.047 | 2.99±.013 | 0.71±.012 |
| 5 | 1A | 0.74±.003 | 1.40±.008 | 2.63±.015 | 3.35±.014 | 3.51±.020 | 3.44±.025 | 2.25±.049 | 0.51±.020 |
| 18 | 1A | 0.44±.003 | 1.10±.017 | 2.12±.015 | 3.15±.010 | 4.11±.012 | 4.19±.005 | 4.52±.018 | 3.72±.017 |
| 19 | 1A | 0.44±.009 | 1.32±.020 | 2.36±0.18 | 3.20±.018 | 3.68±.008 | 2.36±.065 | 2.19±.053 | 0.73±.032 |
| 20 | 1A | 0.46±.008 | 1.52±.018 | 2.90±.028 | 3.46±.020 | 3.59±.017 | 2.83±.078 | 2.34±.254 | 1.11±.048 |
| 21 | 1A | 0.43±.007 | 1.80±.045 | 3.05±.018 | 3.79±.009 | 3.95±.064 | 3.88±.078 | 3.12±.016 | 1.11±.072 |
| 22 | 1A | 0.48±.002 | 1.74±.007 | 2.63±.011 | 3.53±.013 | 3.53±.008 | 3.40±.045 | 2.04±.083 | 0.87±.050 |
| 23 | 1A | 0.37±.008 | 1.74±.007 | 2.29±.024 | 3.16±.015 | 3.38±.021 | 2.93±.078 | 2.10±.054 | 1.07±.061 |
| 24 | 1A | 0.38±.007 | 1.73±.022 | 2.74±.004 | 3.32±.025 | 3.61±.026 | 3.29±.049 | 2.16±.070 | 0.70±.028 |
| 6 | 2A | 0.76±.009 | 1.41±.014 | 2.82±0.19 | 3.90±.017 | 4.42±.016 | 4.27±.007 | 3.45±.029 | 1.06±.048 |
| 7 | 2A | 0.40±.003 | 1.35±.022 | 2.86±.018 | 3.94±.015 | 4.35±.008 | 4.05±.006 | 3.41±.026 | 0.34±.029 |
| 8 | 2A | 0.43±.010 | 1.43±.020 | 2.83±.023 | 3.99±.016 | 3.88±.031 | 3.55±.106 | 2.89±.058 | 0.47±.037 |
| 9 | 2A | 0.36±.006 | 1.63±.030 | 2.72±.019 | 3.98±.012 | 4.49±.016 | 4.30±.022 | 3.48±.032 | 1.21±.014 |
| 10 | 2A | 0.36±.008 | 1.52±.016 | 2.55±.015 | 3.87±.017 | 4.10±.012 | 4.22±.004 | 3.32±.020 | 0.88±.022 |
| 11 | 2A | 0.55±.011 | 1.65±.018 | 2.74±.017 | 3.91±.014 | 4.38±.029 | 4.35±.004 | 3.41±.036 | 0.88±.057 |
| 12 | 2A | 0.52±.010 | 1.63±.012 | 2.52±.030 | 3.74±.037 | 4.17±.009 | 4.30±.014 | 3.50±.022 | 1.00±.031 |
| 13 | 2A | 0.43±.005 | 1.26±.009 | 1.78±.020 | 3.12±.010 | 3.80±.021 | 4.09±.009 | 4.43±.026 | 3.52±.005 |
| 14 | 2A | 0.57±.020 | 1.64±.017 | 2.75±.014 | 3.75±.018 | 4.06±.048 | 3.90±.011 | 2.79±.017 | 0.75±.013 |
| 15 | 2A | 0.46±.002 | 1.41±.022 | 2.72±.013 | 3.83±.009 | 3.99±.009 | 3.73±.026 | 2.33±.117 | 0.62±.026 |
| 16 | 2A | 0.48±.004 | 1.36±.013 | 2.45±.024 | 3.58±.020 | 4.15±.031 | 4.14±.010 | 3.01±.039 | 0.80±.018 |
| 17 | 2A | 0.58±.010 | 1.44±.010 | 3.11±.013 | 4.14±.010 | 4.78±.010 | 4.44±.015 | 3.21±.010 | 0.87±.015 |

From this particular study 24°C was the optimal temperature for *in vitro* growth of most isolates from both VCG strains. 16 isolates had greatest growth at 24°C. Isolate 22 (VCG 1A) grew similarly at 20°C and 24°C. Three isolates (1A isolate 3, 1A isolate 4and 2A isolate 8) had greatest growth rate at 20°C, two isolates (2A isolates 10 and 12) had greatest growth rate at 27°C and 2 isolates (2A isolate 13 and 1A isolate 18) had greatest growth rate at 30°C. Isolates 13 (VCG 2A) and isolate 18 (VCG 1A) were quite different from the other isolates, growing better at 27°C and 30°C and still growing well at 32°C when most of the other isolates had minimal growth. Unfortunately the incubator used could not be adjusted to go any higher than 32°C, so it could not be established what happened with growth rate at even higher temperatures.

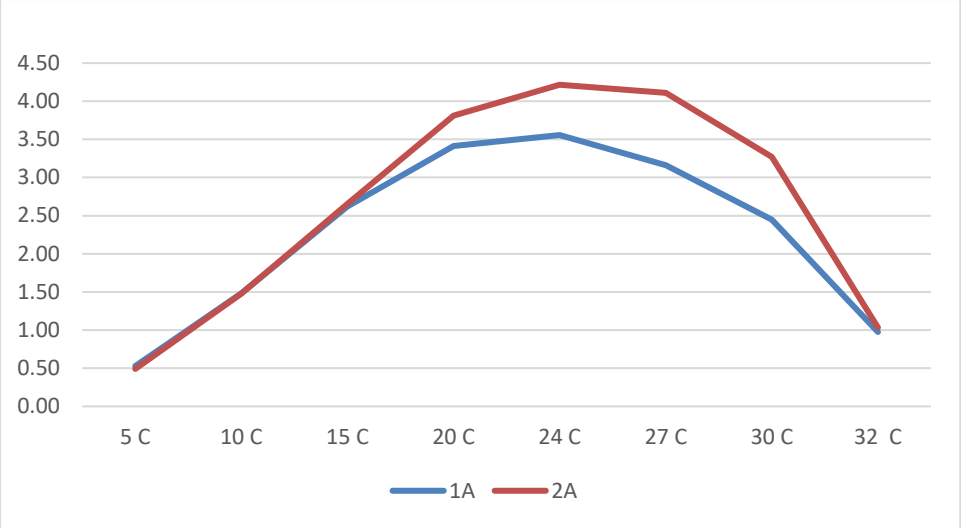



**Figure 128.** Average daily radial growth of all VCG 1A and 2A isolates between 5°C and 32°C

When the mean of all twelve 1A and twelve 2A isolates was graphed (Figure 128), mycelial growth was observed to be very similar between 5°C and 15°C. Between 20°C and 30°C mycelial growth of the 2A isolates on average was greater than the 1A isolates. At 32°C both isolates on average were very similar again. This observation is different from what has commonly been observed overseas where temperatures of 27-28°C are considered more favourable for VCG 1A strains whilst lower temperatures of 24-25°C are considered more favourable for other strains. It is apparent from this study and others previously conducted by NSW DPI that the Australian isolates behave differently from those overseas.

This experiment was only conducted once and should be replicated in future studies and include virulence and pathogenicity tests on all isolates especially those that grew quite differently such as isolates 13 and 18 in this study. Whether these two isolates are more aggressive in plants would need additional confirmation. It is unknown without further testing whether higher growth *rates in vitro* would correspond to higher infection rates in the field, given there are other variables that influence pathogen infection and development such as inoculum density, pathogen virulence, soil moisture, UV radiation and other soil microorganisms. Studies by ElSharawy et al (2015) reported that growth rate was a key factor for pathogenicity. Aggressiveness *in vitro* and *in vivo* tests revealed the highest growth rate was correlated with the highest disease.

ElSharawy, A.A, Xiaoping, H. and Jiarong, Y. (2015). Trade-offs between growth rate, sporulation and pathogenicity in *Verticillium dahliae*. Journal of Agricultural Science, 7, pp 35-41.

**2.4 Communicate research outcomes.**

Research findings from this project have been presented at grower meetings, conferences and industry meetings throughout the project. Data is currently being drafted, aiming for inclusion in a publication dedicated to Verticillium management in the journal *Plant.*

3. **Research question: What is the economic impact of reniform nematode and how do we manage it?**
   Reported in final report DAQ1803

4. **Research question: What is the disease suppression potential of cotton soils from different cotton growing regions.** (Prepared by Gupta Vadakattu)

4.1 Determine the composition and abundance of soil fungal communities in surface soils from different cotton growing regions.

**Objective 1. Disease suppressive microbial communities – Soil fungi: long-term cropping system experiments at ACRI, Narrabri**

Briefly, there were a total of 370 fungal genera in all the cotton soils and the top 25 genera accounted for the major portion of total fungal community. There were significant differences in the composition and genetic diversity of soil fungi between the different field sites (Figure 129). A significant variation in the relative abundances of fungi belonging to a number of families was observed between the KK (conducive) and F6E (suppressive) fields which were <500M apart but under different management practices, e.g. rotation and fertilizer application. The F6E and D1 field experiments varied in tillage practices, i.e. crop residues were incorporated in the F6E experiment whereas they were left on the surface with minimum tillage in D1, which would have contributed to the variation in the fungal community composition. Tillage and crop-based variation in the abundance and composition of fungi has been shown in other cropping systems (4).

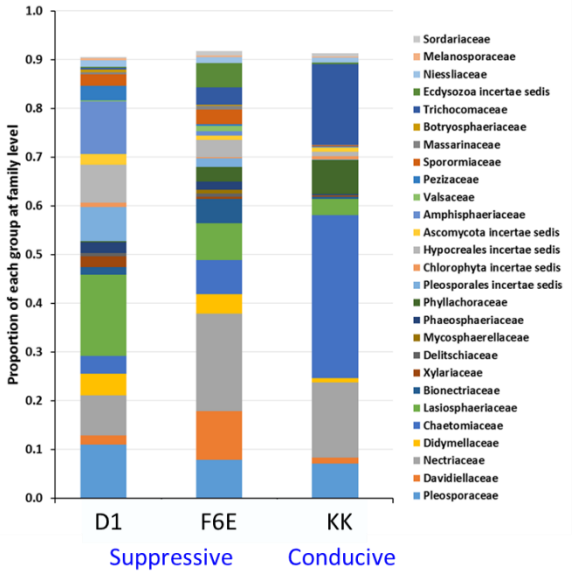



**Figure 129.** Fungal community composition (graph above) and diversity indices (d=species richness; j=evenness; H=Shannon index) in the surface 10 cm soils from field experiments at ACRI, Narrabri. Soils were characterized suppressive/conducive based on disease incidence history.

Results from network analysis showed significant differences in a number of network properties for fungal communities in the soils considered disease suppressive (F6E) and conducive (KK). Fungal networks varied between suppressive and conducive soils both in terms of modularity and interactions between the modules. Suppressive fungal communities were characterized by significantly higher diversity, higher connectedness, and significantly more nodes (Figure 130) indicating resilience to change. Node.degree and clustering coefficient were higher in conducive soils ($p<0.001$) compared to that in suppressive soils, whereas, Node.between and modularity were higher in suppressive soils ($p<0.001$). Suppressive community was also characterized by higher number of modules and greater modularity compared to communities in conducive soils (Figure 131). A smaller path distance in non-suppressive communities indicates a “smaller world” but with lower linkage efficiencies i.e. “disconnectedness”. It is suggested that in highly connected biological networks, efficient resource exploitation by all species can occur thus reducing opportunities for pathogen growth and disease (5, 6). Additionally, analysis of different sample sizes indicated that more abundant OTUs found to be located in “hot spots/microsites” within the soil; the smaller sample sizes access the locally abundant but spatially rare members of the community (7).

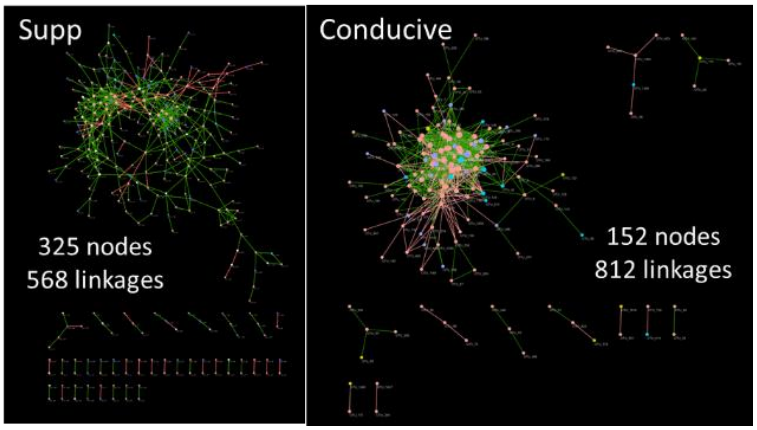



**Figure 130.** Fungal community network plots for disease suppressive (F6E) and conducive soils (KK). Nodes represent species that are connected by pairwise interactions.

Network analysis of microbial community data provides information that delineates the community structure about potential linkages and interactions between its members. It is suggested that in a community network, the nodes (members of the community) that are highly connected with other members and the connectors (those linking modules) have important roles in maintaining the network integrity; that is, they serve as putative keystone taxa that provide stability to the microbial community (Gupta et al. 2019). Overall, these results suggest that soil ecological and environmental factors and filtering processes related to substrate quality and availability, spatially and temporally, play a significant role in shaping soil fungal communities and their functionality. The high level of organization along with higher diversity in the soil fungal community in the suppressive soils would provide the cotton plant with a stable microbial reservoir across varied seasonal environmental conditions. Also, the ability to form hyphal networks across longer spatial scales provides some level of stability in the general soil fungal community across seasons making it important to the natural suppression potential of soils (Penton et al. 2014). It is generally considered that seasonal variation in community composition is generally greater for soil bacteria than that for fungal communities.

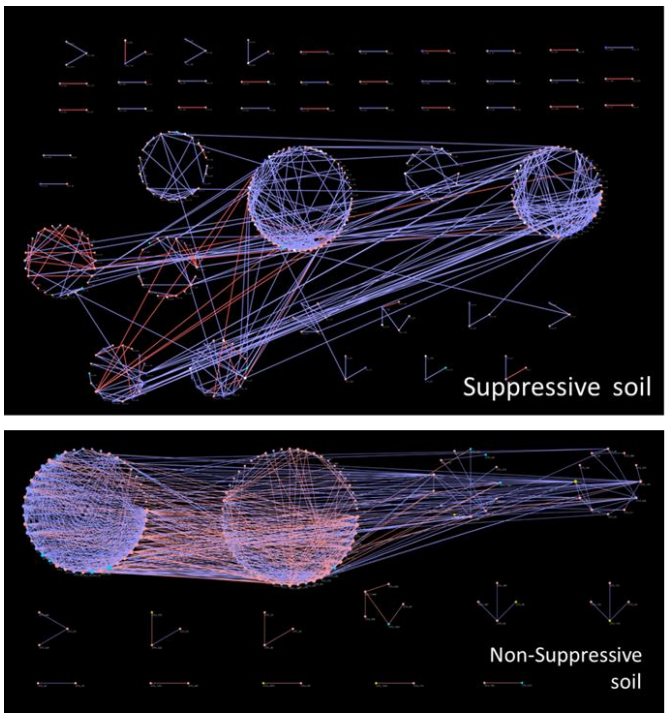



**Figure 131.** Fungal community network plots: modularity and connectedness in suppressive (F6E) and conducive soils (KK). Modules refer to groups of taxa that are correlated with one another.

To summarize, results from the long-term experiments indicate that (i) the diversity and abundance of soil fungal community varied significantly by crop management history and (ii) fungal communities in suppressive cotton soils were characterized by higher diversity and higher connectedness.

**Objective 2. Crop rotation effects on composition of soil microbial communities, microbial activity and potential implications to biological suppression**

Crop rotation significantly influenced total microbial activity and catabolic diversity (in terms of carbon substrate utilization pattern) in surface soils which could be attributed to microbial response due to differences in the quality and quantity of carbon inputs from rotation crops. For example, surface soils from continuous cotton and cotton-fallow rotations showed significantly ($P<0.001$) lowest microbial activity (4.7-8.2 µg $CO_2$/g) and catabolic diversity (CMD - 9 to 14) of soil microbial communities compared to that after sorghum and corn (12 to 17 µg $CO_2$/g and CMD of 16 to 19) (Figure 132). These results confirm previous observations from the long-term crop rotation experiment (F6E) compared to soils from the Disease block (e.g. Biofumigation experiment, KK) at ACRI, which showed distinctly different carbon substrate utilization pattern and lower catabolic diversity compared to that in the soils from the nearby long-term cropping systems experiment (F6E) in which crop residues were incorporated annually (Gupta et al. 2018). Reports from the grain cropping systems in southern Australia have indicated that management practices that supply higher levels of biologically-available C over long periods (>5-7 years) and maintain higher levels of microbial C turnover can result in changes to the composition and activity of the soil microbial community and consequently increase suppression (Gupta et al. 2019). In the rotation treatments with Sorghum and Corn, the supply of crop residues during non-cotton periods seems to have stimulated general microbial activity that reduced the ability of soilborne pathogens like *V. dahliae* grow from either competitive exclusion or inhibition. Data on the abundance of *V. dahliae*, measured using DNA techniques indicated lower levels of the pathogen in Sorghum and Corn rotations compared to that under Continuous cotton treatment (Figure 133). Additionally, the Sorghum-Cotton rotation showed highest total fungal populations supporting the hypothesis of potential for competitive exclusion of the pathogen *V. dahliae*.

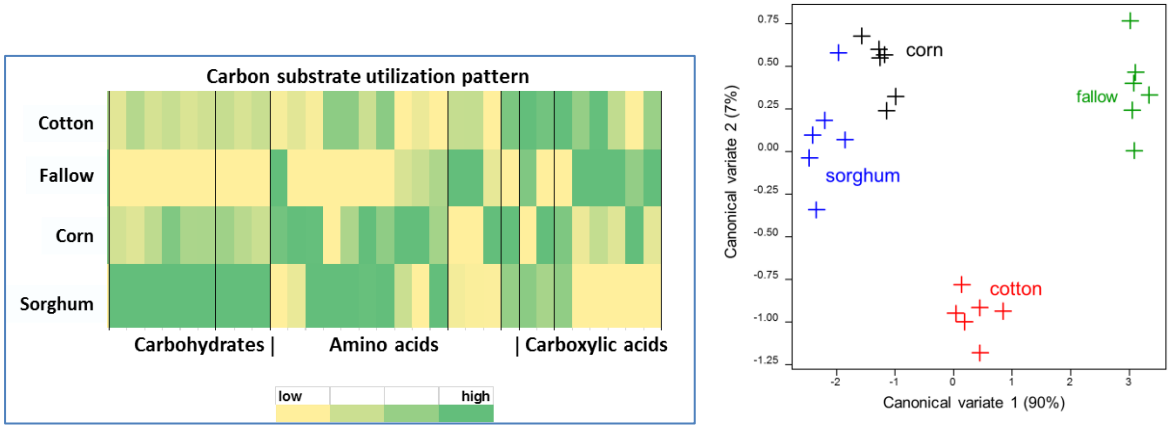



**Figure 132.** Heatmap for the microbial carbon substrate utilization profiles as influenced by crop rotation in surface soils collected at the time of 2017 cotton planting in the Northstar experiment.

Unlike the treatments with the rotational crops, microbial activity and catabolic diversity were lowest in the Fallow treatments both in the 2017-18 and 2018-19 seasons. Surface soils in the experiments have in general lower levels of soil organic carbon levels (~1%) hence C inputs from roots, root exudation and crop residues are the main source of carbon for microbial populations. Crop residues and rhizodeposition are the main sources of carbohydrates and amino acids for microorganisms. Soils from the Fallow treatment showed lowest catabolic activity measured with such substrates both in the 2017-18 and 2018-19 suggest that Fallowing effects on general soil biological activity persists even after the cotton crop.

At the start of 2017-18 season, crop rotation also significantly influenced population abundances of total soil bacteria, fungi and *V. dahliae* pathogen levels (Figure 133). Additionally, rotation had a significant effect on the populations of *Pseudomonas* spp, one of the bacterial groups with potential to impact on disease incidence. The general trends with total bacteria, fungi and Pseudomonas populations remained

same at the start of 2018-19 season, especially the effects of Fallow and Sorghum rotation treatments (data not shown). For example, *V. dahliae* accounted for 1.6% of total fungal population in the Continuous Cotton soils compared to 0.45% and 0.25% of total fungi in the Fallow and Sorghum treatment soils, respectively. These results clearly indicate that rotations treatments not only influence *V. dahliae* population levels, they can modify the overall populations of bacteria and fungi and therefore affecting the pathogen's ability to grow and/or reach roots to cause disease. The combined effect of Corn and Sorghum to reduce the pathogen inoculum level combined with maintaining higher levels of general microbial populations seem to have a positive effect on overall lower disease incidence. Although the Fallow treatment reduced the inoculum level, it also reduced the overall microbial populations and activity and thus may be weakening the biological buffer to reduce the impact of the disease on cotton growth.

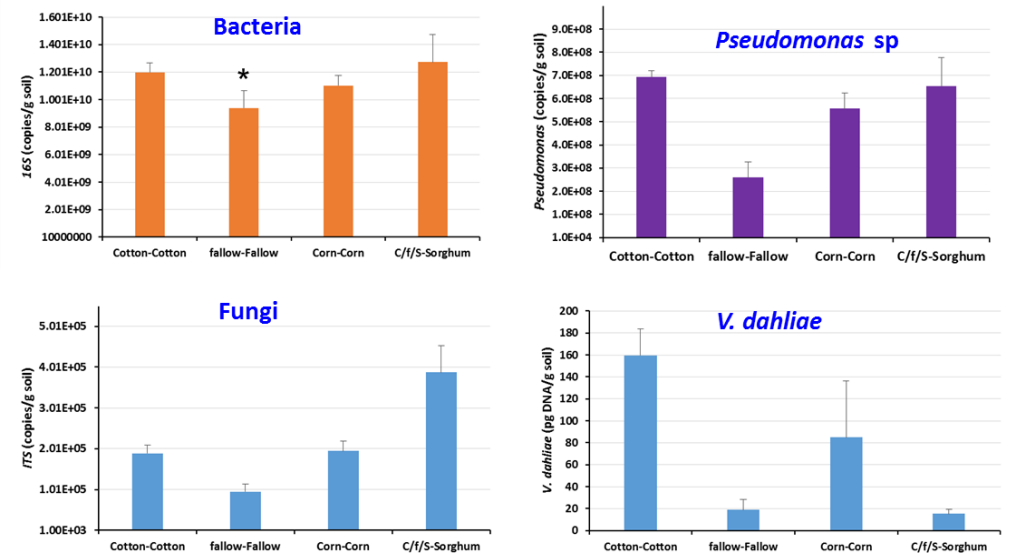



**Figure 133.** Populations of soil bacterial, fungi, pseudomonads and Verticillium pathogen levels as influenced by crop rotation at the Northstar experiment during 2017-18 season.

The potential contribution of soil microbiome involving specific groups of bacteria, actinobacteria and fungi in disease suppression has been observed for a number of soilborne fungal pathogens including Verticillium wilt in broccoli, strawberry, celery etc (e.g. Inderbitzin et al. 2018). Results for the genetic diversity of soil fungal and bacterial communities showed significant differences between the different crop rotation treatments. At the start of the 2017 season, soils under rotation crops Sorghum and Corn generally showed significantly higher species richness and diversity of both bacteria and fungi suggesting that cotton plants would have a diverse reservoir of microorganisms to access for beneficial interactions. Whereas, the fallow treatment showed lowest diversity of soil microbial communities mainly from the reduction in total microbial populations due to lack of C inputs from a growing crop. The effect of rotation treatment persisted even after the cotton crop i.e. at the start of 2018 season, especially the lower bacterial diversity in the Fallow treatment. The diversity of fungi after the 2017-18 cotton crop probably due to the high disease levels. Results from the ACRI experiments indicated that fungal communities in suppressive cotton soils were characterized by higher diversity and higher connectedness indicating a microbial community network that is resilient to change and pathogen invasion. The lower diversity of fungi under Continuous cotton suggests a fungal community that is not resilient for pathogen invasion. This is supported by the observation of lower pathogen suppression measured in the laboratory-based pathogen suppression assay.

It is known that even healthy plants are colonized by a diverse array of bacterial and fungal communities internally (endophytes) and in the rhizosphere. Adams and Kloepper (2002) reported that cotton plants are capable of establishing a carrying capacity for communities of endophytic bacteria following seed germination, most of them originating from the soil. Thus differences in soil microbial community based on rotation and other management practices can modify the microbes associated with the new cotton crop. The occurrence of verticillium disease in Strawberry has been shown to significantly modify root and rhizosphere fungal community structure generally reducing the root inhabiting fungi

(Nallanchakravarthula et al. 2014). At the Northstar experiment, the effect of rotation treatments persisted even after the cotton crop during 2017-18 i.e. significant variation in the bacterial and fungal community composition was seen at the start of 2018-19 season although some of the specific members of the community were not the same in the two seasons. While the microbes varying in 2017-18 seasons would have contributed to the suppression of disease, the differences in 2018-19 season would have been influenced by the diseased cotton roots.

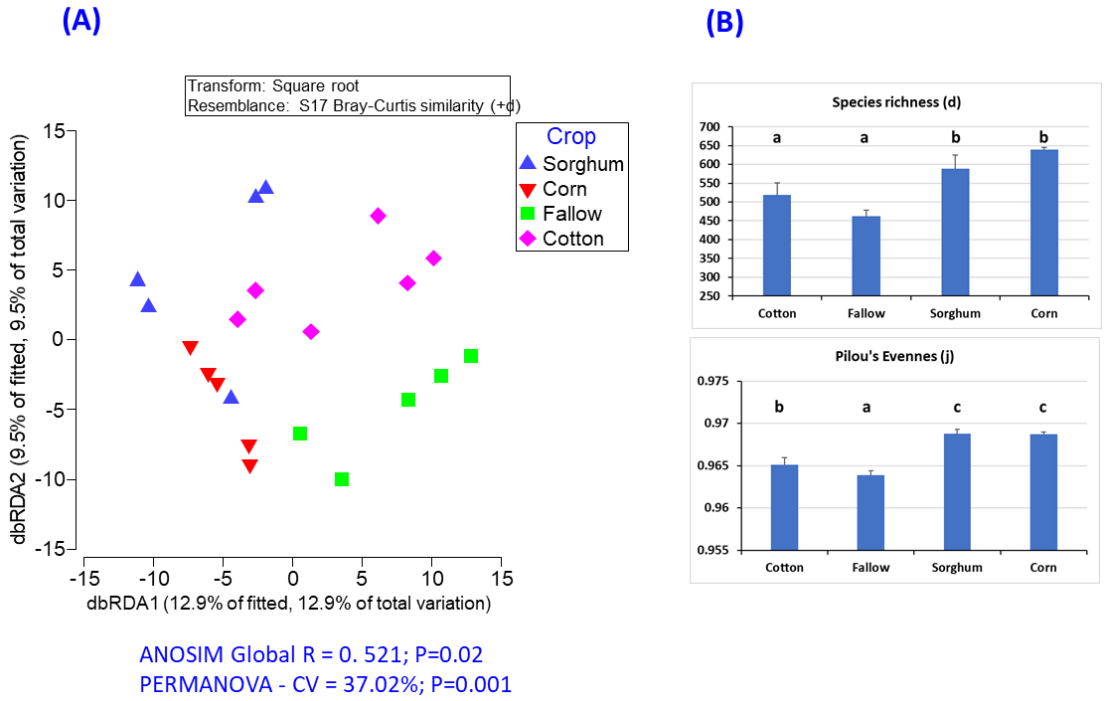



**Figure 134.** Genetic composition of soil bacterial communities measured using 16S rDNA-sequencing analysis in the surface soils collected early in 2017-18 cotton season in the Northstar experiment. (A) Results from redundancy analysis at OTU level showing treatment induced grouping of bacterial community, data points closer to each other show greater similarity (B) microbial diversity indices as influenced by crop rotation.

The impact of soil microbiome structure on disease suppression can also be attributed to the presence and/or higher abundances of specific groups or members of microorganisms. Results for the soil bacterial community at the start of 2017-18 season indicate higher relative abundances of bacterial belonging to families such as Streptomycaceae, Pseudonocardiaceae, Micromonosporaceae, Methylobacteriaceae, Oxolobactereraceae, Rhodobactereaceae, Chitinomonadaceae, Microbacteriaceae in soils under Sorghum and Corn rotation compared to that in the Continuous Cotton and Fallow treatments (Figures 135 and 136). A number of bacterial genera in these Families are known to have antifungal antagonistic and plant growth promoting capabilities suggesting that the presence of these groups of bacteria may have contributed to lower disease incidence in these rotation treatments (Inderbitzin et al. 2018). Fallow treatment seems to have resulted in lower abundances of a number of such bacterial groups compared to the Sorghum rotation treatment (Figure 134).

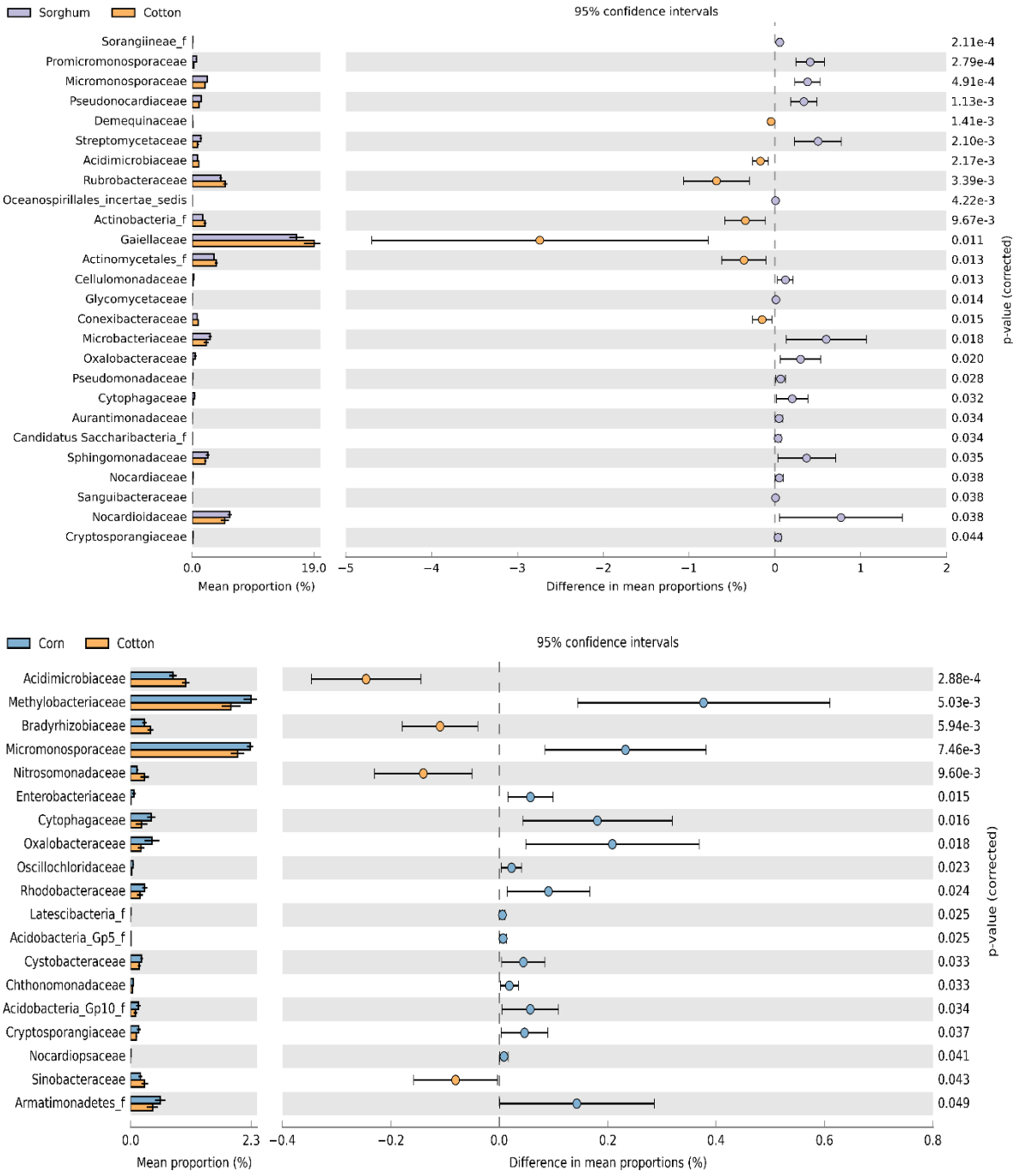



**Figure 135.** Specific members of soil bacterial community, at Family level, showing significant differences between crop rotation treatments involving Cotton, Corn and Sorghum at Northstar experiment for samples collected during 2017-18 season.

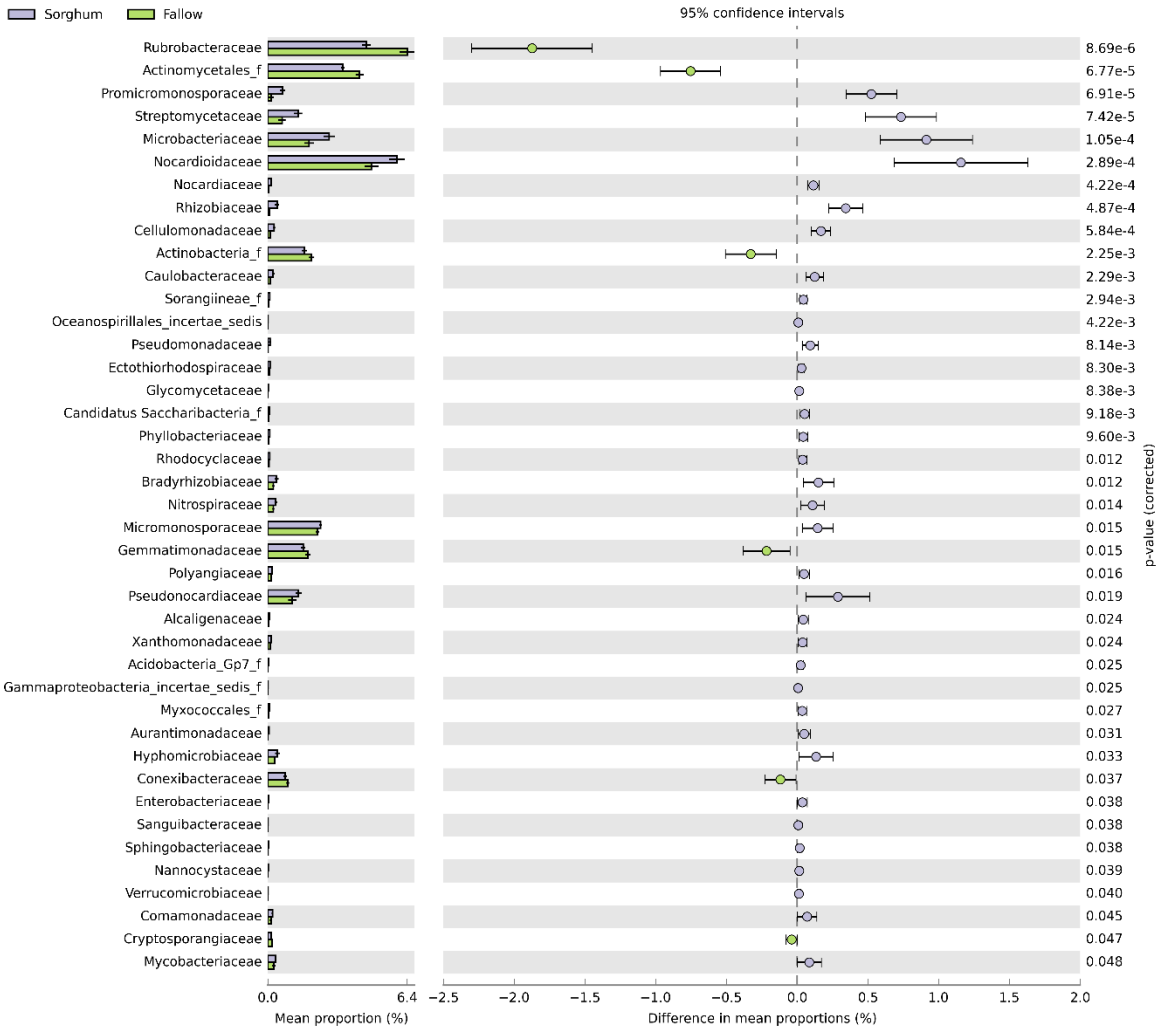



**Figure 136.** Specific members of soil bacterial community, at Family level, showing significant differences between crop rotation treatments involving Cotton and fallow at Northstar experiment for samples collected during 2017-18 season.

Results for the genetic composition of soil fungal community also showed significantly higher diversity in the rotation crop treatments, esp. Sorghum compared to the Fallow and Continuous cotton treatment (data not sown). These results are in agreement with the findings from the ACRI experiments showing lower diversity of fungi in the disease conducive soils in the Disease block (KK) compared to that in the long-term crop rotation experiment that adds large amounts of crop residues annually (F6E). Similarly, research in the grain cropping systems in South Australia indicate that soil fungi play an important role in the suppression of soilborne fungal diseases caused by *Rhizoctonia solani*, *Fusarium* crown rot and Take All in cereal crops (Penton et al. 2014). Soil fungi can form hyphal networks and seem to be less prone to short-term changes in community structure. Thus, long-term adoption of conservative crop management practices including growing different plant types can promote greater diversity of fungi and the development of a resilient beneficial fungal community (Gupta et al. 2019).

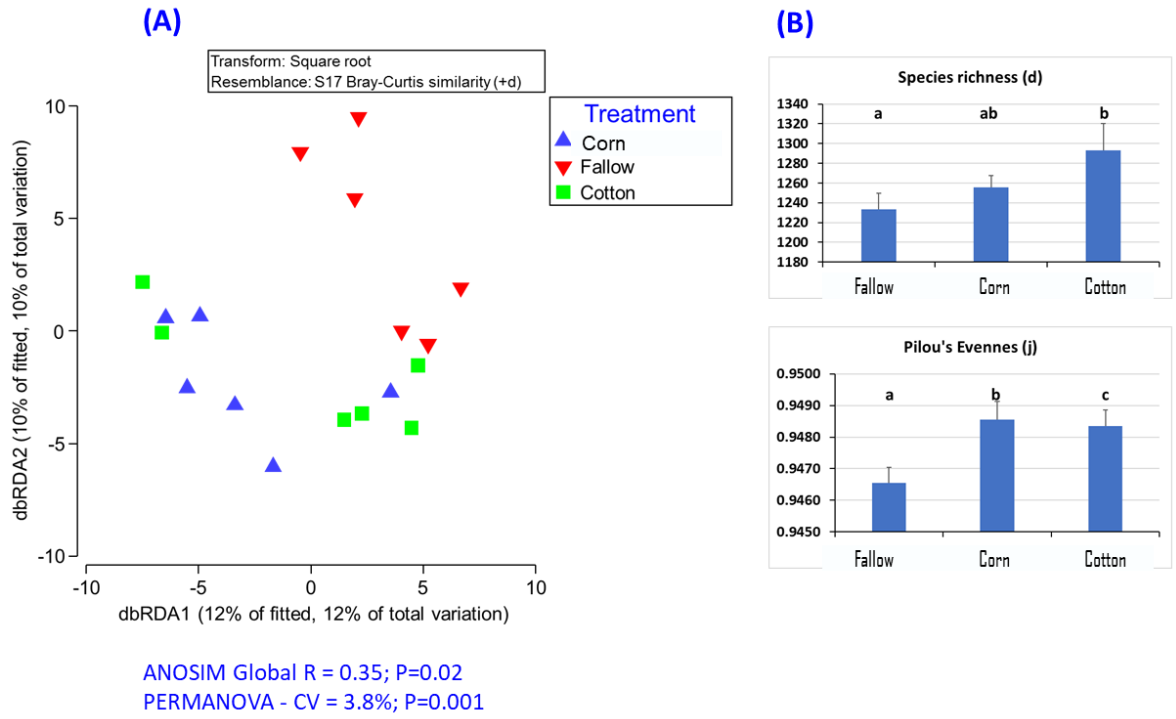



**Figure 137.** Genetic composition of soil bacterial communities measured using 16S rDNA-sequencing analysis in the surface soils collected early in 2018 cotton season in the Northstar experiment. (A) Results from redundancy analysis at OTU level showing treatment induced grouping of bacterial community, data points closer to each other show greater similarity (B) microbial diversity indices as influenced by crop rotation.

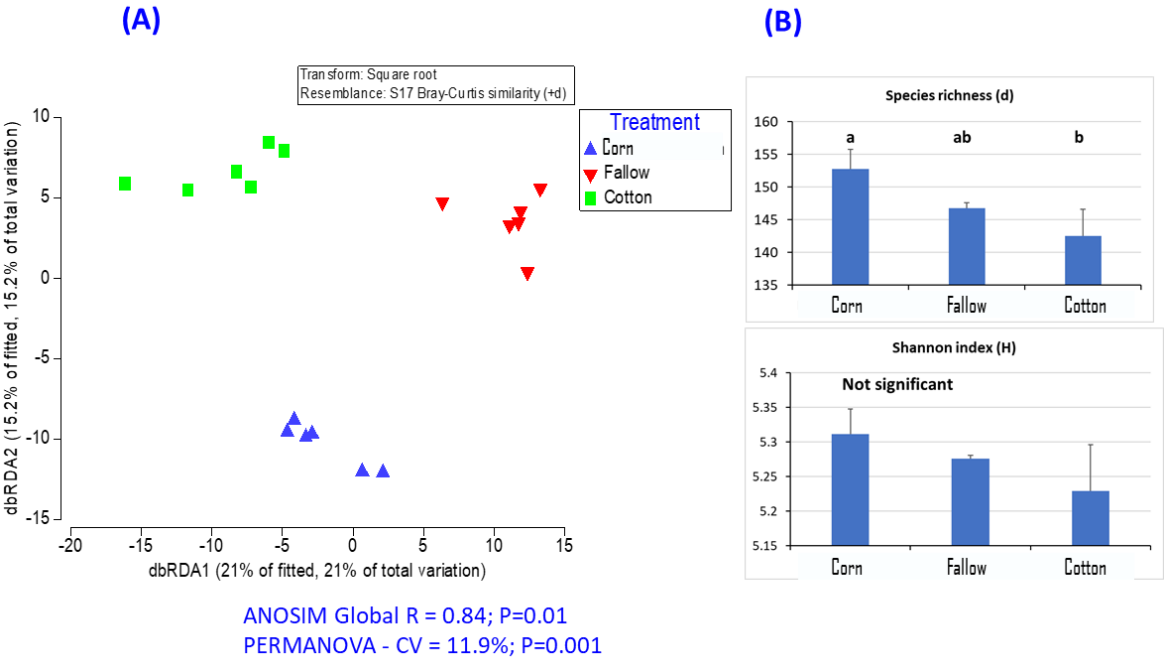



**Figure 138.** Genetic composition of soil fungal communities measured using ITS region-sequencing analysis in the surface soils collected early in 2018 cotton season in the Northstar experiment. (A) Results from redundancy analysis at OTU level showing treatment induced grouping of fungal community, data points closer to each other show greater similarity (B) microbial diversity indices as influenced by crop rotation.

Overall, observations on the changes in the microbial diversity and activity in the short-term rotation experiment at Northstar, NSW clearly indicated the significant and important contribution of soil

microbiome for the suppression of verticillium disease in cotton. The influence of rotation crops such as sorghum and corn could be attributed to (i) increased microbial catabolic diversity and activity (ii) higher diversity of bacteria and fungi, (iii) increased abundances of specific groups of microorganisms involved in antibiosis, antifungal (cell-wall degradation) and plant growth promoting capabilities, and (iv) lower pathogen levels. These changes would have contributed to the suppression of the pathogen, disease incidence and impact. Whereas the fallow treatment caused a significant decline in the total microbial activity and catabolic diversity, genetic diversity of bacteria and fungi resulting in lower pathogen suppression capacity. Although the lower pathogen levels would help in the reduction of disease incidence, long-term adoption of such management practices would not benefit in maintaining or improving the overall soil biological health. The traditional continuous cotton system seems to promote the growth of pathogenic fungi such as *V. dahliae* and result in lower microbial diversity and abundances of beneficial microorganisms.

Biocontrol of plant diseases through the addition in individual microbes often show promise in the controlled environment conditions where soil and environmental conditions are less varying. However, in field situations where soils are heterogenous and environmental conditions are dynamic, the success of such inoculants have been inconsistent and not very successful. Additionally, the control of soilborne fungal pathogens and their disease impacts involves multiple interactions and over a long period i.e. reduction of pathogen levels, growth of pathogen from source of inoculum to the host root, infection and disease incidence etc. Therefore, natural disease suppression involving a group of microorganisms with diverse range of capabilities have a greater chance of reducing the overall disease impact. For this, harnessing the potential of the microbiome indigenous to cotton soils through application of specific management practices that modify the local microbial community promoting beneficial microorganisms would be a better way to decrease the impact of plant diseases and increase plant health (Gupta et al. 2011; Mazzola and Freilich 2017).

In addition to the plant type-based variation in the microbial communities, crop rotation effects are attributed to the differences in the quality and quantity of carbon inputs i.e. a substrate mediated phenomenon. Recently it has been suggested that addition of organic amendments such as chitin amendments can modify soil bacterial communities promoting a wide array of genera that can suppress disease caused by *V. dahliae* in Eggplant (Inderbitzin et al. 2018). Similar reports have been reported for other diseases by the addition of composts (Hoitink and Boehm, 1999; Mazzola et al. 2015). Previous research has shown that addition of composts @10 t/ha to cotton soils only caused a short-term increase in microbial activity (Gupta et al. 2016). Thus, the addition of such treatments could alter microbial composition and activity in the short-term, to be effective and cause significant changes over long-term would require addition of large amounts of material and multiple applications to maintain the short-term boost in the activity and cause true changes in the microbial community composition. At present, there is no information on the effect of such amendments on soil microbial community related to disease suppression in Australian cotton soils.

Finally, in view of the field/region-based variation in soil microbial communities (esp. fungi) in cotton soils, the specific groups of microbes that are influenced by rotation need to be verified to determine the magnitude of benefit that could be harnessed to reduce disease impacts.

**Objective 3: Genetic composition and diversity of soil fungal communities in cotton fields**
Research from the previous project showed a location based (soil type and environment) variation in fungal communities in cotton soils i.e. diversity and abundance of soil fungal community varied significantly in experimental plots located at ACRI, northern NSW and Qld. This has implications to the development of management options to manipulate both beneficial and pathogenic fungi.

Results:
Results from the genetic analysis of fungal community in soils from the three seasons indicated an average of 316 genera (ranging from 297 to 326) covering 432 OTUs/species (ranging from 149-697) in farmer fields from 8 cotton growing regions. These results confirm previous findings from experimental fields at ACRI and northern NSW and southern Qld. The presence of a diverse fungal community indicates the potential to utilize the general fungal community to manipulate biological functions including disease suppression, plant nutrition etc.

Results from the three cotton seasons have shown significant differences in the population abundance, diversity and genetic composition of fungal communities in cotton soils both at the field level and cotton growing region. For example, genetic diversity of fungal community indicated by alpha-diversity measures such as Shannon index (H) values were significantly generally lower in the soils from St. George and Emerald regions compared to that in the Darling Downs and Namoi region soils and similar trends were observed for fungal community species richness (d) values (data not shown). it is known that crop diversity would have a significant effect on the diversity of soil microbes including fungi. Preliminary analysis of data for 2018-19 season indicated that alpha-diversity measures were generally higher in low-disease field compared to their companion/nearby high-disease fields (e.g. McIntyre-Boggabilla, Verticillium; St George-Fields 1 and 4, Fusarium) confirming the trends observed in the field experiment at Northstar, NSW.

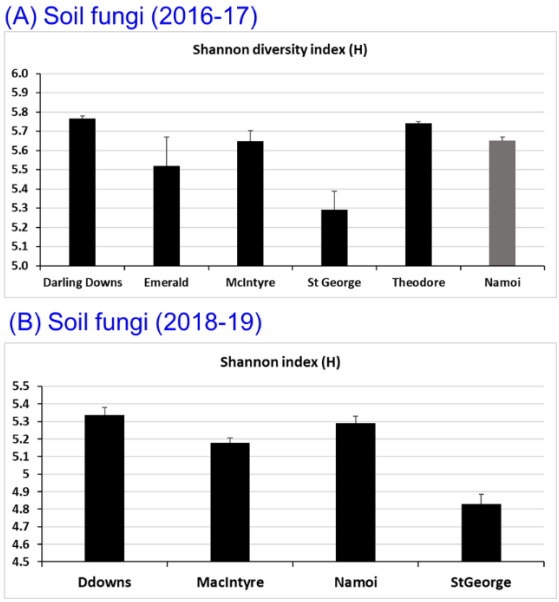



**Figure 139.** Genetic Diversity of soil fungi in surface soils from different cotton growing regions.

In general, fungal abundances significantly ($P<0.01$) varied between fields in all regions and in all three seasons (Figure 140). There were varying degrees of seasonal variation in the fungal abundances in the different regions which could be attributed to varying cropping and management histories (Figure 140). Overall, there was no was no clear relationship between the abundance of fungi and the level of disease incidence, for example during 2018-19 season fields from McIntyre and St George with varying levels of disease incidence didn't not show any systematic variation in the fungal abundance. This is not unexpected as pathogenic fungi are also included in the total fungal abundance. It is known that crop rotation, tillage and agrochemical application can significantly influence soil fungal communities (Gupta et al. 2019).

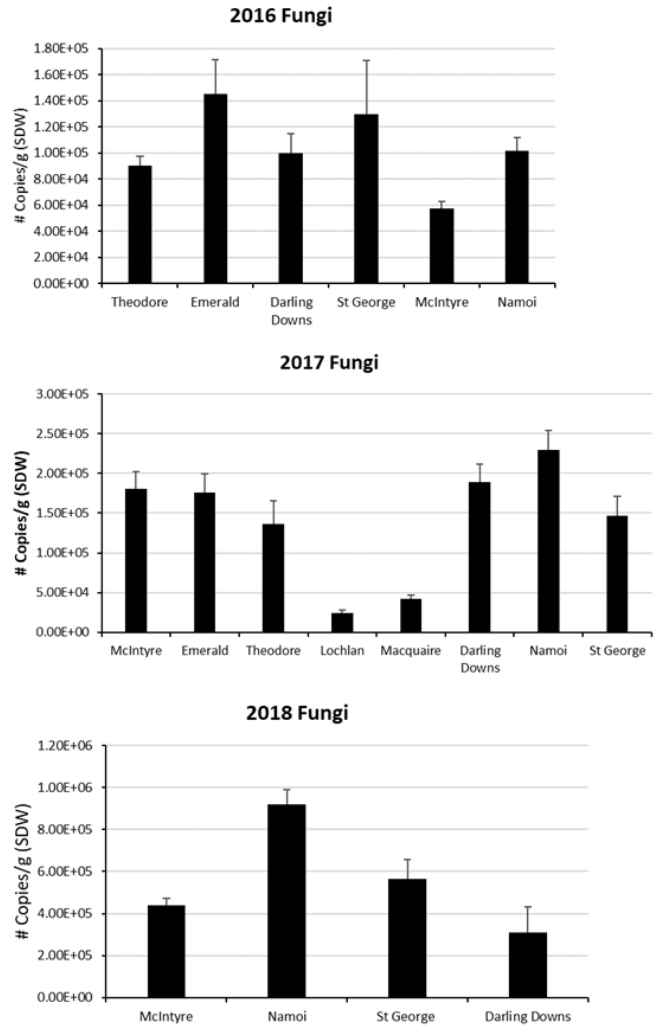



**Figure 140.** Abundance of soil fungi in surface soils from different cotton growing regions.

**Beneficial fungi - Mycorrhizal fungi (Phylum: Glomeromycota):**
The importance of mycorrhizal fungi (VAM) to cotton plant nutrition esp. P and Zn is well known. While the various management practices have been suggested to affect mycorrhizal colonization there is no information about population structure of mycorrhizal fungi. Fungal species belonging to Glomeromycota are considered as mycorrhizal fungi. Results from the three seasons indicated that Glomeromycota species accounted for 0.1 to 0.2% of total fungal communities in the cotton soils which is less than that previously found in the remnant bush soils from the ACRI in Narrabri (>2.2%) (Gupta et al. 2016). This could be partly attributed to the P fertilization in cropping fields compared to no P application in the native vegetation. Also, it is known that Glomeromycota generally account for 1-2% of total fungal communities in agricultural soils, in particular in non-rhizosphere soils. Our results are for soil near the plant row and not specifically rhizosphere soil. A total of 5 genera (*Glomus, Rhizophagus, Funneliformis, Diversispora and Claroideoglomus*) and 18 species were observed in all our soil sample. *Glomus* and *Funneliformis* were the most abundant genera. Mycorrhizal colonization of host species has been reported to be influence by management practices such as intensive tillage, repeated application of agrochemicals etc. In view of the limited number of genera/species observed, further research on which species of fungi actually colonize modern cotton varieties and identify management practices that promote their populations.

**Table 48.** Genera and species of mycorrhizal fungi observed in cotton soils.

| Genus | Species |
|---|---|
| *Diversispora* | *Paraglomus occultum* |
| *Funneliformis* | *Glomus dimorphicum* |
| *Glomus* | *Glomus monosporum* |
| *Rhizophagus* | *Glomus aggregatum* |
| *Claroideoglomus* | *Glomus custos* |
| | *Glomus indicum* |
| | *Glomus irregulare* |
| | *Glomus_hoi(JF439206)* |
| | *Glomus_geosporum(AJ319796)* |
| | *Glomus_sp_8_SUN_2011(JF439098)* |
| | *Rhizophagus custos* |
| | *Rhizophagus diaphanus* |
| | *Rhizophagus fasciculatus* |
| | *Rhizophagus_irregularis(FJ009607)* |
| | *Rhizophagus_intraradices(FM865568)* |
| | *Funneliformis_caledonium(JQ048773)* |
| | *Funneliformis_mosseae(JQ048808)* |
| | *Diversispora_sp_JP_2011(FR873629)* |

**Beneficial fungi - Nematophagous fungi:**

Nematophagous fungi are one group of beneficial soil fungi that feed/predate on nematodes and are considered to be beneficial as they could help reduce the impact of plant parasitic nematodes. Recently *Arthrobotrys* species have been suggested as biological inoculants to reduce some parasitic nematode impacts. Interrogation of the sequence data identified that there are five *Arthrobotrys* species in the cotton soils analysed i.e. *A. cladodes*, *A. thaumasia, A. conoides, A. oligospora*, *A. superba* and *A. cladodes* was the most abundant in the majority of soils (Figure 141). Several members (>700) belonging to phyla *Ascomycota, Basidiomycota, Chytridiomycota* and *Zygomycota* have been suggested to have Nematophagous properties (Soares et al. 2018). Most of the research in Australian cotton systems is on the plant parasitic nematodes. This is the first reported observation about the presence of this group of nematodes in Australian cotton soils. Soil nematodes represent a suitable group for monitoring soil environments due to their sensitivity to perturbations, nutrient conditions, and toxic elements. Further analysis of the data is required to properly identify if specific factors affecting the true diversity of this group of nematodes in cotton soils.

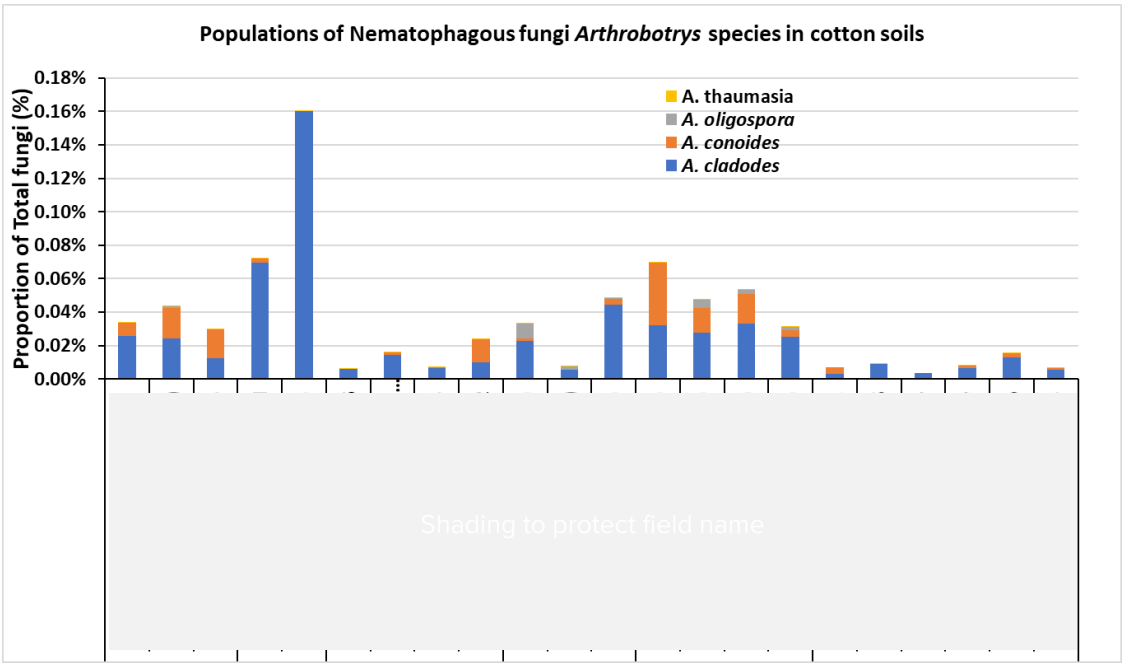



**Figure 141.** Relative abundance of Nematophagous fungi *Arthrobotrys* species in farmer field soils from different cotton growing regions collected during 2016-17 season.

**Genetic composition of soil fungal community:**
Ascomycota were the most dominant group of fungi in all the soils accounting for 49-98% of total fungi followed by Basidiomycota (1-47%), Zygomycota (1-9%) and as indicated before Glomeromycota were accounted for <0.5% in all three seasons. While the absolute abundances of individual groups of community varied between seasons, the relative abundances and trends remained the same in cotton fields from all regions. A comparison of fungal community in the cotton fields with that from a nearby native vegetation, at ACRI, showed significant differences in the community composition in terms of relative abundances of various groups (Gupta et al. 2016). Results from this study clearly indicated that fungal community in cotton fields is generally dominated by the Ascomycota group whereas members of phyla Basidiomycota and Zygomycota are lower than seen in undisturbed native vegetation soils. In the remnant field soil *Zygomycota* and *Basidiomycota* were a significant part of the soil fungal community (Gupta et al. 2016). While there was some variation in the proportion of different phyla, soils from St George region generally showed higher proportion of Basidiomycota in all seasons compared to that in soils from Namoi and McIntyre regions. Between seasons, the relative abundance of Ascomycota group was highest in 2018-19 season 78-93% (ave. 89%) compared to 32-90% (ave. 70%) in 2017-18 and 68-78% (ave. 75% in the 2016-17 season which could be attributed to the seasonal environmental conditions.

Within the two dominant phyla, fungal genera belonging to the Classes Sordariomycetes, Agaricomycetes and Dothideomycetes were the most abundant groups in all regions and all three seasons. For example, during 2016-17 season fungal general belonging to the Classes Sordariomycetes (43 to 53%), Agaricomycetes (9-21%) and Dothideomycetes (5 to 17%) were not only the major groups but also showed distinct differences between locations (Figure 142). Differences in fungal community composition between fields and regions extends Family level composition. Although the community in cotton soils shows a broad diversity of Families, i.e. 105 to 140 families in different seasons, 25 to 30 of the most abundant families accounted for >90% of the total population and the most abundant 10 families accounted for 60-86% of the community (Figures 143 and 144). Some of the examples of Families showing significant variation between regions and fields include: Trichochomaceae, Chaetomiaceae, Nectraceae, Didymellaceae, Lasiosphaeriaceae, Hypocreaceae etc. A number of genera/species belonging to the families Chaetomiaceae and Hypocreaceae have been reported posses hypoparasitic and biocontrol capabilities and shown to be part of microbial communities in disease suppressive soils (Penton et al. 2014). In spite of the differences in the community composition between fields in all seasons, there is an identifiable variation in the composition at family and Class level. Additionally, this variation in relative proportion of various groups extended at genus levels, with distinct differences in a number of groups between soils however significant genus level variation was also observed (data not presented). The presence of such fungal species in cotton soils and that they seem to be responding to differences in management practices suggest the potential to manipulate them to harness their beneficial capabilities. Future research investigating the changes in specific groups due to management practices involving rotation and application of fertilizer, compost or agrochemicals is needed to modify their abundance and associated functions.

Comparison of fungal community composition from beta-diversity analysis (generated using the Bray-Curtis distance matric) showed significant dissimilarity between different cotton fields and different fields within each region. PERMANOVA analysis showed that region-based variance explained 26% and 22% of variation (P=0.001) during 2016-17 and 2018-19 seasons, respectively suggesting distinct region-based similarity in the community composition (Figure 145). Field based variance explained 33% and 30% of variation (P=0.001) in fungal community during 2016-17 and 2018-19 seasons, respectively (Figure 146). In spite of field-based variation significant region-based variation persisted in all seasons. For example, large differences in fungal community composition between fields in St George vs. Namoi and Darling Downs regions, whereas there were similarities in community composition in McIntyre region compared to that with some fields in St George and Namoi (Figure 146). Results from the analysis of community composition at different taxonomic levels indicated that significant region-based variation at OTU/species level extended to Genus and Family level grouping (Figure 147), although field level variation at higher taxonomic level was smaller. Based on the significant PERMANOVA results, the nature of the region or field-based differences and their relationships to soil physicochemical properties (as predictor variables) were tested using distance based linear model (distLM) analyses using the BEST solutions option to identify variables that explained statistically significant proportion of variation in fungal

community structures (Clarke and Gorley, 2006). Results indicated that pH, Exchangeable Ca, Mg, organic C, Ca:Mg ratio, CEC, Zn and %silt were the significant ($P<0.001$) variables explaining the variation (predictors) in fungal composition between sites.

It is generally considered that soil microbial community can show large variation at smaller spatial scales (microsite) within a field would mask differences at field or region scale therefore a comprehensive sampling protocol is required to reduce within a field variation masking the effects of management history and climate. We have implemented a systematic sampling protocol and multiple replicates (a minimum of 3 in each field) and soil samples (8-10 samples in each replicate) when collecting soils to cover smaller scale variation within a field. This has resulted in consistent observation of significant differences between fields in fungal community composition in all seasons i.e. replicate based variation didn't mask field-based differences (Figure 147). Results from the microbial community and activity analysis obtained indicate that the sampling protocol implemented has reduced the effects of within a field (site) variation allowing us to use microbial and molecular methods to measure soil biological properties in farmer field soils.

Overall, this study demonstrated the presence of a genetically diverse fungal community in cotton soils and distinct variation in the community composition and diversity between fields within each region and between regions. The region-based similarity was observed in all three cotton seasons. Despite large diversity, a majority of the fungal community (>60%) was accounted by a small number of Family level groups which were also found to vary between fields and seasons suggesting that management practices (rotation, fertilizer application etc) may have significant impact on the community composition. Similarly, large field-based differences were observed in the abundance of fungi in all seasons which could be attributed to both soil/edaphic and management factors. Results from the Northstar rotation experiment indicated that the significant effects of crop rotation on the diversity, abundance and genetic composition of soil fungi. For example, treatments with Sorghum and Corn as rotation crops supported more diverse fungal community compared to that in the Continuous cotton and fallow treatments. Treatments with Sorghum and Corn also showed lowest verticillium disease incidence. These results confirm the observations from ACRI experiments that the diversity and abundance of soil fungal community varied significantly by crop management history and fungal communities in suppressive cotton soils were characterized by higher diversity and higher connectedness. Additionally, it was evident that although rotations with fallow could decrease pathogen inoculum level and in turn reduce disease incidence, fallow treatments caused a significant decline in the abundance and diversity of soil fungal communities. Also, the effect of fallow didn't disappear after the cotton crop suggesting a longer-term negative impact on soil microbial community and associated biological functions.

Data for the genetic composition and relative abundances of beneficial fungi indicated that members of fungal community known as mycorrhizal fungi (Glomeromycota) only accounted for <0.1% of total community and only a small group of genera (5) / species (18) were observed suggesting the necessity to monitor management effects in order to full harness the benefits from mycorrhizal association with cotton plants. Another surprising finding is the observation of the wide spread presence of the six species of the genus *Arthrobotrys* a putative Nematophagous fungi in >80% of fields analysed. The functional significance of this group of fungi in controlling plant parasitic nematode effects is not known.

Additionally, this study is mostly a descriptive genomics-based investigation identifying the nature of the community in Australian cotton soils and influencing factors and it should be extended to understand the functional role of the different groups present to understand the functional importance of the specific members of microbial community.

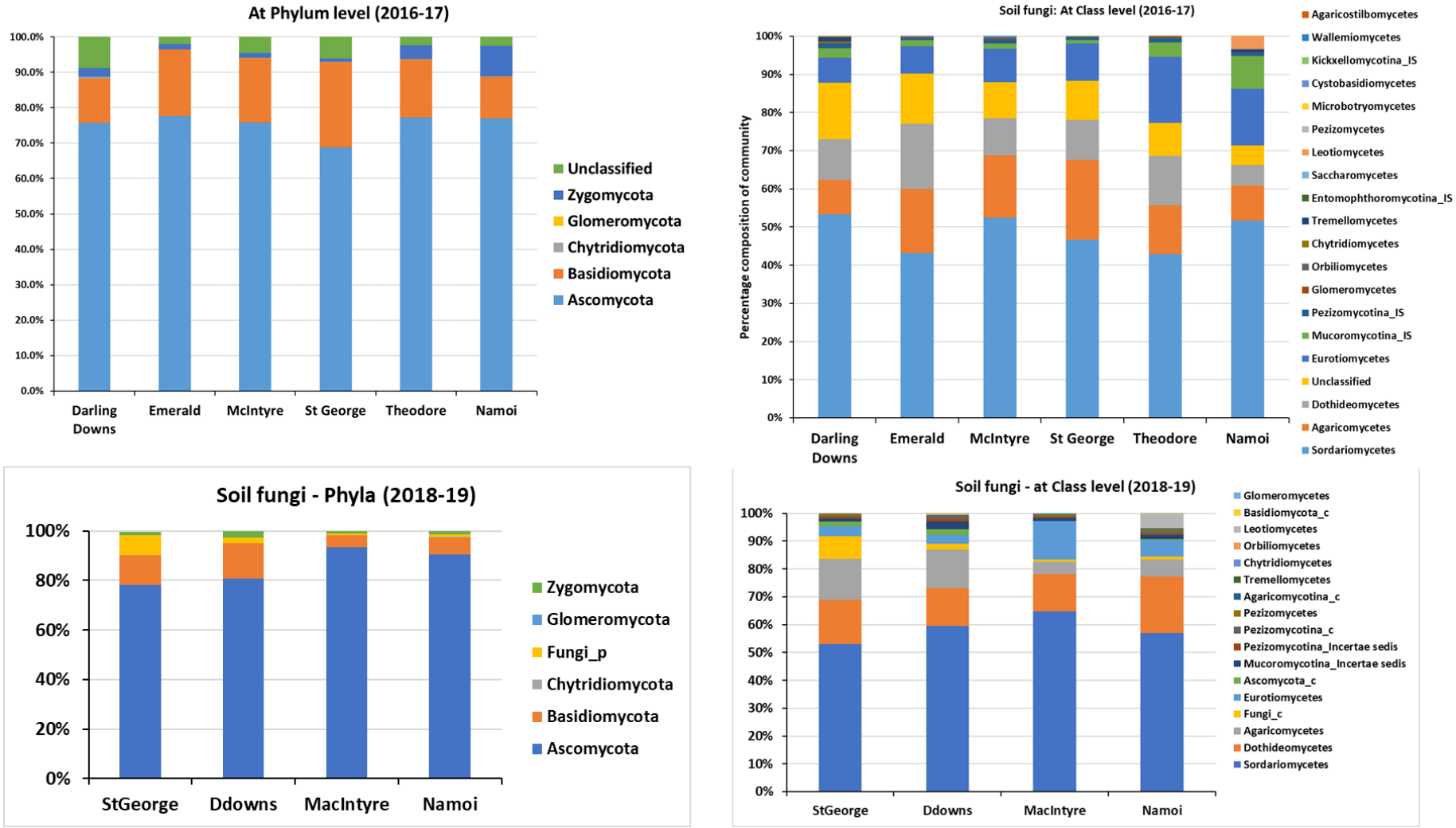



**Figure 142**. Relative abundance of soil fungi at Phylum and Class level in farmer field soils from different cotton growing regions collected during 2016-17 and 2018-19 seasons.

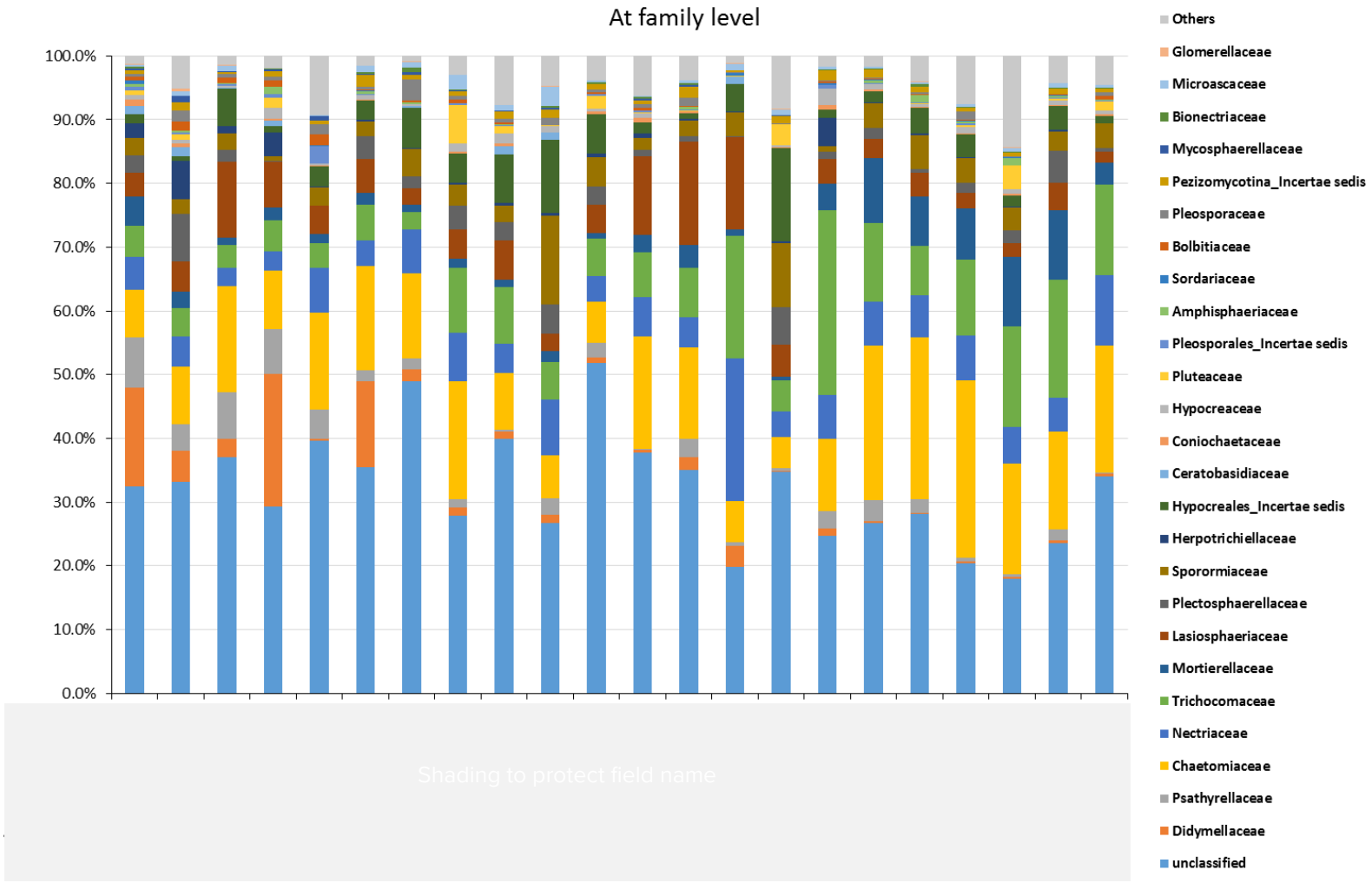



**Figure 143.** Relative abundance of soil fungi at Family level in farmer field soils from different cotton growing regions collected during 2016-17 season.

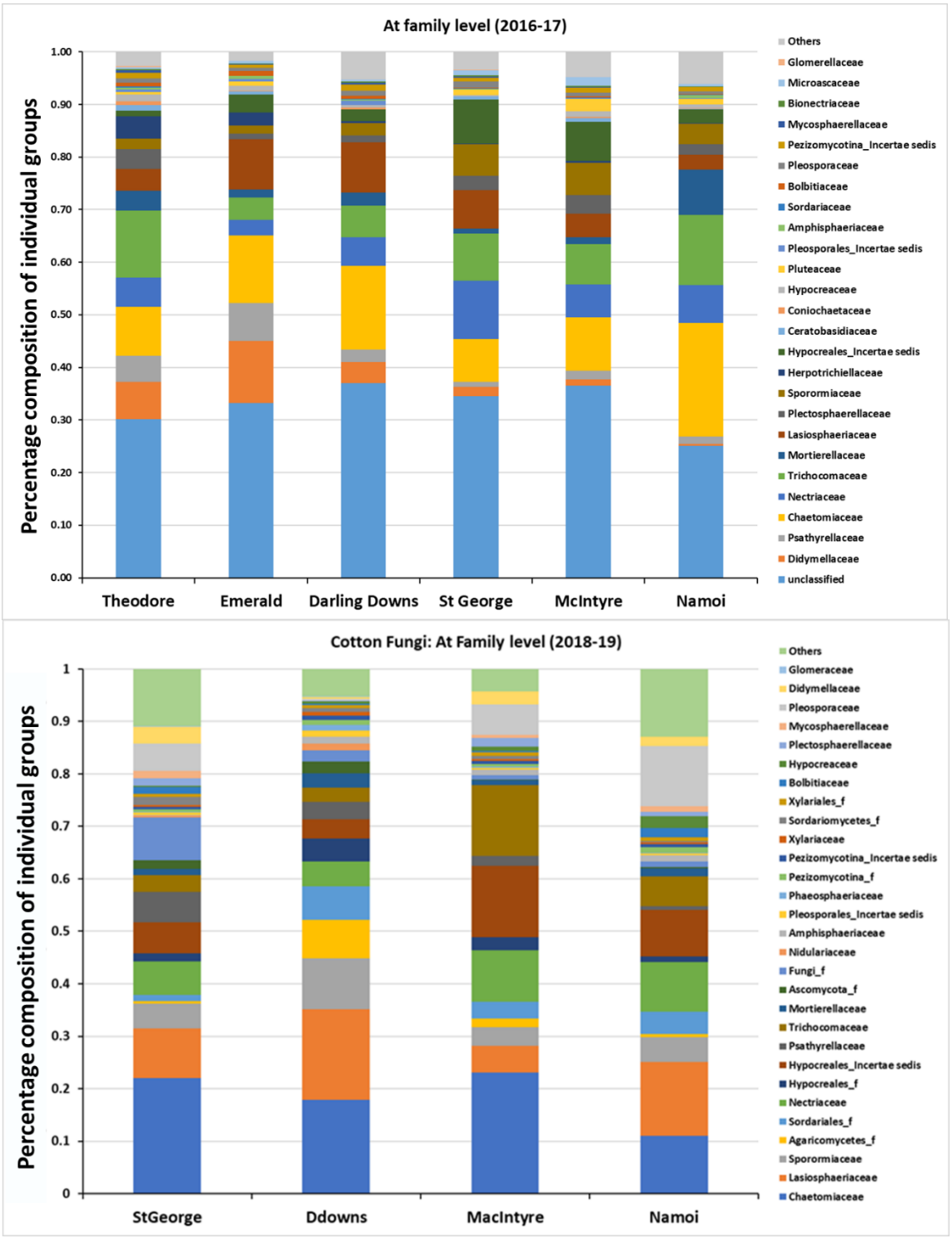
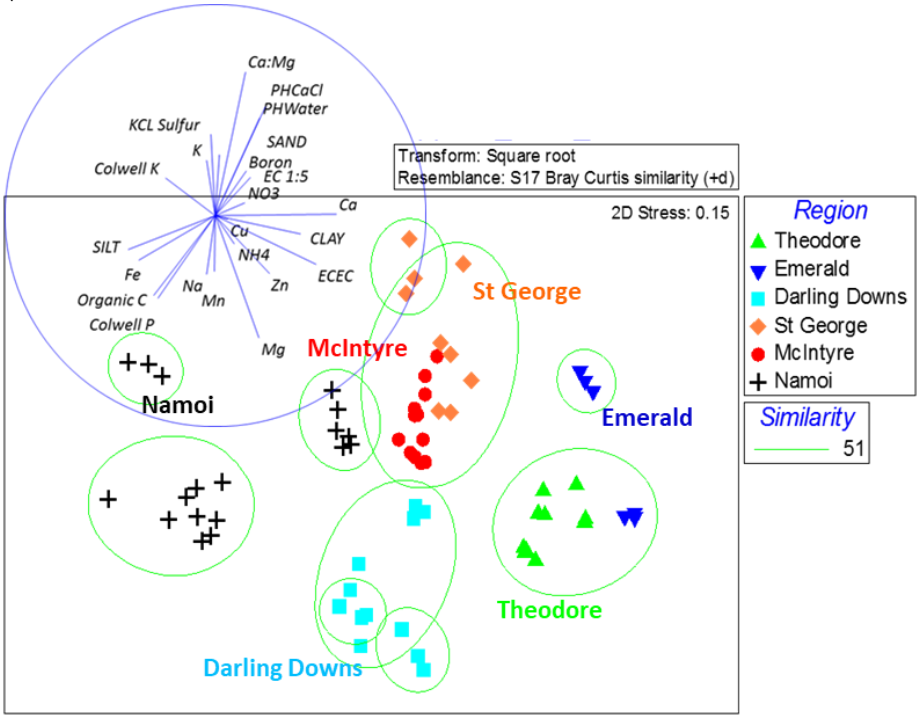



**Figure 144.** Relative abundance of soil fungi at Family level in farmer field soils from different cotton growing regions collected during 2016-17 and 2018-19 seasons.

(A) 2016-17 season




(B) 2018-19 season

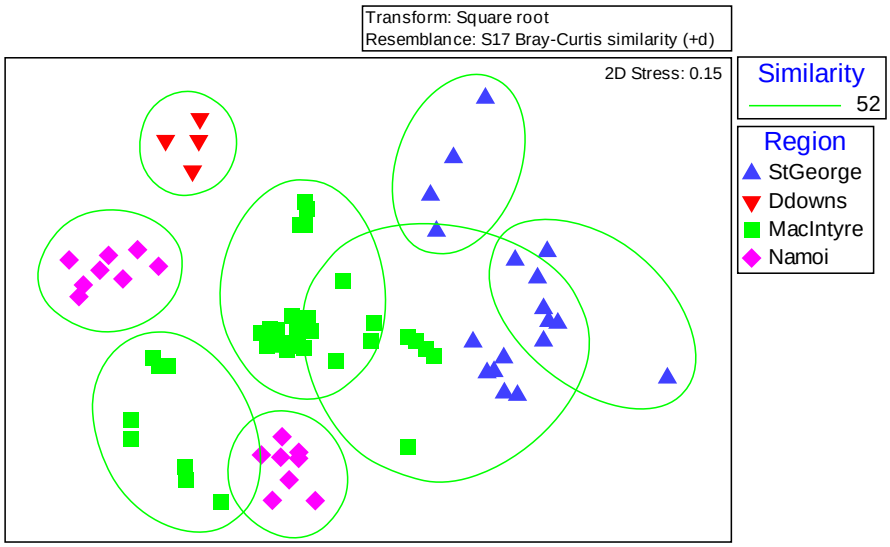



**Figure 145.** Genetic composition of soil fungal communities measured using ITS region-sequencing analysis in the surface soils collected from farmer fields and experiments in different cotton growing regions. Data points closer to each other show greater similarity.
2016-17: Region based variation – PERMANOVA CV= 26%, P=0.001
2018-19: Region based variation – PERMANOVA CV=22%; P=0.001

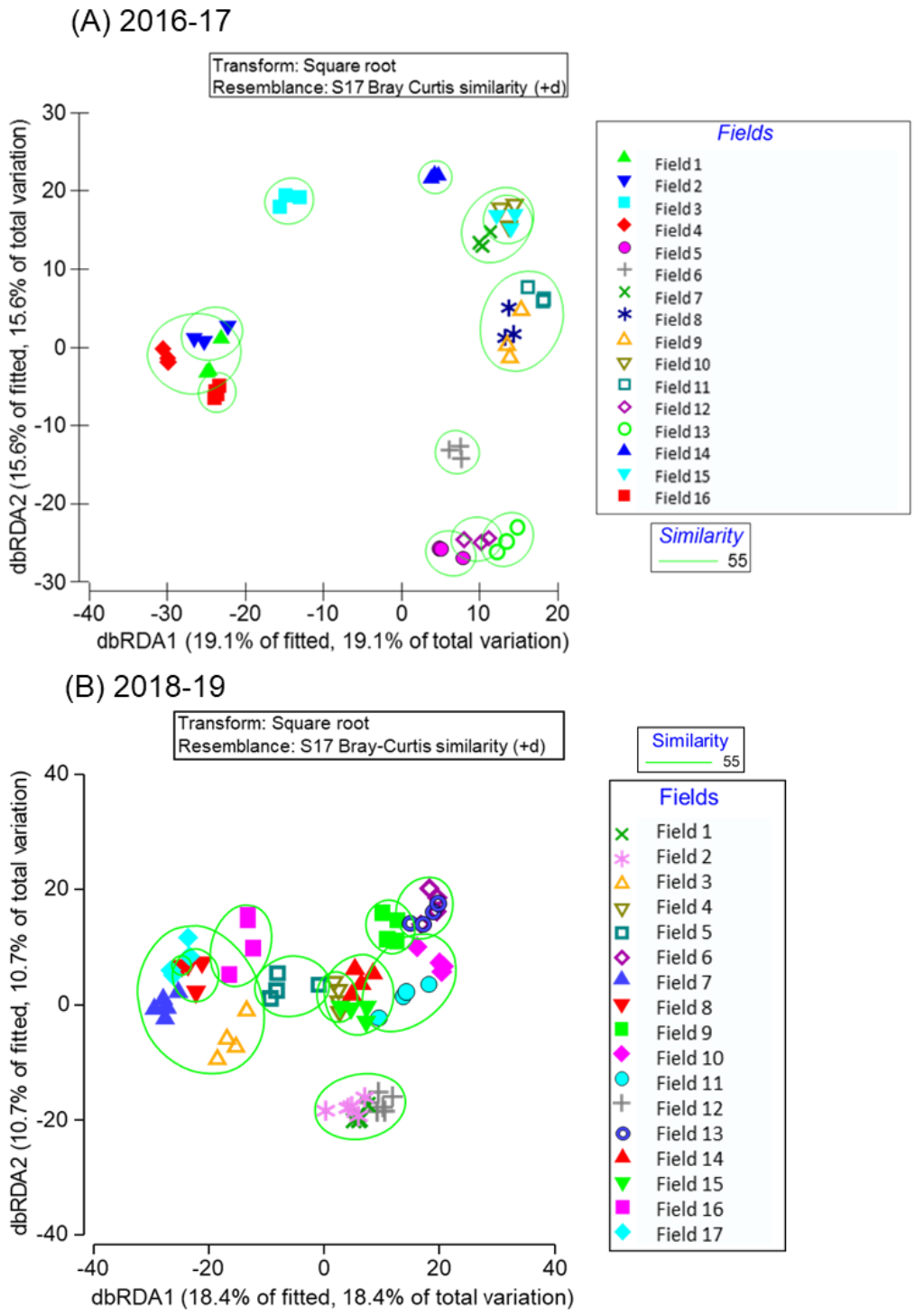



**Figure 146.** Genetic composition of soil fungal communities measured using ITS region-sequencing analysis in the surface soils collected from farmer fields and experiments in different cotton growing regions. Data points closer to each other show greater similarity.
2016-17: Region based variation – PERMANOVA CV= 33%, P=0.001
2018-19: Region based variation – PERMANOVA CV=30%; P=0.001

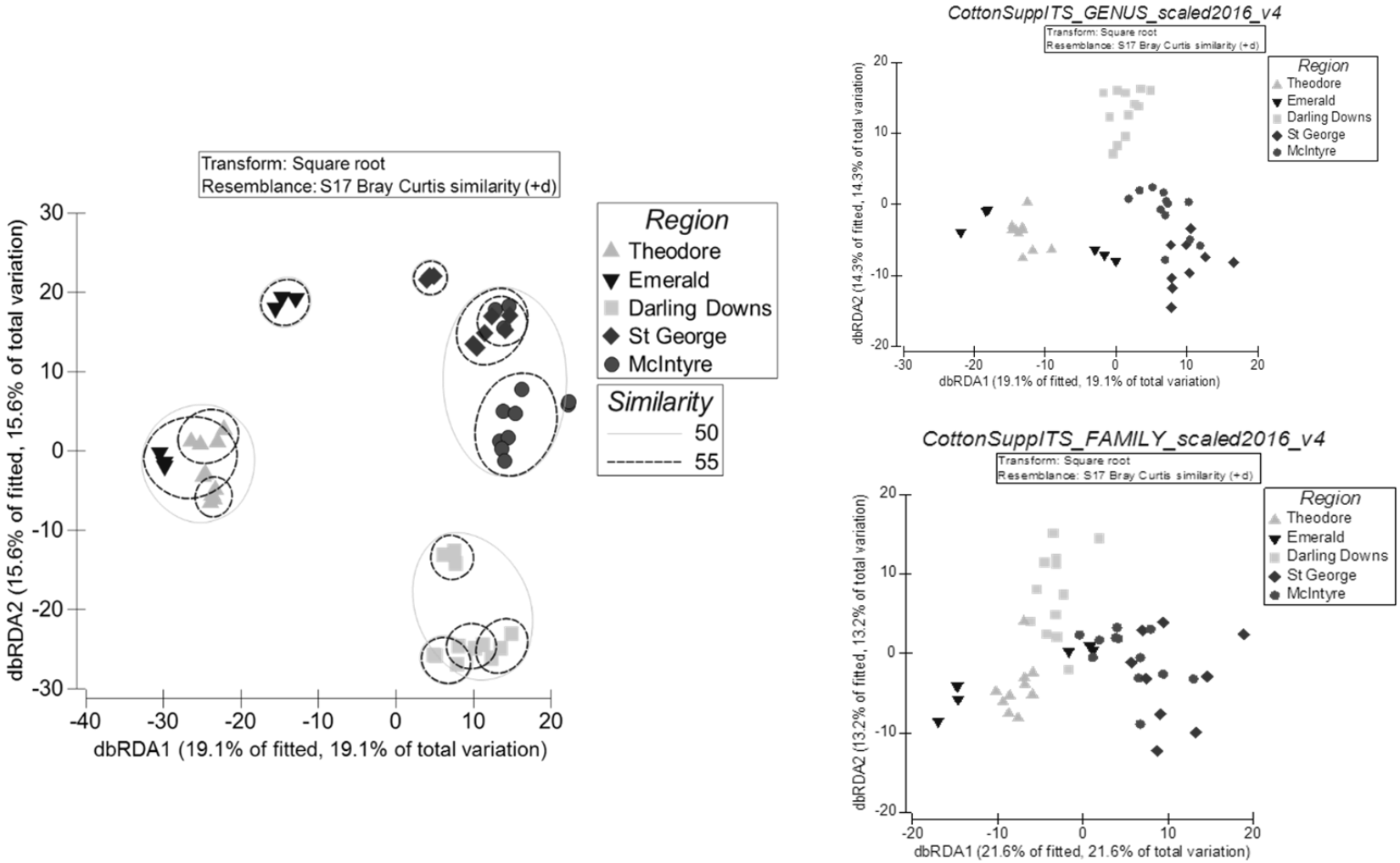



**Figure 147.** Genetic composition of soil fungal communities in the surface soils collected from farmer fields in different cotton growing regions in Queensland. Left – Individual fields at OTU/species level, Top right – at Genus level; Bottom right – at Family level.

## 4.2 Evaluate the impact of management on total microbial catabolic activity and beneficial microbial community composition in different soil types.

**Milestone 4.2 Catabolic diversity and microbial biomass**

Soilborne plant pathogens interact with general soil microbial community both during the non-crop phase affecting their survival, growth and their ability to reach host plant root. Additionally, interactions between general microbial community and plant can impact plant nutrition and ability to tolerate biotic and abiotic stress thereby influencing the impact of the disease, its severity and overall plant health. Cropping practices that affect soil habitat structure (e.g. Tillage), availability of food sources (C) from crop residues (e.g. residue retention and rotation) and agrochemical application are known to influence the total microbial biomass (MB) and its ability to response to favourable conditions. For example, the size of total microbial biomass and activity are significantly influenced by soil physico-chemical properties (Gonzalez-Quiñones et al. 2011; Gupta et al. 2019). While soil MB, the living component of SOM, comprises a small percentage of total soil organic matter, it serves as the engine for all biological functions. Previous research has shown that microbial catabolic diversity (based on carbon substrate utilization profiling) was significantly different between disease suppressive and conducive soils (Gupta et al. 2009).

Results:

Surface 10 cm soils from cotton fields and experiments generally contained 0.8 to 1.2% of soil organic C with a C:N ratio between 11.4 to 12.7. Mineral nitrogen concentrations (nitrate and ammonium N) in the surface 10 cm soils ranged between 4 and 212 kg N / ha with an average of 64 kg N/ha. Dissolved organic N (DON) accounted for 29±0.01% of total soluble N in soils indicating that inorganic N accounts for major part (3:1 ratio of DON:min N) of soluble N pool. Soil microbial biomass levels in the three seasons were 327±21, 404±32 and 335±23 μg C / g soil in the samples from 2016-17, 2017-18 and 2018-19, respectively. There was no systematic trend for the amount of MB in fields between regions although there were significant differences between field within a region. For example, during 2016-17 season MB values ranged between 193 to 590 μg C/g soil (Figure 148). During 2017-18 season, MB levels were generally lower in farmer field samples compared to a research experiments, in particular in experiments with rotational crops including millet, sorghum, forage sorghum, corn. Soils from Fallow treatments (e.g. Northstar experiment) and some farmer fields generally showed significantly lower MB (>30%) compared to that under rotational crops. The term Microbial quotient (MQ) representing the amount of MB per unit soil organic C is used in representing large variation in MB values and for comparing soils across varying textures and regions i.e. as microbial index for soil biological fertility or health of soil. MQ values for the cotton soils measured ranged between 3.1 to 3.9 in the three seasons. Crop rotation treatments such as fallow in the Northstar experiment showed lowest MQ 2.2 to 2.3% whereas soils under the rotation crops Sorghum, Corn and Forage sorghum showed higher MQ values (3.0 to 3.9%) indicating that microbial populations in cotton soils at these sites are constrained by the availability of biologically available carbon.

The amount of soil MB is generally positive correlated with soil organic C levels and the strength of the relationship is considered as an indicator of short-term C inputs regulating the dynamics of microbial populations. Data in this study showed a weak relationship between MB and SOC (R=0.16) when all the three season results were included, however during 2018-19 the relationship was significantly positive (R=0.57, P<0.05). No significant relationships between MB levels and mineral N or DON levels.

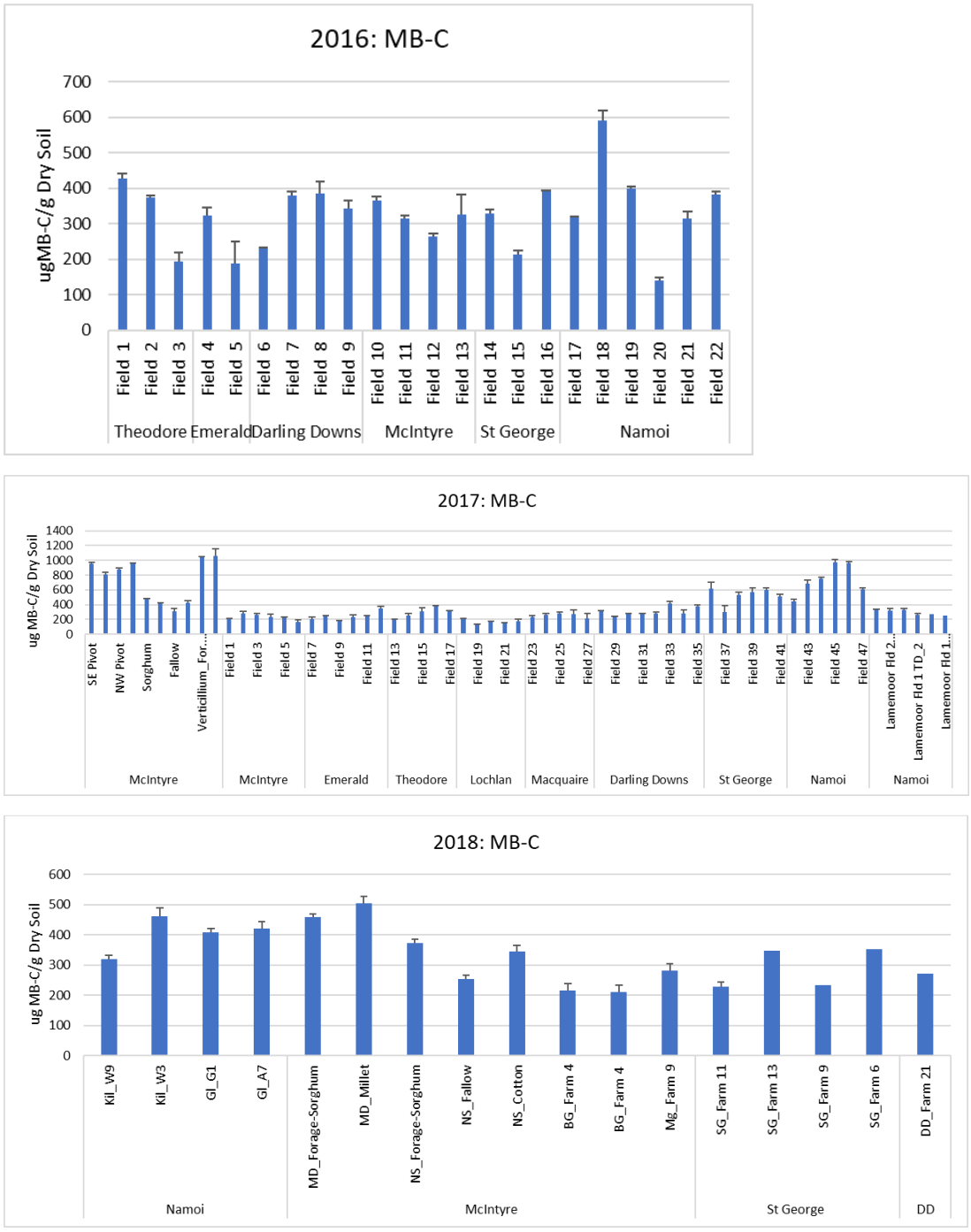



**Figure 148.** The amount of microbial biomass C in surface soils from cotton fields from different cotton growing regions. Data includes results for soils from farmer fields and experimental plots.

During 2018-19, a significant positive relationship was also observed between MB and dissolved organic C levels (R=0.68, P<0.05) indicating that availability of C was the major factor influencing the amount of MB. These results suggest that either SOC values are not a true reflection of biologically available organic matter or the presence of other constraints for the build-up of MB. In many Australian soils a significant pool of recalcitrant C due to a history of burning has been reported (Skjemstad et al. 1999) and in such soils, relationships between SMB and soil organic C tend to be less well defined.

Reports from a wide range of agricultural and Horticultural soils across Australia and overseas suggests that a MQ value of 5% is considered as *attainable* in disturbed agricultural systems and could be used as

a simple 'rule of thumb' for *attainable* level of microbial populations provided there are no other constraints. In this study, MQ was generally lower than this value in approx. 84% samples, except for a very few soils from rotation crop fields (Figure 149), suggesting other constraints other than C availability may be restricting the build-up of MB in these cotton soils. Previous research in grain cropping systems indicated that soils where MB is limited by C availability, soilborne pathogens may have competitive advantage resulting in increased disease impacts.

The size of total soil organic C is not considered as a good indicator of C availability to microorganisms and short-term changes in soil quality/health, whereas MB and MQ which responds to short-term management practices and thus a good indicator representing microbial dynamics and biological functional capability including biological disease suppression potential.

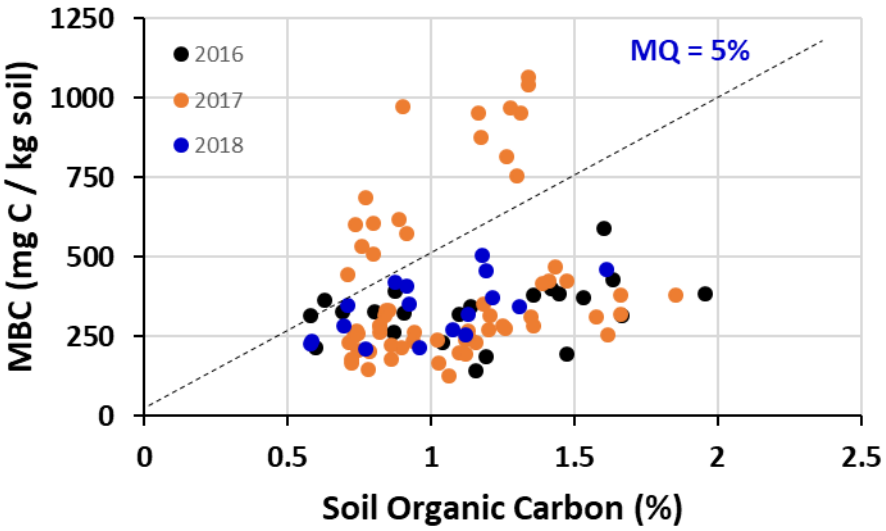



**Figure 149.** Soil microbial biomass C (MBC) plotted against total soil organic C (%) for all cotton soils from different regions including farmer fields and experimental plots from the three seasons. Dotted line indicates a microbial quotient at 5%.

Results from the activity-based assay measuring multiple C substrate utilization capacity provided an indication of the responsiveness of the different members of microbial community to C from SOM and management practices that add external C sources. Microbial activity and catabolic diversity (ability of microbes to respond to availability of carbon substrates) are generally influenced by soil type, C availability and management practices (Coleman et al. 2010). Results from these analyses indicated significant differences in total microbial activity and catabolic diversity between soils from different cotton growing regions and between farmers' fields (Tables 49 and Figure 150). For example, data shown in Table 49 indicates significant variation in microbial response to different types of C substrates. Soils from St George region generally showed lower respiratory response to carbohydrates and amino acid substrates but highest response to Carboxylic acids, whereas response was opposite in the soils from Namoi region. Such variations could be attributed to differences in the genetic composition of microbial groups, soil chemical properties such as organic matter quality and type of C substrates added (e.g. Crop rotation). Additionally, there was significant variation in the catabolic diversity index (CMD) between soils within a region and between regions (Figure 150). However, there was no significant seasonal variation in the CMD values (ranged between 7.3±0.7 and 8.4±0.5).

**Table 49.** Heat map showing comparisons in carbon substrate use efficiency for 31 different carbon substrates by microbial communities in soils from different cropping regions collected during 2016-18 seasons.

| | C substrate | Darling Downs | Emerald | Theodore | McIntyre | St George | Namoi | |
|---|---|---|---|---|---|---|---|---|
| | **Water** | | | | | | | |
| **Carbohydrates** | **Arabinose** | | | | | | | |
| | **Fructose** | | | | | | | |
| | **Galactose** | | | | | | | |
| | **Glucose** | | | | | | | |
| | **Xylose** | | | | | | | |
| | **Mannose** | | | | | | | |
| | **Maltose** | | | | | | | |
| | **Sucrose** | | | | | | | |
| | **Raffinose** | | | | | | | |
| **Aminoacids** | **Hydroxy-L-proline** | | | | | | | |
| | **Glycine** | | | | | | | |
| | **Asparagine** | | | | | | | |
| | **Valine** | | | | | | | |
| | **Serine** | | | | | | | |
| | **Alanine** | | | | | | | |
| | **Glutamine** | | | | | | | |
| | **Tryptophan** | | | | | | | |
| | **Leucine** | | | | | | | |
| | **Phenylalanine** | | | | | | | |
| | **Lysine** | | | | | | | |
| | **Arginine** | | | | | | | |
| | **Histidine** | | | | | | | |
| | **Aspartic** | | | | | | | |
| | **Methionine** | | | | | | | |
| | **Cysteine** | | | | | | | |
| Carboxylic acids | **Fumaric acid** | | | | | | | |
| | **Malic Acid** | | | | | | | |
| | **Malonic Acid** | | | | | | | |
| | **Oxalic Acid** | | | | | | | |
| | **Succinic acid** | | | | | | | |
| | **Tartaric Acid** | | | | | | | |
| | | | | | | | | **LSD(P<0.05)** |
| **Average Metabolic Response (AMR)** | | 5.613 | 10.662 | 9.574 | 5.335 | 7.834 | 6.173 | **2.60** |
| **Community Metabolic diversity (CMD)** | | 2.911 | 6.190 | 4.929 | 3.470 | 5.010 | 3.841 | **1.34** |
| | | | | | | | | |
| | | low | | high | | | | |
| | | | | | | | | |

Note: For each substrate, differences between treatments are shown through colour variation


**Figure 150.** Community metabolic diversity (CMD) values for based on microbial response to 31 different C substrates for soils from different farmer fields and experimental sites collected during three cotton seasons.

Previous research has shown significant differences in soil microbial catabolic diversity and potential in soils from rotations with legume crops compared to those from fallow-cotton and continuous cotton rotations (Experiments at Northstar and ACRI) and CMD was generally higher in soils under treatments with rotation crops compared to Continuous cotton or Cotton-Fallow rotation. In general, fallow treatment significantly reduced CMD and overall catabolic potential (or microbial activity). The observed differences between soils from different cotton growing regions could be attributed to different in soils type and management practices including crop rotation, agrochemical application. Additionally, crop rotation practices including number of cotton crops grown during the last 5-10 years and other management

practices vary between cotton growing regions which would have also contributed to the observed region-based variation.

Overall, surface 10 cm soils in farmer fields from different cotton growing regions contained moderate levels of microbial biomass levels with microbial quotient (MQ) values ranging from 3.1 to 3.9%. There were significant differences between fields demonstrating the effect of management and soil factors, but no systematic region-based variation was observed. Since a 5% MQ value is considered as a minimum attainable level of microbial biomass, the general lower levels of MB suggest that in these low SOC soils, levels of biologically available carbon along with other constraints may be present restricting the build-up of microbial populations. Additionally, observation of higher MB and MQ values in experimental plots and fields with rotational crop that add large amounts of crop residues e.g. sorghum, corn etc suggest further support the potential limitation for microbial activity. Research from grain cropping systems in South and Western Australia indicated that management practices that increase C inputs (e.g. crop residues) and turnover over a number of years (5-7 y) will improve disease suppression and reduce the impact of rhizoctonia disease. The observation of higher pathogen and/or disease suppression potential in treatments with rotation crops where large amounts of crop residues are added, e.g. F6E experiment at ACRI and rotation experiment at Northstar support the hypothesis that in the lower organic carbon cotton soils (i.e. average SOC levels of 0.8 to 1.2%) may be partly restricting the development of agronomically useful disease suppression potential. Rotation crops also modify the C substrate utilization profiles (i.e. catabolic diversity) reflecting the changes in the genetic composition of bacterial and fungal communities. The significant reduction in the MB level, catabolic diversity and genetic diversity of fungi in soils under Fallow further emphasizes the critical role of soil microbial communities in providing beneficial functions such as disease suppression in Australian cotton soils. Finally, in the lower SOC cotton soils in Australia, the adoption of management practices that can improve the amount of microbial biomass and its catabolic potential may be one of the practical and simpler ways to increase biological suppression potential against soilborne plant pathogens.

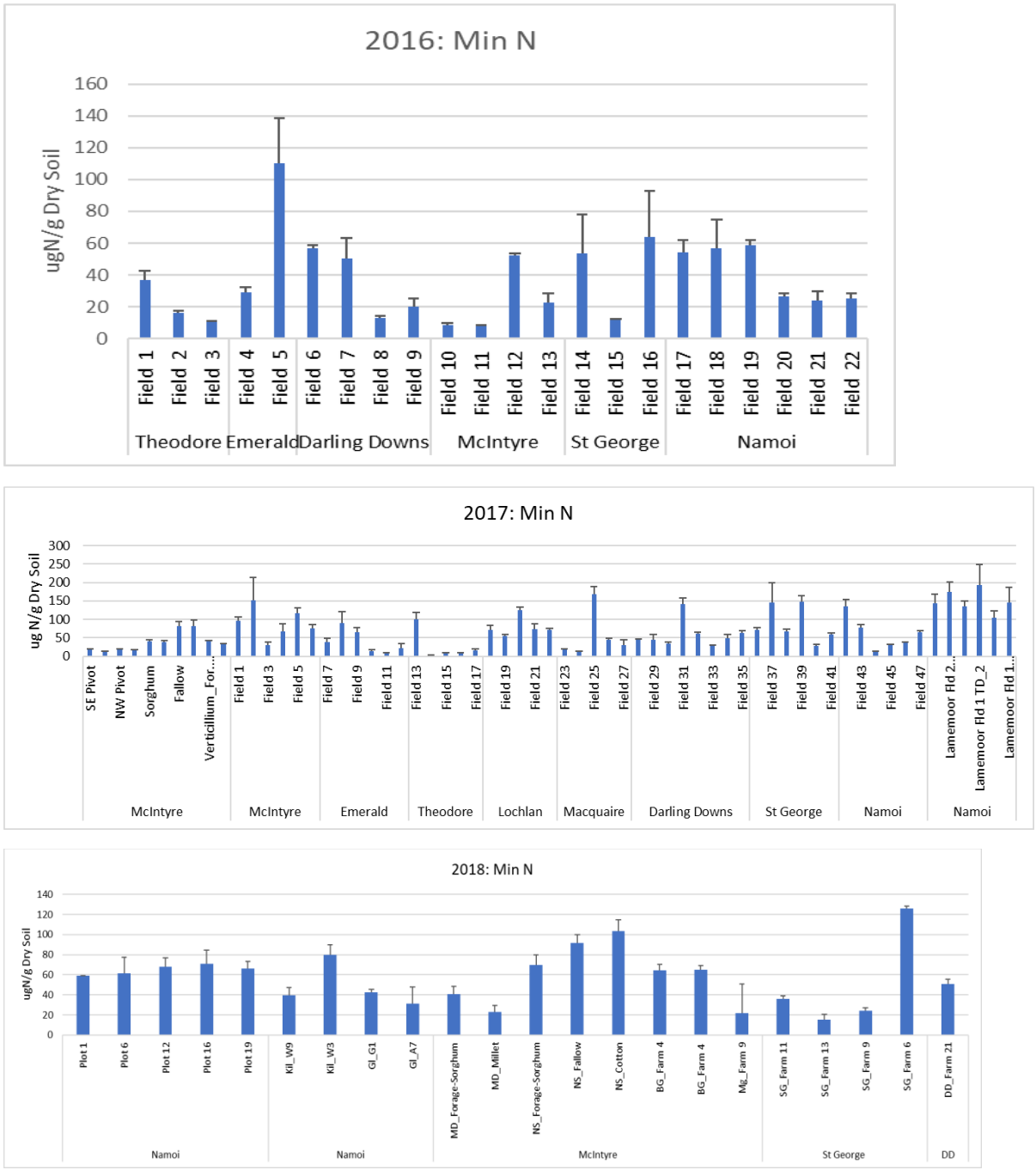



**Figure 151.** Mineral N (nitrate and ammonium N) concentrations at the time of collection for soils from different farmer fields and experimental sites collected during three cotton seasons.

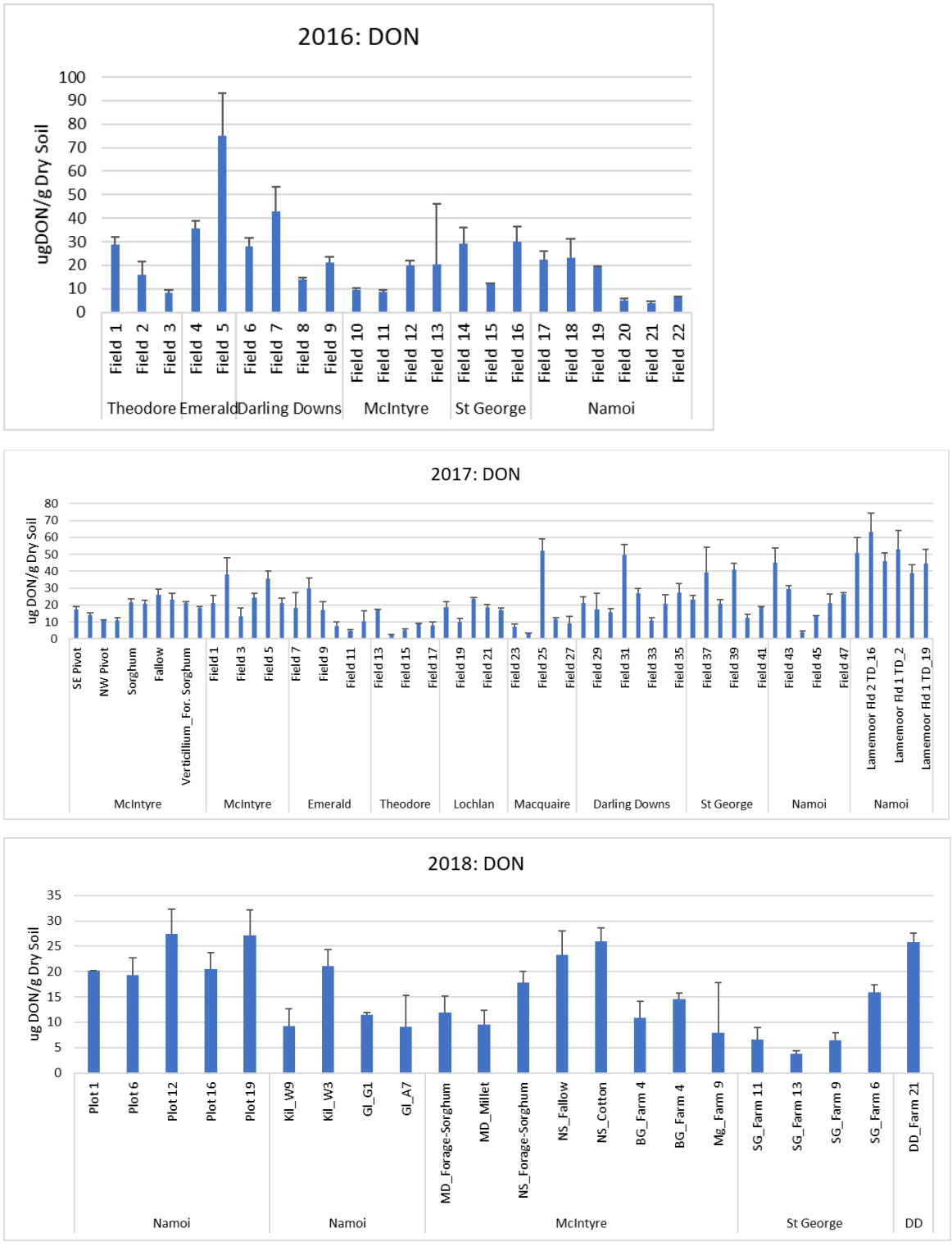



**Figure 152.** Dissolved organic N concentrations at the time of collection for soils from different farmer fields and experimental sites collected during three cotton seasons.

**Bacterial diversity and abundance in cotton soils**

Bacterial diversity in cropping soils has been linked to a number of processes key to plant health, nutrition and overall soil quality. Currently little is known about the composition of bacterial communities in cotton soils. The main objective of this project is to determine the genetic composition of soil bacterial communities and abundances of key bacterial groups in cotton fields across different cotton growing regions. For this soil samples from cotton fields from different regions, assessed for disease incidence as part of annual disease survey, were analysed for the diversity and abundance of soil bacterial groups

using next generation sequencing and DNA based quantitative assays. Also, the effect of rotation crops on bacterial genetic composition were measured for the field experiment at Northstar (Getta Getta). Results were interrogated to identify the key groups of bacteria present in cotton soils from different regions and their responses to crop rotation with an aim to identify factors that regulate bacterial diversity and any linkages with disease incidence and plant health.

Results:
Results from the genetic analysis of bacterial community in soils from the three seasons (67, 185 and 74 samples during 2016-17, 2017-18 and 2018-19 seasons) indicated an average of 272 genera (ranging from 171-566) in farmer fields from 8 cotton growing regions. Data from the three cotton seasons showed significant differences in the population abundance, diversity and genetic composition of bacterial communities in cotton soils both at the field level and cotton growing regions and between regions (Figure 4.6.1). During the three seasons abundances of soil bacteria ranged between $2.2 \times 10^{10}$ to $4.21 \times 10^{10}$ (gene copies) per gram soil and abundances of bacteria were significantly ($P<0.01$) different between fields in all regions and in all three seasons and there were no consistent trends between regions and seasons, for example the regions with highest abundances varied between the three regions (Figure 153). There were varying degrees of seasonal variation in the bacterial abundances in the different regions which could be attributed to varying cropping and management histories. It is generally known that bacterial abundances generally vary with season, management, plant growth stage and environmental factors. Overall, there was no was no clear relationship between the abundance of bacteria and the level of disease incidence.

The genetic diversity of bacterial community indicated by alpha-diversity measures such as Species richness (d) and Pilou's evenness (j) values showed significant variation between regions (Figure 154). But region-based differences in Shannon index was only significant in 2017-18. Unlike the fungal diversity there was no consistent region-based variation in bacterial diversity. Similar to bacterial abundance, diversity of bacteria has been reported to be influenced by crop type (rotation), management (tillage, agrochemical application), soil type and climatic factors hence between season and within seasonal variation in the diversity is commonly observed in a wide range of crops and cropping regions (Bissett et al. 2016). For example, results from the Northstar field experiment during 2017-18, i.e. prior to cotton, showed higher diversity of bacteria after rotational crops Corn and Sorghum compared to Fallow and Continuous Cotton.

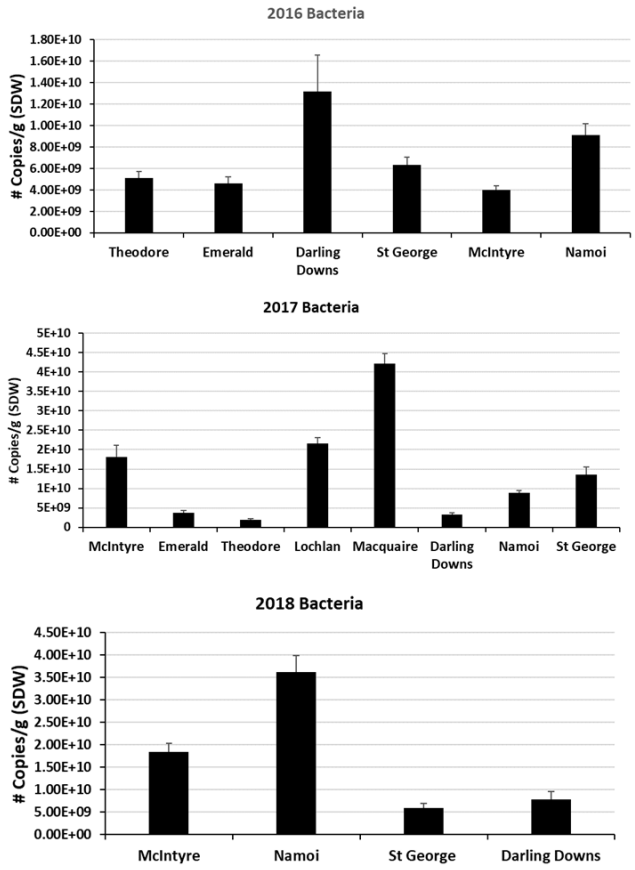



**Figure 153.** Abundance of soil bacteria in surface soils from different cotton growing regions.

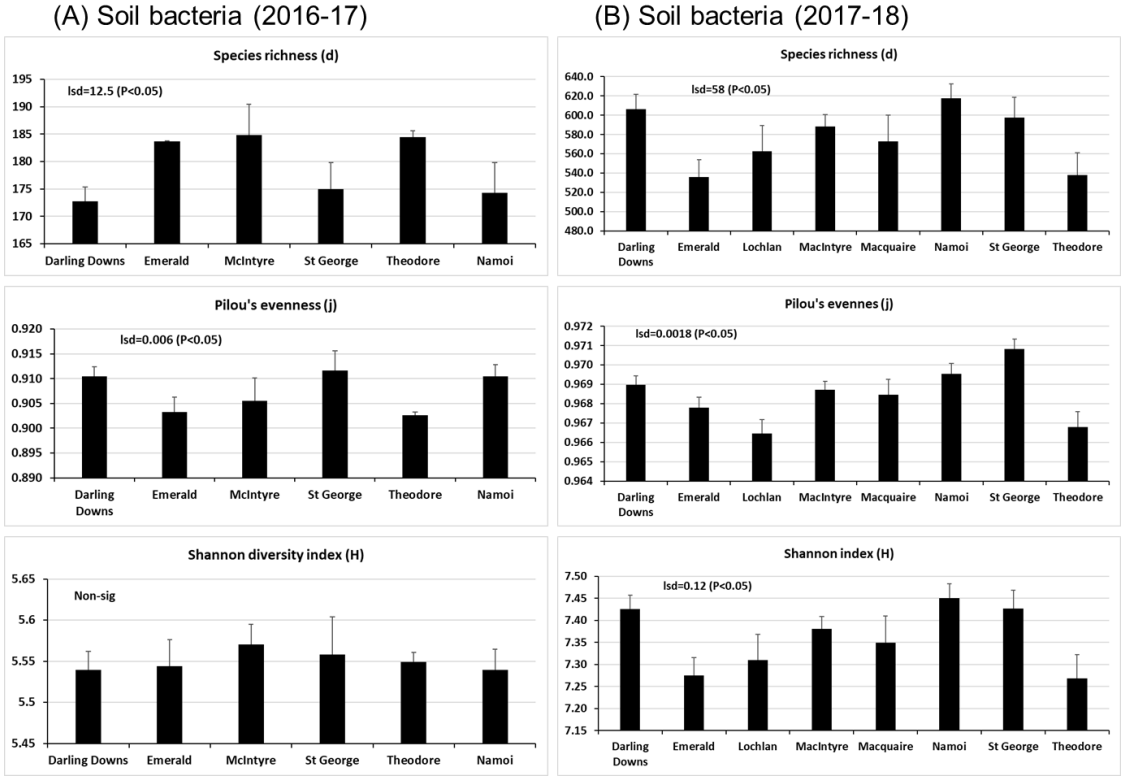



**Figure 154.** Genetic Diversity of soil fungi in surface soils from different cotton growing regions.

Comparison of soil bacterial community composition from beta diversity analysis (using the Bray-Curtis distance metric) showed significant dissimilarity between regions and fields (Figure 155 and Figure 156).

For example, bacterial community composition in soils from the Namoi region was distinctly different from that in fields from Emerald and Theodore regions, whereas bacterial community in soils from St George and MacIntyre were more similar particularly during 2016-17 (Figure 155).

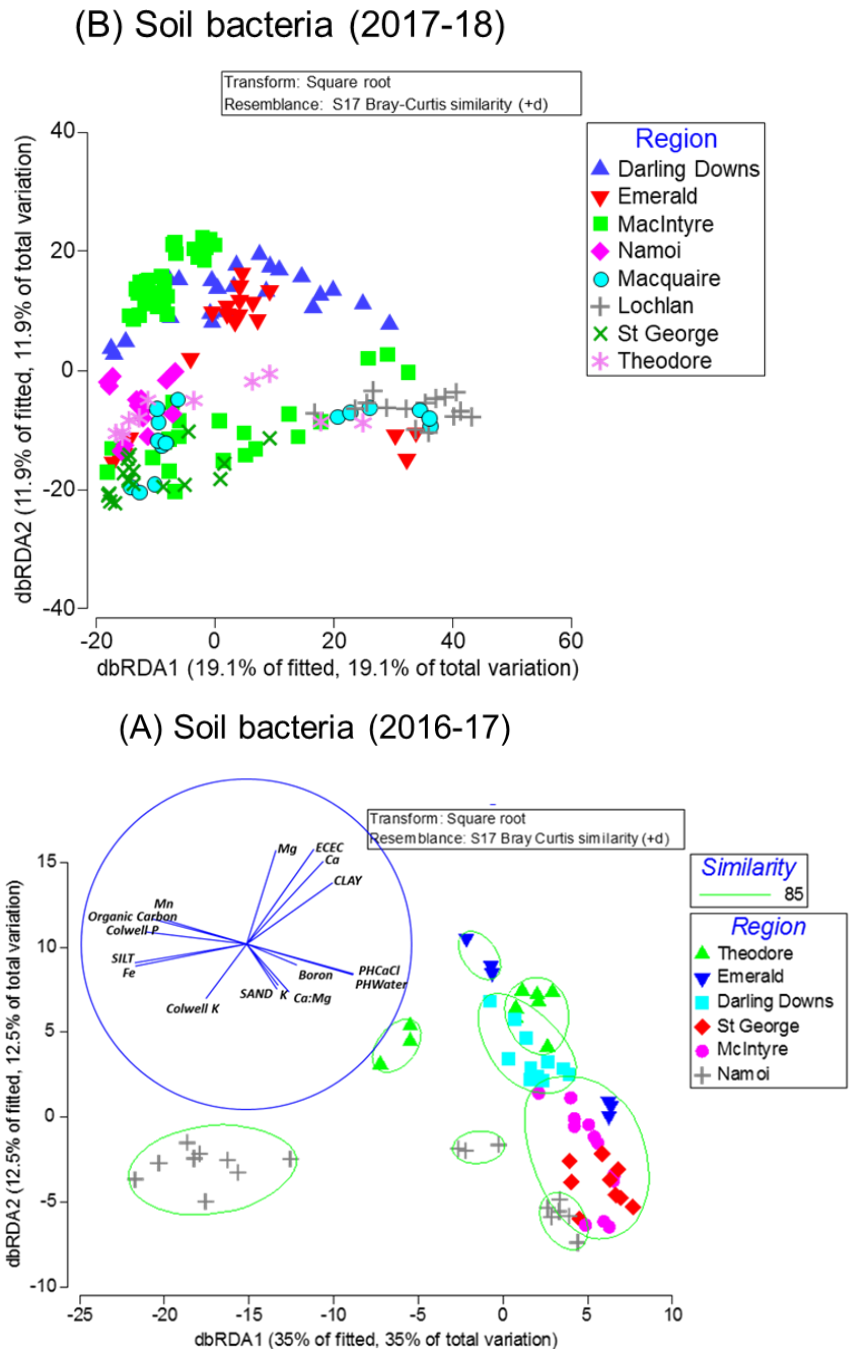



Figure 155. Genetic composition of soil bacterial communities measured using 16Sr RNA sequencing analysis in the surface soils collected from farmer fields and experiments in different cotton growing regions. (A) at OTU level during 2017-18 and (B) at Genus level during 2016-17. Data points closer to each other show greater similarity.
2017-19: Region based variation – PERMANOVA CV=21.5%; P=0.001
2016-17: Region based variation – PERMANOVA - CV = 9.7%; P=0.001

Within each region, field-based variation in community composition was only seen in some regions which could be attributed to the differences in previous seasons' cropping systems and management practices and associated soil physico-chemical properties. Analysis to identify relationships between the variation in bacterial community composition in different fields or regions and soil physico chemical properties

(BEST) showed that soil pH was the major factor. This concurred with the general observation of soil pH as the most dominant factor influencing bacterial community composition in wide ranging ecosystems within Australia and worldwise (Bissett et al. 2016; Fierer et al. 2012). On a regional scale, habitat and environment (pH, ECEC, texture (silt), Boron, exchangeable Mn, Fe and Ca) significantly ($\rho$ = 429, P = 0.01) influenced the genetic composition of bacterial community in cotton soils. Additionally, application of DistLM analysis (distance based linear models) indicated additional soil variables such as Exchangeable Mg, Na and organic C and Colwell P also contributed to the variation in bacterial community composition in cotton soils. Soil variables including pH, Exchangeable Ca, Zn, CEC and silt content were the best 5 variables explaining the differences in bacterial community composition. Additional analysis of dissimilarities in bacterial community composition with differences in management practices could help identify specific factors affecting bacterial composition provided seasonal influences and the randomness in field selection allow enough comparisons between different factors. Within a region/field (location and soil type) based variation in bacterial community short-term management and even varietal based differences can have significant effect on bacterial community composition (Bissett et al. 2016; Donn et al. 2016). Results from the Northstar experiment clearly indicated the significant effect of crop rotation on bacterial community composition, specifically the increased abundance of putative beneficial bacterial groups following rotation crops such as Sorghum and Corn.

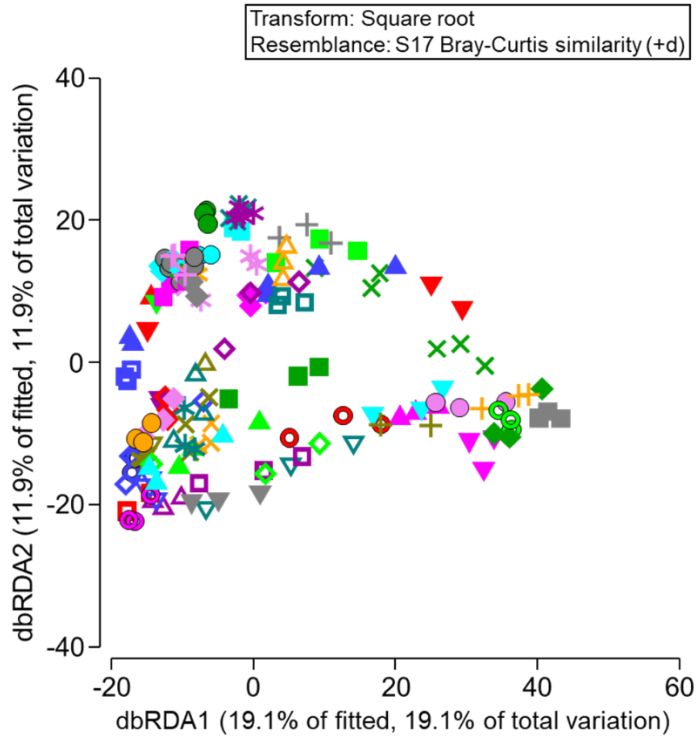



**Figure 156.** Field-based comparison of the genetic composition of soil bacterial communities measured using 16Sr RNA sequencing analysis in the surface soils collected from farmer fields and experiments during 2017-18. Different fields are represented by different symbols. PERMANOVA – CV=29.7%, P=0.001.

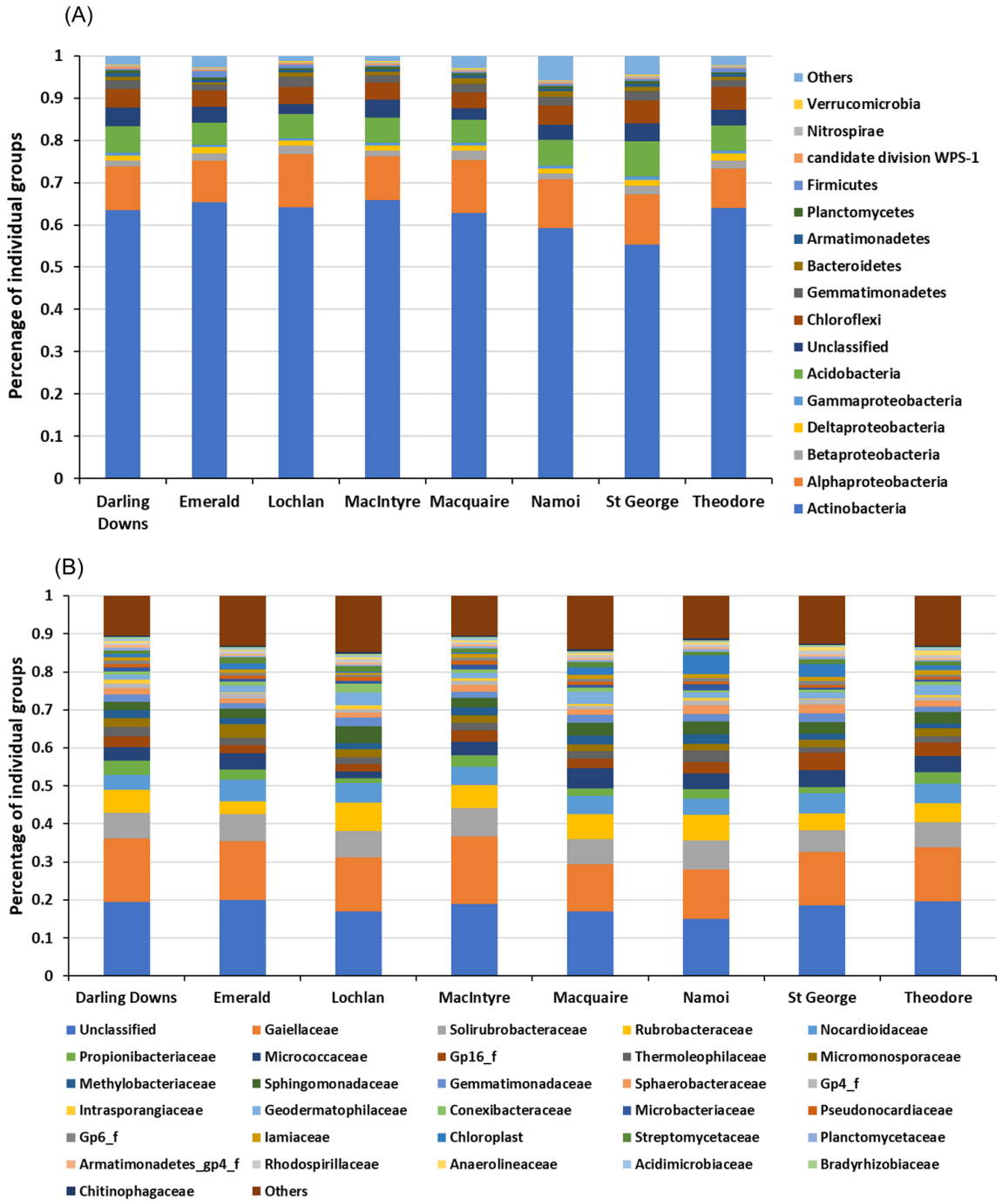



**Figure 157.** Relative abundance of soil bacteria at (A) Phyla and Class and (B) Family levels in farmer field soils and experiments from different cotton growing regions collected during 2017-18 season.

In general, bacteria belonging to the phylum Actinobacteria were the most dominant group among the 21 phyla of total bacterial community followed by Proteobacteria (Figure 157). For example, Actinobacteria accounted for 53.4±1.4% and 62.5±1.3% whereas Proteobacteria accounted for 17.5±0.72 and 15.7±0.4% of overall community during 2016-17 and 2017-18 seasons, respectively. Other major groups included members belonging to phyla Chloroflexi, Acidobacteria, Gemmatimonadetes and Bacteroidetes (Figure 157). Within the phylum Proteobacteria, members belonging to the Class $\alpha$ -Proteobacteria were the most abundant (11-15%) followed by $\beta$ -Proteobacteria (1.7-2.3%), $\delta$ delta-Proteobacteria (1.3-17%) and $\gamma$-Proteobacteria (0.6-1.2%) of total soil bacteria. Bacterial species belonging to the phylum Firmicutes which include well known plant growth promoting and biocontrol bacteria such as *Bacillus* accounted for 0.4 to 0.6% of total bacterial community only. Despite the broad diversity of bacteria, the most abundant

30 families covered over 85% of the total soil bacterial community indicating the dominating effect of a few groups in cotton soils. This observation matches with general observation for soil bacterial community in agricultural soils. A global analysis of soil bacterial communities across six continents found that only 2% of bacterial phylotypes consistently accounted for almost half of the soil bacterial communities worldwide (Delgado-Baquerizo et al. 2018). The top 5 phyla in this study matched with our findings although the sequence of abundance varied. One of the significant differences between the bacterial communities in cotton soils with that reported for other agroecosystems, both in Australia and worldwide, is with the abundance of Actinobacteria which ranged between 44-75% of total soil bacterial community. In an Australian continental wide survey of soil actinobacterial composition showed that actinobacteria generally dominate in soil environments that are exposed to extreme climatic factors (temperature and moisture) and soil (e.g. salinity and nutrient) characteristics (Araujo et al. 2020). Bacterial communities in Australian cotton soils are exposed to extreme dry and hot conditions along with flooding or saturated soil moisture conditions for extended periods which could be contributing to the dominance of Actinobacteria. Proteobacteria are generally reported to be the dominant group of bacteria colonizing the plant rhizosphere and endosphere environments and a wide range of bacterial species belonging to this phylum have been reported as plant growth promoting and biocontrol bacteria. In view of the finding that Actinobacteria are overwhelmingly dominant in cotton soils and actinobacterial genera found to be responsive to crop rotation in the Northstar experiment, future research to identify specific bacterial genera that colonize cotton plant in relation to its health is recommended.

Overall, results from this project are the first industry wide knowledge on the bacterial community composition in cotton soils including from farmer fields. The finding that Actinobacteria as the most dominant group and crop rotation can modify bacterial composition including actinobacterial species suggests that this group may have potential for manipulation to improve beneficial functions related to disease control and plant health. The new information on the bacterial abundance and diversity complements results on soil fungal community providing a comprehensive data set to determine the difference in the overall microbial community in cotton soils from different cotton growing regions.

**Milestone 4.3: Disease suppression potential**

**Objective 1**: To develop / standardize a laboratory 'soil plate assay' to quantify pathogen suppression potential (PSP) of cotton field soils.

Results shown in Figure 158 show a clear and significant difference between the soils from the F6E and KK fields, e.g. the growth of the *V. dahliae*-ND strain was higher in the KK field soil considered disease conducive compared to low disease KK field soils. The differential growth of the pathogen was mainly seen in the zone close to the inoculum plug and no significant variation was seen in the outer zone in the test plate. Repeated analysis of these soils in multiple assays confirmed the findings indicating the significant difference in the pathogen suppression potential in the soils from these two field sites.

Growth of fungi in soils can be influenced by their chemical and biological properties, e.g. soils with higher general microbial activity and/or specific microbial (suppressive) communities are known to inhibit fungal growth. Thus, results from the soil-based PSP assay provides a quantitative measure of pathogen suppression potential of cotton soils.

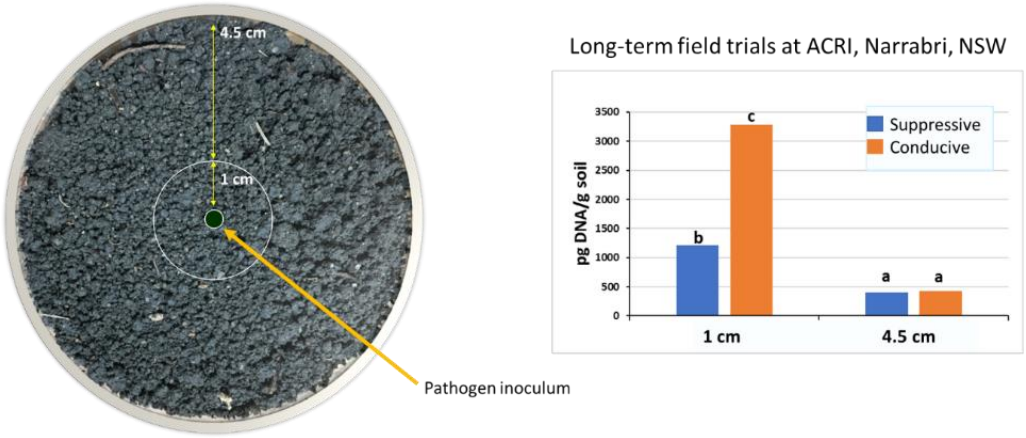



**Figure 158.** Results for growth of *V. dahliae*-D strain in soils from the long-term cropping system experiments at ACRI (Suppressive – F6E, long-term crop rotation experiment of I. Rochester; Conducive – KK, disease block experiments maintained by Karen Kirkby). Columns for each zone with different letters are significantly different at $P<0.05$.

Results for the surface soils from the crop rotation experiment at Getta Getta in NSW showed significant differences in the growth pattern of both the defoliating (D) and non-defoliating (ND) strains of *V. dahliae* in soils from the different rotation treatments (Figure 159). For example, pathogen growth was generally higher in the soils from Continuous cotton treatment and lowest in the treatment with Corn. Also, for the ND strain the field treatment effects are generally seen in the two zones close to the inoculum plug only and no variation in the outer zone was observed whereas the effects for the D-strain were seen in all the zones. Results from the PSP assay using ND strain also showed similar trend although the absolute concentrations of the pathogen varied between the D and ND strains. For example, the relative growth of D-strain was generally lower than the ND-strain (Figure 159).

The soil-based laboratory assay was also tested for the effects of incubation time (1 vs. 2 weeks) using soils from the Getta Getta experiment and addition of C substrates (using soils from Glencoe, NSW). Briefly, although the general trends with the treatment differences in pathogen growth in Getta Getta soils were similar with the one-week test, a two-week incubation showed higher sensitivity (data not shown). Soilborne necrotrophic pathogens such as *V. dahliae*, *R. solani* and *Fusarium* sp. are known to grow in the presence of decomposing plant residues hence cropping system treatments like fallow can result in a reduction in the abundance of fungal pathogen concentration. For example, results for the *V. dahliae* pathogen level in the Getta Getta field experiment soils from 2017 and 2018 seasons measured using the SARDI diagnostic centre showed significantly higher levels of the pathogen in soils from

Continuous cotton plots compared to the in the Fallow treatment. Similar results were observed for the fungal pathogen *R. solani* AG8 in fields in NSW and SA (Gupta et al. 2016).

The suitability of the PSP assay was tested in farmer field soils from Boggabilla, NSW that showed different disease history. Results indicated that pathogen growth was significantly higher in soils from High-Verticillium disease history compared to that in the field soils with Low-Verticillium disease history e.g. 30-135 vs 1-20 pg DNA per gram soil in the High and Low disease history fields, respectively.

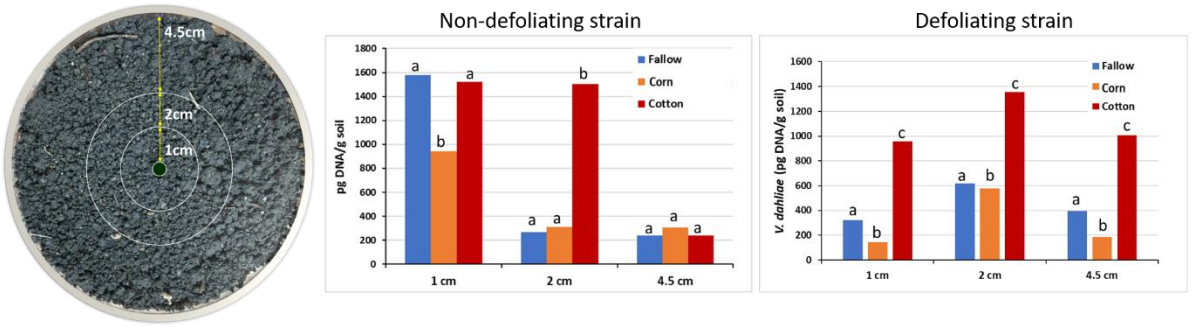



**Figure 159**. Results for the growth of D and ND strains of *V. dahliae* in the surface 10 cm soils from the crop rotation experiment at Getta Getta, NSW. Columns for each zone with different letters are significantly different at P<0.05.

Overall, results from the limited tests using the soils from cropping system experiments at ACRI and Getta Getta and farmer fields near Boggabilla clearly indicated that the laboratory-based PSP assay can provide a quantitative measure of the ability of soils from cotton fields to support/inhibit the growth of soilborne fungal pathogens such as *V. dahliae*. Additionally, the PSP assay seem to provide information for both the ND and D strains of the pathogen suggesting its general suitability for the different pathogen strains.

Future: Since this lab-based bioassay is a short-term assay (1 to 2 weeks) and uses actual soils, unlike laboratory media-based culturing methods, it should be amenable for high-throughput screening of field soils that provides an integrated measure on the effects of soil biology on pathogen growth as part of its ability to alter disease incidence. Currently the test has only been standardized for the pathogen *V. dahliae* and needs further testing for other soilborne pathogens of cotton crop. The PSP assay could also be modified to test the effects of additives (e.g. crop residues, chemicals etc) on soilborne pathogens.

*Soil collection for microbiological analyses*

Figure 160 shows the locations in Qld and NSW for samples collected and used for microbiological analyses. Samples were collected during disease surveys and from experimental trial sites.

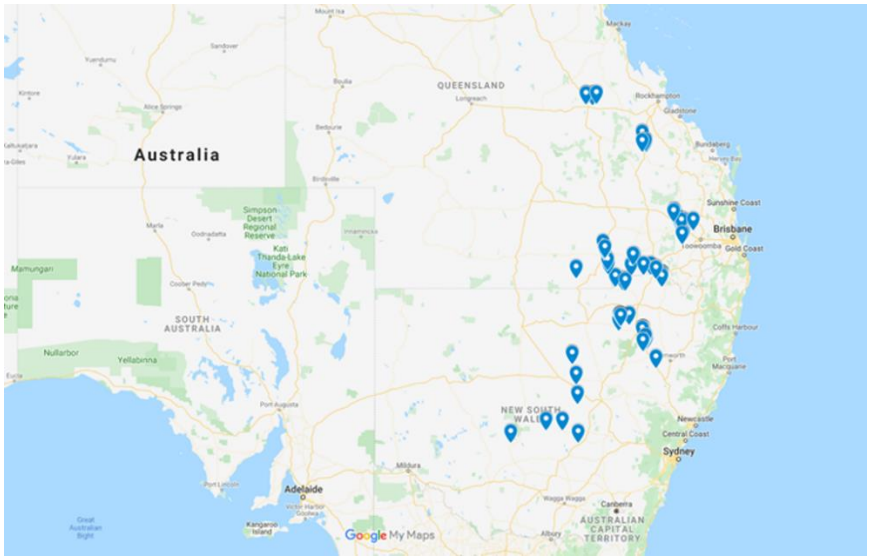



**Figure 160.** Locations in Qld and NSW for samples used for microbiological analyses

**Objective 2**: To develop / standardize a plant growth assay to quantify pathogen suppression potential of cotton field soils.

Millet seed inoculum

No external symptoms developed and no internal or external symptoms developed after 10 weeks. It transpired that inoculum should have been air-dried for a much shorter period, 24 hours, and excessive drying likely killed the pathogens. The millet seed inoculum test will be repeated and drying time of inoculum rectified to ensure viability.

Cornmeal sand mix (CMS) inoculum

Verticillium was only able to be isolated from two of seven plants where there was a 10 g layer of inoculum. One of these plants was asymptomatic while the other had slight vascular stem browning. The plants growing where the inoculum was mixed through remained disease free, Fusarium was unable to be isolated from where there was the layer of inoculum. Where the inoculum was mixed through the pot at 4.3% Fusarium was recovered from two of seven plants, both symptomatic.

Different CMS inoculum was prepared and used in a second test. The second test involved adding the inoculum to pots at the rate of either 5.7% or 11.4% mixed thoroughly into sterilised m-mix. At twelve weeks only the Fusarium inoculations had been successful in producing disease with Fusarium re-isolated from all plants at both rates. Where Verticillium inoculum had been incorporated no pathogen was able to be re-isolated.

The lack of uniformity in disease using these techniques could be related back to the inoculum needing to be air-dried quickly overnight not left for a few days to dry out, especially for Verticillium. (Additional testing in a separate experiment has since proven this). Problems encountered will be rectified.

Agar plugs (AP) from culture plates

No external disease symptoms developed and no vascular discolouration was observed after 10 weeks. One week was possibly not enough time for colonisation of soil by the pathogens. If agar plugs are to be used, a longer colonisation time will be needed before sowing given the soil plate assay has since determined that pathogen growth on soil was only 2cm from inoculation point after two weeks.

**Figure 161.** Seedling growth 10 days after sowing into potting mix inoculated using the Agar Plug method

**4.1 Communicate research outcomes**.
A total of 8 presentations given at the cotton industry meetings, cotton research conferences, national and international scientific meetings.

Manuscript 1 - The refereed conference paper on 'Disease suppression: soil fungal community diversity and interactions' presented at the 10th Australasian Soilborne Disease Symposium held in Adelaide in 2018 is being finalised as a scientific paper to be submitted to the Journal 'Phytopathology' by March 30th, 2020.

Manuscript 2 - Information on the diversity, genetic and catabolic composition of soil bacterial and fungal communities in the rotation experiment at NorthStar, presented under Milestone 4.1.2 is being converted into a manuscript titled "Crop rotation effects on composition of soil microbial communities, microbial activity and potential implications to biological suppression" (abstract accepted) for submission to the Journal MDPI Plants – Special issue on Management of Verticillium disease, Abstract accepted manuscript to be submitted by April 30th, 2020.

**5. Research question: What percentage of growers adopted practices that reduce the incidence of diseases and pests on their farms at project completion?**

5.1 Adoption pathways and measurement of adoption

The involvement of CottonInfo on surveys has resulted in a successful collaboration in which CottonInfo have a better understanding of cotton diseases and Pathology teams have a better understanding of regional issues directly resulting in quick and effective dissemination of research findings back to the cotton community. This has been through regional newsletters, field day presentations (CottonInfo and Pathologists), research updates at annual general meetings and CCA meetings, CottonInfoENews, industry articles and CottonInfo presentations at CSD roadshows.

Initial results of survey evaluation form
An evaluation form was developed and sent to growers involved in annual disease surveys at the end of the 2018/19 survey. A small number of responses to survey have been received, however waiting on further responses to finalise data.

Initial results indicate that 1) The surveys should be conducted, 2) They inform about disease trends, and 3) Represent the valley; all receiving either 4 or 5 rating where 5 is the highest score.

Comment about usefulness of the surveys: The value is when the seasons promote diseases and now with the cotton being grown in many states the risks are getting greater so biosecurity focus on presence absence I find very worthwhile.

Growers were positive about the farm report they received, and some suggested changes/additions include:

- Would like to see reports provide a historical trend of fields surveyed over time.
- Holistic report of the survey conducted.

Changes made to manage diseases on farm include:

- Come Clean Go Clean; Machinery onto fields are clean, restricted access to farm.
- Solarisation of Fusarium infected stubble for 90 to 120 days for 5 years when detected which has managed the disease
- Crop rotation
- None

***Outcomes***

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

**Outcome 1. Research question: How is the incidence and severity of cotton diseases changing?**
**Outcome 1.1** *Survey methodology considers industry needs and delivers and ensures science outcomes can be met. Clear roles and responsibilities for project team established; CottonINFO team personnel identified and agreed regarding the adoption pathways for the project.*

**Output 1.1** *Protocols for disease surveys developed and agreed upon by industry and project partners. Protocols establish clear roles and responsibilities for project and CottonINFO team; personnel identified and agreed regarding the adoption pathways for the project. Rules around privacy and use of survey data are established in agreement with industry and partners.*

Protocols for disease surveys were developed and agreed upon by industry and project partners. Protocols were developed to establish clear roles and responsibilities for project and CottonINFO team. Rules around privacy and use of survey data were established in agreement with industry and partners. Any changes were agreed upon prior to implementation.

**Outcome 1.2** *An understanding of the factors that contribute to pathogen occurrence and development of epidemics. Quantify the relative performance of disease threats and ensure an adequate response by the cotton industry, communities and government agencies.*
**Output** *Disease surveys and summary of findings completed for Qld & NSW, reviewed by project leader, communicated to industry.*

Cotton is susceptible to many yield-limiting diseases. To understand the importance and impact of diseases present, biannual disease surveys are conducted to monitor the distribution and incidence of diseases. The surveys also maintain the surveillance for exotic pathogens. Historically, disease surveys were undertaken separately within NSW and Qld. This project is taking a new approach, to bring together data across the industry in a national project.

Different regions are being affected by different pathogens. Black Root Rot is a limiting factor in fields in southern NSW because of its cooler climatic conditions. The highest incidence in the 2018/19 season was in the Gwydir; however, severity was mild, mostly below 15% as indicated by the percentage of the taproot surface that was blackened.

Alternaria leaf spot is developing as an early season disease of concern in NSW. Serious infection of young seedlings were observed in the Lachlan and Murrumbidgee regions in early December 2017. Research was conducted to better understand the species of *Alternaria* contributing to this leaf spot disease. In the 2018/19 season the mean disease incidence (MDI) of seedlings infected was 59%, 23% and 21.5% in the Gwydir, Namoi and Murrumbidgee respectively; however disease severity was generally low due to less favourable conditions than previous seasons.

Fusarium wilt (FW) remains a key disease for St George and the Darling Downs, detected in 50% and 65% of fields' surveyed early season. A high incidence was detected in some fields late season in St

George, Darling Downs and Macquarie. There was likely an impact on yield for some fields. It is imperative not to become complacent of this disease, or relying solely on host resistance. An integrated approach to management is extremely important to manage this disease, as it is with all soil-borne pathogens.

Verticillium wilt (VW) continues to be a major disease of concern in the Border Rivers and Namoi, with a MDI of 21.5% and 20% respectively in the 2018/19. This is concerning given that the environmental conditions were not considered conducive to Verticillium wilt development. The Border Rivers and Darling Downs were the only regions with an increase in the mean incidence of disease compared to the 2016/17 season. There were no significant rain events January/February of 2019, which are generally associated with development of disease symptoms. Alternatively, the high temperatures and dry conditions at this time led to an intense irrigation schedule on farms where water was available, which may have provided a cooling effect in the soil and therefore conditions conducive to the development of VW.

In the Gwydir, 30% of fields' surveyed had both wilt diseases. This poses a significant challenge in managing crop residues, crop rotations and cultivar selections to minimise disease impacts.

Environmental conditions are the biggest factors in determining infection rates of boll rots. Persistent rain, moisture and/or cloudy weather with cooler temperatures are huge contributors to boll rot incidence, especially when occurring at boll cracking. Boll rots were prevalent in some fields in all regions except the Lachlan and Murrumbidgee in the 2017/18 season. The average incidence of boll rots was recorded as 2.6% boll rot, 1.5% tight lock and 2.6% seed rot. However, given boll rot development is linked to environmental conditions, timing of disease surveys is important and only captures the disease incidence at that time. The drastic decrease in temperatures plus significant rainfalls during the first week of February provided conducive conditions for boll rot development in some regions, and in particular tight-lock. For example, a significant amount of boll rot and tight-lock was observed in the Ambassador sites and variety trials with a range of 8-20 bolls/m affected by a combination of boll rot and/or tight-lock. There was also anecdotal evidence that boll rot and tight-lock was the cause of significant yield reduction in the Macintyre.

Detection of Cotton Bunchy Top and mealy bugs highlights the need to ensure that ratoon or volunteer cotton and weed hosts are eliminated on farm, particularly over winter.

The surveys also served as a vehicle for the collection of soil samples for analysis of plant-parasitic nematodes. New farms in Emerald were confirmed to have reniform nematode. To date, this nematode has only been detected in cotton in CQ.

Results demonstrate the necessity of general multi-pest surveillance systems in cotton in providing an ongoing evaluation of the distribution and impact of key endemic pests.

Disease surveys and summary of findings were completed for Qld and NSW and communicated to industry. An additional communication of results as of 2017/18 season was the presentation of regional survey updates by CottonInfo as part of the CSD roadshow and publication of survey results in the CSD Trial results USB booklet.

**Outcome 1.3** *Report on enhanced benefit from geospatial analysis, and recommendation on data gaps required to assess management impact of disease.*
**Output** *Initially a review of suitable digital approaches for collecting and collating geospatial data across commodities will need to be undertaken. Relevant historical data included.*

The disease survey team have utilised Fulcrum mobile-applications since 2017 to facilitate geospatial data capture. Fulcrum is a web-based application, offering customisable forms for mobile data collection in a geospatial context, such as plant disease surveys. To date, the project field staff have retained the existing physical-field form for raw data capture. Within Fulcrum, a photograph of the completed field form during a survey creates a digital copy, linked to the geotagged record to be synced and stored in the cloud. This significantly improves data handling where loss or alteration of the original occurs, and in

the case of field surveys where large volumes of data are generated by multiple users. The Fulcrum desktop app is a powerful visual tool in monitoring field activities across the cotton production regions of NSW and Qld. Filters allow records to be visualised specifically to region, farm, user or year.

To date, survey data is manually transcribed into a Microsoft Excel worksheet before analyses can be conducted, which is extremely time consuming and delayed analyses of data. Discussions with Adam Sparkes suggest that some data might be able to be recorded in Fulcrum at time of surveys if worksheet is appropriately modified. This will be investigated for future surveys.

**Outcome 1.4** *Development of disease management strategies reported annually.*
**Output** *Analysis of data for evaluating trends and management impact on disease. Three years of field assessed cotton pathology data, management practices, composition and abundance of microbial communities will be collated and analysed to address systems questions on disease management.*

Three years of field assessed pathology data for NSW and Qld was evaluated for trends and reported.

**Outcome 1.5** *Improved collection of grower information/ Better understanding of factors contributing to pathogen occurrence leading to better management strategies.*
**Output** *Data collection template for growers developed. CottonInfo to distribute prior to annual surveys to inform growers about the type of information and provide background to the surveys.*
*Pathologists to follow up on grower surveys and enter grower survey data into database.*

A grower information template was developed and emailed to each grower whose field was surveyed, with the aim to capture additional information that could be considered in the data analyses to provide a better understanding of factors that influence disease incidence.

Obtaining information from growers was very time consuming and often difficult due to how busy growers are. In addition, an understanding of the benefits the additional information would provide the grower was a difficult message to get across and may have prevented some growers not prioritising filling in the form. However, now that an initial analyses has been conducted of the data with significant findings, when published, will provide evidence of the benefit in providing requested information. This I believe will encourage all participants in the surveys to provide requested information.

**Outcome 1.6** *Scientific publication/s based on analysis. Improved decision support information packages for growers to choose best options to manage disease.*
**Output** *Analysis of survey data completed and implications for integrated disease management published.*

Multivariate analysis of survey data was performed by Dr Adam H. Sparks (USQ, Toowoomba) on Queensland and New South Wales cotton production survey data for the seasons 2016-2017, 2017-2018 and 2018-2019. Analyses were performed to test for any effect of previous crop and cotton trash present on early and late season diseases. Further correlation network analyses were performed on the data to identify relationships between diseases and yield and diseases themselves in the whole data set and in each state.

<u>Significant Findings</u>

- Verticillium wilt was significantly higher in winter cereal crops than cotton but no different in other previous crops.
- Seed rot was significantly lower in fallow and winter cereals than cotton but no different in other previous crops.
- Boll rot was significantly higher in summer grains than cotton but no different in other crops.
- Tight-lock was significantly lower in fallow and winter cereals than cotton but no different in other previous crops.
- Tight-lock had the strongest negative relationship with yield in the data set.
- Early season Alternaria had a strong correlation with early season Black root rot.
- Clusters of positively associated diseases in the entire data set included early Alternaria and Black root rot
- Clusters in the Queensland only data showed weaker relationships

    - Early season Fusarium wilt and late season Fusarium wilt being clustered and positively correlated.
    - Tight-lock, Boll rot and Seed rot were clustered with weakly positive correlations
    - Early season Alternaria and late season Verticillium wilt clustered with negative correlations to tight-lock and boll rot
- Clusters in New South Wales showed some stronger correlations than Queensland
    - Early Alternaria clustered more closely with Black root rot than in Queensland

Non-significant Findings
The amount of cotton trash present in the paddock did not have any significantly detectable effect on disease in the analyses performed on these data.

These findings provide direction for research to investigate cropping rotations that potentially will decrease/increase disease incidence of Verticillium wilt and Boll rot/Seed rot/Tight-lock.

The research finding that winter cereals increase the incidence of Verticillium wilt is an important finding because a common crop rotation implemented to manage Verticillium wilt is a fallow-wheat-cotton rotation. Anecdotally in the Macintyre valley, this strategy has not managed Verticillium wilt and disease incidence has continued to rise. Further research is required through field trials to confirm this finding and to determine the mechanism(s) involved. A better understanding of relationships among cereal crops and *V. dahliae* may allow us to use crop rotations more effectively in efforts to reduce soil inoculum levels as an efficient management practice.

Given that yield data was limited in the database, an unexpected finding is Tight-lock having a strong negative relationship with yield in the data set. There are currently no management strategies for Tight-lock in Australian cotton. Further investigation is required to ascertain if crop rotation effects Tight-lock incidence.

There is much further work that can be done with these data including but not limited to:
- Analysing the effects of weather provided by BOM from the nearest station for each field
- Cluster analysis to determine clusters of areas acting in a similar or dissimilar fashion
- Analyse effects of the following on disease e.g. Variety, Seed rate, Configuration, irrigation, Fertilisation regime and Soil type

**Outcome 1.7** *To keep team members informed of research progress. To highlight and address issues or concerns. To assist in delivery of milestones in a timely manner.*
**Output** *Survey Team to discuss research progress quarterly either via telephone conference call or physically in January, April, July and October.*

The survey team met as regularly as possible to discuss progress. Options included face-to-face at a specifically designated location, at industry meetings (eg. FUSCOM, CCA meetings) and conferences (e.g. The Australian Cotton Growers Conference, AACS Australian Cotton Research Conference).

A fortnightly skype/teleconference commenced in 2019, which has helped to keep everyone informed of project progress, and provide an opportunity to discuss project work as a team across locations.

**Outcome 1.8a** *Cotton growers and consultants have simple processes to submit diagnostic samples regardless of location. Diagnostic samples are processed and reported in timely and efficient manner.*
*Information and samples from cotton growers are managed to maximise benefit to the industry.*
**Output**
- *All samples from NSW to be sent initially to EMAI.*
- *All samples from QLD to be sent initially to ESP*
- *Flow chart and chain of custody procedures developed for all Australian cotton pathology inquiries including*
- *Clear points of contact for all cotton industry pathology inquiries*
- *Procedures in place to ensure that regardless of where a sample is submitted, it can get to appropriate scientists for diagnosis. NSW samples for Fusarium and virus to be sent to Qld.*

- *Agreed process for sub-sample and sharing of samples/results established where there is research benefit for industry.*
- *Identification and distribution of material and results communicated according to agreed confidentiality and processes including timeframes.*
- *Clear point of contact and process for growers and consultants is communicated to industry.*

The aim of milestone 1.8 was develop a sample diagnostic flow chart/chain of custody procedures for all diseases and virus samples to ensure that the diagnostic process was consistent between NSW DPI and DAF. A process for diagnostics was discussed and developed between pathology teams in NSW DPI and DAF, and CottonInfo. Once decided upon, information was disseminated to all concerned. Updates and changes to the process were made as required, after discussion and agreement from survey participants.

**Outcome 1.8b** *Survey and industry sample diagnostics completed and reported appropriately.*
**Output** *Samples processed and identification and distribution of results in accordance with agreed procedures (2.1)*
Survey and industry sample diagnostics were completed and reported in accordance with agreed procedures (2.1).

**Outcome 2**

**2.1 Outcomes:** *Better understanding of host resistance of crop rotation crops to different strains of V. dahliae. Improved decision support information packages for growers to choose best options to manage Verticillium wilt.*

**Output:** *Trials completed, data collected and statistically analysed.*

This research showed that some crops commonly rotated with cotton, namely mungbean, chickpea, wheat and barley are actually symptomatic hosts being systemically colonised by two different strains of *V. dahliae* in glasshouse studies. While plant isolations from field plants has to date not identified these crops as being susceptible in field situations, it will be of great importance in the future to strategically monitor these crops growing in Verticillium infested fields to assess their potential susceptibility.

Crop rotation trials at "Getta Getta" has provided valuable data on rotations to non-host crops and their potential as a management strategy for Verticillium. In highly infested fields more than one year of rotation to a non-host crop or fallow is required to reduce disease levels in the next cotton crop. This trial site also provided useful information on changes in the soil microbial populations reported under milestone 4.

**2.2 Outcomes:** *Increased knowledge on potential modes of spread. If V. dahliae is seed-borne in cotton this will influence seed production site location, production protocol and seed treatment.*

**Output:** *Collect seed from V. dahliae infected plants, receive seed from CSIRO trials at Narrabri nursery, surface sterilise and plate onto semi-selective medium. Incubate plates and observe for growth of V. dahliae, isolate, single-spore and characterise using PCR and VCG analysis.*

The recovery of *V. dahliae* (VCG 1A) from 0.025% of acid-delinted seed identifies that the pathogen can be seed borne in cotton. This represents a small risk to introducing the disease into new, previously disease free regions. While acid-delintng seed should effectively remove *V. dahliae* it appears that it is not 100% effective. Fungicidal seed treatments may be of some value but would only eliminate the pathogen from the seed surface. The recovery of the pathogen from surface sterilised seed suggests that the fungus was inside the seed. Ideally site selection for seed production in the future should be reviewed to identify disease free areas to try to reduce this risk to zero.

**2.3 Outcomes:** *Increase knowledge on potential of strain to cause disease under different environmental conditions. Some strains may be more of a threat in a particular region.*

**Output:** *Optimum growth temperatures for each strain determined.*

The optimal temperature for mycelial growth *in vitro* for most isolates of both VCG 1A and 2A in this study was 24°C, with on average VCG 2A strains growing faster at this temperature. There was variability among isolates within each strain, however, highlighting that individual isolates rather than specific VCG

groups, have the potential to grow differently and potentially be more of a threat in certain regions. For example, two isolates in this study, one belonging to VCG 1A and one belonging to VCG 2A had optimal growth rate at 30°C, indicating that these are potentially more of a threat in warmer regions. Unfortunately due to issues gaining timely access to growth chambers and time commitments to other areas of this project, virulence testing on cotton using the different isolates was not completed.

**2.3 Outcomes:** *Environmental influence on Verticillium strains and potential to cause disease in Australian cotton; host range of different strains of Verticillium used in rotation with cotton; potential for V. dahliae to be seed-borne in Australian cotton; management strategies for Verticillium.*

**Output:** *Draft journal paper on Verticillium research.*

Research findings from this project have been presented at grower meetings, conferences and industry meetings throughout the project. Data is currently being drafted, aiming for inclusion in a publication dedicated to Verticillium management in the journal *Plant.*

**Outcome 3 reported in DAQ1803**

**Outcome 4. What is the disease suppression potential of cotton soils from different cotton growing regions**
**4.1 Outcomes** *Knowledge on the factors that drive the soil fungal community in cotton soils from different regions and potential link with disease to help identify management options to modify beneficial soil fungi.*
**Outputs** *Complete analysis of abundance and composition of soil fungi in a minimum of 5 fields per region in each of 8 cotton growing regions in NSW and Qld and relationship between soil fungi and disease incidence.*

1. Fungal community network analyses for disease suppressive and disease conducive soils from long-term experiments in fields indicate that (i) the diversity and abundance of soil fungal community varied significantly by crop management history and (ii) fungal communities in suppressive cotton soils were characterized by higher diversity and higher connectedness indicating resilience to change.

Overall, the results suggest that soil ecological and environmental factors and filtering processes related to substrate quality and availability, spatially and temporally, play a significant role in shaping soil fungal communities and their functionality. The high level of organization along with higher diversity in the soil fungal community in the suppressive soils would provide the cotton plant with a stable microbial reservoir across varied seasonal environmental conditions.

2. Observations on the changes in the microbial diversity and activity in the short-term rotation experiment clearly indicated the significant and important contribution of soil microbiome for the suppression of Verticillium disease in cotton. The influence of rotation crops such as sorghum and corn could be attributed to (i) increased microbial catabolic diversity and activity (ii) higher diversity of bacteria and fungi, (iii) increased abundances of specific groups of microorganisms involved in antibiosis, antifungal (cell-wall degradation) and plant growth promoting capabilities, and (iv) lower pathogen levels. These changes would have contributed to the suppression of the pathogen, disease incidence and impact. Whereas the fallow treatment caused a significant decline in the total microbial activity and catabolic diversity, genetic diversity of bacteria and fungi resulting in lower pathogen suppression capacity. Although the lower pathogen levels would help in the reduction of disease incidence, long-term adoption of such management practices would not benefit in maintaining or improving the overall soil biological health. The traditional continuous cotton system seems to promote the growth of pathogenic fungi such as *V. dahliae* and result in lower microbial diversity and abundances of beneficial microorganisms.

In addition to the plant type-based variation in the microbial communities, crop rotation effects are attributed to the differences in the quality and quantity of carbon inputs i.e. a substrate mediated phenomenon.

In view of the field/region-based variation in soil microbial communities (esp. fungi) in cotton soils, the specific groups of microbes that are influenced by rotation need to be verified to determine the magnitude of benefit that could be harnessed to reduce disease impacts.

3. Results from the genetic analysis of fungal community in soils from three seasons indicated an average of 316 genera (ranging from 297 to 326) covering 432 OTUs/species (ranging from 149-697) in farmer fields from 8 cotton growing regions. These results confirm previous findings from experimental fields at ACRI and northern NSW and southern Qld. The presence of a diverse fungal community indicates the potential to utilize the general fungal community to manipulate biological functions including disease suppression, plant nutrition etc.

Results from three cotton seasons have shown significant differences in the population abundance, diversity and genetic composition of fungal communities in cotton soils both at the field level and cotton growing region. In general, fungal abundances significantly ($P<0.01$) varied between fields in all regions and in all three seasons. There were varying degrees of seasonal variation in the fungal abundances in the different regions which could be attributed to varying cropping and management histories. Overall, there was no was no clear relationship between the abundance of fungi and the level of disease incidence, for example during 2018-19 season fields from McIntyre and St George with varying levels of disease incidence didn't not show any systematic variation in the fungal abundance. This is not unexpected as pathogenic fungi are also included in the total fungal abundance. It is known that crop rotation, tillage and agrochemical application can significantly influence soil fungal communities.

Data for the genetic composition and relative abundances of beneficial fungi indicated that members of fungal community known as mycorrhizal fungi (Glomeromycota) only accounted for <0.1% of total community and only a small group of genera (5) / species (18) were observed suggesting the necessity to monitor management effects in order to fully harness the benefits from mycorrhizal association with cotton plants. Another surprising finding is the observation of the wide spread presence of the six species of the genus *Arthrobotrys* a putative Nematophagous fungi in >80% of fields analysed. The functional significance of this group of fungi in controlling plant parasitic nematode effects is not known.

Additionally, this study is mostly a descriptive genomics-based investigation identifying the nature of the community in Australian cotton soils and influencing factors and it should be extended to understand the functional role of the different groups present to understand the functional importance of the specific members of microbial community.

**4.2 Outcomes** *New knowledge on the elements (e.g. biological components and soil and environmental factors) of soil-borne disease suppression in cotton farming systems that would assist in developing improved management options that promote suppression.*
**Outputs** *Complete analysis of surface soils from farmer fields in different cotton growing regions and under varying management practices for the total microbial activity (catabolic activity and diversity) and composition of beneficial microbial community (phenotypic microarray analysis for beneficial bacteria/functions) – minimum of 25 fields in two seasons.*

Previous research has shown that microbial catabolic diversity (based on carbon substrate utilization profiling) was significantly different between disease suppressive and conducive soils. Hence, cotton soils from farmer fields in different cotton growing regions and under varying management practices were analysed for the total microbial activity (catabolic activity and diversity) and relationships determined.

Overall, surface 10 cm soils in farmer fields from different cotton growing regions contained moderate levels of microbial biomass levels with microbial quotient (MQ) values ranging from 3.1 to 3.9%. There were significant differences between fields demonstrating the effect of management and soil factors, but no systematic region-based variation was observed. Since a 5% MQ value is considered as a minimum attainable level of microbial biomass, the general lower levels of MB suggest that in these low SOC soils, levels of biologically available carbon along with other constraints may be present restricting the build-up of microbial populations. Additionally, observation of higher MB and MQ values in experimental plots and fields with rotational crop that add large amounts of crop residues e.g. sorghum, corn etc suggest further support the potential limitation for microbial activity.

Research from grain cropping systems have indicated that management practices that increase C inputs (e.g. crop residues) and turnover over a number of years (5-7 y) will improve disease suppression and

reduce the impact of rhizoctonia disease. The observation of higher pathogen and/or disease suppression potential in treatments with rotation crops where large amounts of crop residues are added, e.g. F6E experiment at ACRI and rotation experiment at Northstar support the hypothesis that in the lower organic carbon cotton soils may be partly restricting the development of agronomically useful disease suppression potential. Rotation crops also modify the C substrate utilization profiles (i.e. catabolic diversity) reflecting the changes in the genetic composition of bacterial and fungal communities. The significant reduction in the MB level, catabolic diversity and genetic diversity of fungi in soils under Fallow further emphasizes the critical role of soil microbial communities in providing beneficial functions such as disease suppression in Australian cotton soils. Finally, in the lower SOC cotton soils in Australia, the adoption of management practices that can improve the amount of microbial biomass and its catabolic potential may be one of the practical and simpler ways to increase biological suppression potential against soilborne plant pathogens.

Results from this project are the first industry wide knowledge on the bacterial community composition in cotton soils including from farmer fields. The finding that Actinobacteria as the most dominant group and crop rotation can modify bacterial composition including actinobacterial species suggests that this group may have potential for manipulation to improve beneficial functions related to disease control and plant health. The new information on the bacterial abundance and diversity complements results on soil fungal community providing a comprehensive data set to determine the difference in the overall microbial community in cotton soils from different cotton growing regions.

**4.3 Outcomes** *Improved knowledge on management practices that promote biological disease suppression in cotton soils to complement Integrated Disease Management in cotton farming systems.*
**Outputs** *Analysis of surface soils from farmer fields (a minimum of 25) with contrasting management history (e.g. nutrition, irrigation) from different regions for their disease suppressive potential using bioassay completed.*

Objective 1: A laboratory 'soil plate assay' to quantify pathogen suppression potential (PSP) of cotton field soils was developed. Results from the limited tests using the soils from cropping system experiments at ACRI and Getta Getta and farmer fields near Boggabilla clearly indicated that the laboratory-based PSP assay can provide a quantitative measure of the ability of soils from cotton fields to support/inhibit the growth of soilborne fungal pathogens such as *V. dahliae*. Additionally, the PSP assay seem to provide information for both the ND and D strains of the pathogen suggesting its general suitability for the different pathogen strains.

Objective 2: The aim this work was to develop / standardize a plant growth assay to quantify pathogen suppression potential of cotton field soils. Previous bioassays to study Fusarium and Verticillium wilts have successfully used a root dip method in which two-week-old seedlings are dip inoculated into a spore suspension then transplanted. The development of a pathogen suppression bioassay requires planting of seed into soil infested with the pathogen at a level that will provide adequate disease incidence, but not too great. There were issues in preparing inoculum that was viable and/or provided sufficiently consistent infection. Research to develop a suitable plant growth assay to quantify pathogen suppression is ongoing.

**4.4 Outcomes** *Integrated dataset on the pathogen, disease incidence, disease suppression and crop yield for field soils from different cotton growing regions.*
**Outputs** *Draft journal paper 1 (soil suppression)*

The refereed conference paper on 'Disease suppression: soil fungal community diversity and interactions' presented at the 10th Australasian Soilborne Disease Symposium held in Adelaide in 2018 is being finalised as a scientific paper to be submitted to the Journal 'Phytopathology' by March 30th, 2020.

**4.5 Outcomes** *Integrated dataset on soil fungi and beneficial microbial community in cotton soils identifying the biological elements of disease suppressive soil*
**Outputs** *Draft journal paper 2 (fungi and beneficial microbial community in cotton soils)*

Information on the diversity, genetic and catabolic composition of soil bacterial and fungal communities in the rotation experiment at NorthStar, presented under Milestone 4.1 Objective 2 is being converted into a manuscript titled "Crop rotation effects on composition of soil microbial communities, microbial activity and potential implications to biological suppression" (abstract accepted) for submission to the Journal MDPI Plants – Special issue on Management of Verticillium disease, Abstract accepted manuscript to be submitted by April 30th, 2020.

**Outcome 5. What percentage of growers adopted practices that reduce the incidence of diseases and pests on their farms at project completion?**

**5.5 Outcomes** *Clear roles and responsibilities for project and CottonINFO team personnel identified and agreed regarding the adoption and pathways for the project. Linkage between disease survey, annual grower survey and CottonInfo and CRDC M&E plans ensures information is collected efficiently and does not duplicate processes.*
**Outputs** *Discussions held with Warwick Waters, manager of CottonINFO Team, and adoption pathway(s) outcomes including monitoring of adoption agreed and reported to CRDC.*

Adoption pathways have focussed on dissemination of research findings by various means such as CottonInfo updates, regional newsletters and industry articles, presentations at field days, grower meetings and conferences etc.

An evaluation form was developed and sent to growers involved in annual disease surveys. Feedback so far has been positive with useful suggestions to improve surveys and reporting of findings to grower.

2. **Please describe any:-**
   a) **technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);**
   b) **other information developed from research (eg discoveries in methodology, equipment design, etc.); and**
   c) **required changes to the Intellectual Property register.**

None

## *Conclusion*

3. **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?**

This project took a new approach, to bring together data across the industry in a national on diseases present and their incidence. These data were used to monitor disease trends over time and was useful for informing industry of disease issues and providing recommendations for disease management.

Identifying disease trends
Disease survey data has been collected across NSW and Qld for many years and has provided an understanding of disease trends. In brief, different regions are being affected by different pathogens. Black Root Rot is a limiting factor in fields in southern NSW because of its cooler climatic conditions. Alternaria leaf spot is developing as an early season disease of concern in NSW. Serious infection of young seedlings were observed in the Lachlan and Murrumbidgee regions in early December 2017. It was confirmed through molecular techniques that *Alternaria alternata* was the species causing severe disease early season in southern NSW.
Fusarium wilt remains a key disease for St George and the Darling Downs with high incidences also being detected in some fields late season in the Macquarie and Macintyre. There was likely an impact on yield for some fields due to Fusarium wilt. It is imperative not to become complacent of this disease, or relying solely on host resistance. An integrated approach to management is extremely important to manage this disease, as it is with all soil-borne pathogens.

Verticillium wilt continues to be a major disease of concern in the Border Rivers and Namoi, which is concerning given that the environmental conditions (due to drought) have not been considered

conducive to Verticillium wilt development. However, the high temperatures and dry conditions at this time led to an intense irrigation schedule on farms where water was available, which may have provided a cooling effect in the soil and therefore conditions conducive to the development of this disease. In the Gwydir, 30% of fields' surveyed in the 2018/19 had both wilt diseases. This poses a significant challenge in managing crop residues, crop rotations and cultivar selections to minimise disease impacts.

When environmental conditions were conducive for the development of boll rots/tight-lock a significant amount was observed (e.g. a range of 8-20 bolls/m affected by a combination of boll rot and/or tight-lock). There was anecdotal evidence that boll rot and tight-lock was the cause of significant yield reduction in these fields.

The surveys also served as a vehicle for the collection of soil samples for analysis of plant-parasitic nematodes. This new approach for detection of plant-parasitic nematodes was successful for detecting new farms in Emerald reniform nematode. To date, this nematode has only been detected in cotton in CQ.

These results demonstrate the necessity of general multi-pest surveillance systems in cotton in providing an ongoing evaluation of the distribution and impact of key endemic pests.

Statistical associations identified in historical datasets

The development of technologies such as regression modelling enables statistical associations to be identified in historical datasets, from which potential causal associations relevant to disease control may then be investigated. To meet this end, multivariate analyses of survey data were performed to test for any effect of previous crop and cotton trash present on early and late season diseases. Further correlation network analyses were performed on the data to identify relationships between diseases and yield and diseases themselves in the whole data set and in each state. Key findings include the impact of previous cropping on disease in the subsequent cotton crop. For example, Verticillium wilt was significantly higher following winter cereal crops than cotton. Seed rot was significantly lower following a fallow or winter cereals, boll rot was significantly higher following summer grains, and tight-lock was significantly lower following fallow and winter cereals, than cotton. Tight-lock had the strongest negative relationship with yield in the data set, providing statistical support of anecdotal findings. The amount of cotton trash present in the field did not have any significantly detectable effect on disease in the analyses performed on these data. Given the impact of previous crop on disease incidence, an estimation of crop residues other than cotton may provide insight to how other crops influence disease, such as maintaining inoculum levels through asymptomatic colonisation or providing a suitable carbon source for saprophytic growth. Hence, these findings provide direction for research to investigate cropping rotations that potentially will decrease/increase disease incidence of important diseases of cotton. The groundwork achieved in this project has provided the knowledge to improve the collection and storage of data, and to build on the analyses already conducted.

Verticillium wilt research

The management of Verticillium wilt requires an integrated approach that ultimately reduces soil inoculum levels. The field trials conducted in this project have shown that rotation can reduce disease but needs to be longer than one year out of cotton where Verticillium levels are high. Two years of rotation to either non-hosts (sorghum and corn) or a bare fallow significantly reduced Verticillium levels compared to growing three years of continuous cotton. One year of rotation (corn, sorghum or fallow) on the other hand was not long enough to significantly lower disease levels. The assessment of microbial changes in the soil under the different rotations sequences suggest that management of this disease through cropping to other non-host crops may be a better option than fallow as they reduce disease incidence but also maintain overall soil biological health. A decline in overall microbial populations in the long term could potentially make soils more conducive to soil borne diseases.

*V. dahliae* has one of the widest host ranges of any fungal pathogen, including over 400 susceptible crop and weed hosts. It may cause classic characteristic symptoms but also has the ability to develop asymptomatic, endophytic infections. The susceptibility of some rotation crops commonly grown in the Australian cotton farming system has been largely unknown to date. Our studies have shown that grain sorghum is a non-host (previous study) and that faba bean (previous study) and cultivars of chickpea,

mungbean, wheat and barley are all susceptible symptomatic hosts with some differential cultivar and strain reactions observed in some of these crops. While the susceptibility of these crops has not been proven under natural field conditions there is clearly the potential for infection to occur and close monitoring of field plants of these alternative hosts should be carefully monitored and assessed when grown in fields known to have a history of Verticillium.

Disease suppressive soils
The successful control of many soil-borne plant pathogens involves management of the pathogen at a combination of different microsites (*e.g.* inoculum source and rhizosphere) in soil and at different time periods (pre-season or in the presence of the susceptible plant). Therefore, *in situ* enhancement of natural disease suppression may be more effective than adding inoculants. To enable the enhancement of natural disease suppression, knowledge on the factors that drive the soil microbial communities in cotton soils from different regions, and how these influence disease, may identify management options to modify beneficial soil microorganisms.

Composition and abundance of microbial communities were analysed for soils collected from different regions with different cropping histories and varying disease incidences. Results from the long-term experiments indicate that (i) the diversity and abundance of soil fungal communities varied significantly by crop management history and (ii) fungal communities in suppressive cotton soils were characterized by higher diversity and higher connectedness. The high level of organization along with higher diversity in the soil fungal community in the suppressive soils would provide the cotton plant with a stable microbial reservoir across varied seasonal environmental conditions.

Changes in the microbial diversity and activity in the short-term rotation experiment clearly indicated the significant and important contribution of soil microbiome for the suppression of Verticillium disease in cotton. The influence of rotation crops such as sorghum and corn could be attributed to (i) increased microbial catabolic diversity and activity (ii) higher diversity of bacteria and fungi, (iii) increased abundances of specific groups of microorganisms involved in antibiosis, antifungal (cell-wall degradation) and plant growth promoting capabilities, and (iv) lower pathogen levels. These changes would have contributed to the suppression of the pathogen, disease incidence and impact. Whereas the fallow treatment caused a significant decline in the total microbial activity and catabolic diversity, genetic diversity of bacteria and fungi resulting in lower pathogen suppression capacity. Although the lower pathogen levels would help in the reduction of disease incidence, long-term adoption of such management practices would not benefit in maintaining or improving the overall soil biological health. The traditional continuous cotton system seems to promote the growth of pathogenic fungi such as *V. dahliae* and result in lower microbial diversity and abundances of beneficial microorganisms.

A laboratory based pathogen suppression potential assay was developed which provides a quantitative measure of a cotton soils ability to support or inhibit soil-borne fungal pathogens such as *V. dahliae*.

Results from this study clearly indicate the presence of a genetically diverse fungal community in cotton soils and distinct variation in the community composition and diversity between fields in different cotton regions. Actinobacteria were the most dominant bacteria in cotton soils and bacterial community composition was significantly different in fields from some regions e.g. Darling Downs vs. Lochlan and Namoi vs. Theodore.

### *Extension Opportunities*

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

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

**(a) to further develop or to exploit the project technology**
A new project will further develop technologies developed in this project as per (c) for future research.

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

Finalise research papers for publication as detailed in report.

**(c) for future research**

**1). Verticillium research**

Future research will include continued assessment of different rotation crops to determine potential host susceptibility and identify potential new hosts. Expanding on the initial experiment reported here, a glasshouse experiment is currently being assessed looking at ten inoculated cultivars of wheat and barley and nine cultivars of chickpea grown to maturity and examining the seed of the test crops to identify any systemic infection through to seed. The potential for field grown cereals and legumes to be infected under natural conditions will be investigated. This information will help growers make more informed decisions regarding crop rotation to manage Verticillium wilt.

Field trials are likely to continue at "Getta Getta" when water is available. This is a valuable field site which has provided extremely useful data to date.

Growth curve studies have revealed some isolates behave slightly differently by growing optimally at higher temperatures. Isolates from all regions should continue to be monitored and virulence testing of isolates completed.

**2). Survey Database**

Improvements to in-field data capture in Fulcrum will assist in more efficient and timely transference to a database for multivariate analyses.

**3). Future Analyses**

Much further work can be done with these survey data including but not limited to:

- Analysing the effects of weather provided by BOM from the nearest station for each field
- Cluster analysis to determine clusters of areas acting in a similar or dissimilar fashion
- Analyse effects of the following on disease:
    - Variety
    - Seed rate
    - Configuration
    - Irrigation
    - Fertilisation regime
    - Soil type

Potential to analyse historical data e.g. 13 years of Qld survey data collected by Stephen Allen, if additional support was provided to transfer data from an Access database to Excel database.

**4) Pathogen Suppression Potential (PSP) assays**

Laboratory based and pot based PSP assays developed further and quantification of the suppressive potential of cotton field soils conducted.

## 9. 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)

*Meetings*

- Crop protection update meetings in Emerald 18th October 2016 and Theodore 20th October 2016
- 16th August 2016 CRDC board tour in Narrabri with Namoi growers to highlight collaborative project between DAF and CottonInfo
- 7th February 2017 Met with CRDC board in Theodore to discuss nematode research.
- 7 - 8 June 2017: presentations on seed treatments and managing seedling diseases to Emerald and Theodore growers during CSD roadshows
- 19th June 2017: presentation to Goondiwindi growers at local meeting organised by Sally Dickinson providing an end of season review
- 9th August 2017: presentations to growers and consultants in the Macintyre Valley (Goondiwindi) at a Verticillium information session (Linda Smith, Season update; Linda Scheikowski, Results from the 'Getta Getta' trial)

- 30th August 2017: presentations made at Auscott and Wee Waa to growers concerning Verticillium (Linda Smith, Verticillium Wilt Update; Linda Scheikowski, Verticillium – "Getta Getta" rotation trial)
- 14th November 2017: informal discussion with Hillston cotton growers and consultants concerning black root rot. Field visits followed with further grower and consultant discussions
- Project summary presented at the Cotton Pathology review meeting on 16th May 2018 in Sydney by Gupta Vadakattu and Linda Smith
- Project update presented Monday 28th May 2018, CSIRO Agriculture and Food, Urrbrae SA – Rural R&D for Profit.
- Research updates presented at the Macintyre Valley End of Season Review in Goondiwindi on the 13th June 2018
  *Disease survey update*: Linda Smith
  *Verticillium wilt research update*: Linda Scheikowski
- Research updates presented at the CCA meeting in Narrabri on the 21st June 2018
  *Disease survey update*: Linda Scheikowski
  *Verticillium wilt research update*: Linda Scheikowski
- Disease survey update and research summary presented at the MCGA meeting in Mungindi on the 14th November 2018
- Linda Smith and Linda Scheikowski presented research update on the use of corn in rotation with cotton to manage Verticillium wilt at Wee Waa, invited by Steve Madden, 6th June 2019
- Linda Smith presented a disease update at the CCA workshop, Jondaryan, Qld 15th August 2019.
- Duy Le presented a disease update at the CCA workshops in Griffith 22nd and Moree 29th August 2019
- Linda Smith and Dinesh Kafle presented a reniform nematode research update at Emerald and Theodore 30 - 31 July 2019
- Provided CottonInfo with regional disease survey results for presentation at CSD roadshow.

*Articles*

- *24th April 2017 CottonInfo e-news: Surveying for disease – an update on early season disease survey results.*
- 6th November 2017 CottonInfo e-news: Spotted anything unusual?
- Article in Spotlight Autumn 2018- Solarisation under the microscope at Garah
- Linda Scheikowski, Linda Smith, Gupta Vadakattu, Tim Shuey and Dinesh Kafle. Longer rotations are required to reduce Verticillium where disease levels are high. Australian CottonGrower Dec 2018 p 14-18.

*Papers*

- Duy P. Le & Aphrika Gregson, 2019. Alternaria leaf spot of cotton seedlings grown in New South Wales, Australia is predominantly associated with *Alternaria alternata*. Australasian Plant Pathology https://doi.org/10.1007/s13313-019-0617-9
- Draft paper: Verticillium wilt of cotton: identification and detection of the causal pathogen and its control. Duy P. Le, Karen Kirkby, Carlos Trapero, Thao T. Tran and Linda Smith

*Industry publications*

- Regional Disease Update 2016 – 2017 for the CRDC Pest Management Guide
- Regional Disease Update 2017 – 2018 for the CRDC Pest Management Guide
- Regional Disease Update 2018 – 2019 for the CRDC Pest Management Guide
- Regional disease survey summary for the 2017/18 CSD Trial Results USB Booklet
- Regional disease survey summary for the 2018/19 CSD Trial Results USB Booklet

*Conferences*

The following presentations were made at FUSCOM (Goondiwindi 7-9 August 2017):

- Linda Smith, National Disease Surveys Update; Quick diagnostic kit for *Verticillium dahliae* based on LAMP technology; Reniform nematode – an update
- Linda Scheikowski, Verticillium – Getta Getta trial
- Gupta, V.V.S.R., Smith, L. and Scheikowski, L., (2017) How different are soil fungal communities in different cotton growing regions. Presentation at the FUSCOM meeting in Goondiwindi, NSW.

Staff presented at the 3rd Australian Cotton Research Conference (Canberra 5-7 September 2017)

- Smith, L. (2017). Plant Disease Epidemiology: The challenges to managing economically important pathogens in Australian cotton. Cotton Research Conference Abstract Book, 3rd Australian Cotton Research Conference, CSIRO Discovery Centre, Canberra, 5-7 September 2017: 17.
- Scheikiowski, L. Smith, L. and Shuey, T. (2017). Vericillium wilt – rotation crops. Cotton Research Conference Abstract Book, 3rd Australian Cotton Research Conference, CSIRO Discovery Centre, Canberra, 5-7 September 2017: 54.
- Gupta, V.V.S.R., Smith, L., Scheikowski, L., Hunter, G. and Greenfield, P. (2017) Region-based differences in the diversity and abundance of fungal communities in cotton soils. Association of cotton Scientists Conference held during Sept 5-7 in Canberra, ACT.

Staff presented the following at the Science Protecting Plant Health Conference (Brisbane, 26-28 September, 2017)

- Smith, L.J., Scheikowski, L.J. and Cobon, J. (2017). Can reniform nematode (*Rotylenchulus reniformis*) in Australian cotton be managed by crop rotation? *Science Protecting Plant Health Conference Handbook*, Brisbane Convention & Exhibition Centre, Brisbane 26-28 September 2017: 69.

International conference presentations:

- Gupta, V.V.S.R., Penton, C.R. and Tiedje, J.M. (2017) Small worlds-big functions: soil fungal networks and plant health. Oral presentation 2nd Global Soil Biodiversity Conference, held during 15-19 October in Nanjing, China.
- Gupta, V.V.S.R. (2019) Australian Cotton Soil microbiome, Invited oral presentation at the International Agricultural Microbiomes RCN workshop (National Science Foundation USA funded) held during on Nov 25th in Melbourne, Australia.

Staff presented the following at the Australian Cotton Conference, Gold Coast 7 - 9 August 2018

- New approaches to disease surveys: Linda Smith (3 minute thesis)
- Two year rotation lowers incidence of Verticillium wilt: Linda Scheikowski (3 minute thesis)
- Gupta, V.V.S.R. (2018) Microbes do bounce back in Cotton soils. 2018 Australian Cotton Conference, Broadbeach, Queensland.

The following presentations were made at FUSCOM (Griffith August 28 - 29, 2018)

- 2017/18 National Disease Surveys- An Update: Linda Smith and Aphrika Gregson
- Verticillium Wilt Update: Linda Scheikowski
- Genetic characterization of black root rot pathogen on cotton in NSW: Duy Le
- Alternaria leaf spot – an emerging disease and pathogen in cotton?; Duy Le
- Cotton disease diagnostics – season 2017/18 update: Aphrika Gregson
- Fusarium species collected from seedling and mature cotton in NSW: Duy Le

Staff presented the following at the Australasian Soil-borne Diseases Symposium, Adelaide, 4 – 7 September 2018.

- Two year crop rotation lowers incidence of Verticillium wilt in cotton. Linda Scheikowski, Linda Smith, Tim Shuey and Dinesh Kafle
- Genetic characterisation of black root rot pathogen of cotton in NSW Australia. Duy Le and Aphrika Gregson
- Gupta, V.V.S.R., Kirkby, K., Smith, L and Penton, C.R. (2018) Disease suppression: soil fungal community diversity and interactions. In: Proceedings of 10th ASDS meeting, Gupta VVSR et al. (eds.). pp. 171-172, Adelaide.
- Gupta, V.V.S.R., Kroker, S.K., Hicks, M., Nidumolu, B. and Weir, D. (2018) Effect of adding compost on microbial activity and composition in Australian cotton soil. In: Proceedings of 10th ASDS meeting, Gupta VVSR et al. (eds.). pp. 161-162, Adelaide.

Staff presented the following at the AACS Cotton Research Conference in Armidale on the 28 – 30 October 2019.

- Duy Le KEYNOTE: Alternaria leaf spot of cotton: a re-emerging disease associated with a new pathogen
- Aphrika Gregson *Colletotrichum truncatum,* a new causal agent associated with cotton boll rot in Australia
- Dinesh Kafle et al. Understanding the ecology of reniform nematodes in Australian cotton
- Linda Scheikowski et al. Host or non-host: study on *Verticillium dahliae*
- Linda Smith et al. Prevalence and distribution of cotton diseases in 2018/19 season
- Gupta VVSR et al. Harnessing beneficial microbiomes in cotton systems.

Staff presented at Australasian Plant Pathology Conference

- Le DP, Gregson A, Ravichander P, Aitken E, Scheikowski L, Smith L, 2019. Fusarium wilt of cotton: surveillance, detection and management. Fusarium Workshop, the 22nd APPS, Melbourne, Australia November 2019 (oral presentation)
- Le DP, Gregson A, Jackson R, Smith L, 2019. Cotton diseases: the big four in NSW. The 22nd APPS, Melbourne, Australia November 2019 (oral presentation)

*Other*

- Verticillium Update – "Strathguyle", Garah, NSW Farm walk Wednesday 24th January 2018. Linda Smith gave an overview of Verticillium wilt research and Linda Scheikowski gave an overview of our solarisation trial to manager Verticillium wilt.
- CottonInfo 'Cotton diseases 101', organized by Sharna Holman, was presented to new local agronomists at Emerald on 9th October 2018 and outlined both common and new cotton disease issues found in the Central Highlands region with advice on how to identify and manage these diseases.
- Disease survey summaries provided to REOs

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

CottonInfo: connecting growers with research (YouTube videos )

- Managing Fusarium wilt (launched June 2018) https://youtu.be/Cygy6XiRDcw
- Reniform nematode in cotton: Linda Smith (launched June 2018) https://youtu.be/QgBn4vfkOzI
- Verticillium wilt in cotton: Linda Smith (launched June 2018) https://youtu.be/hgiatA52nNw
- Biosecurity top tips: prevent disease transfer between fields: Tim Shuey (launched September 2018) https://youtu.be/bXIEsY87CXo
- Biosecurity top tips: disease surveys find out what is (and isn't) in your fields: Linda Scheikowksi (launched September 2018) https://youtu.be/fHWzrImgUy4

## Part 4 – Final Report Executive Summary

*Provide a one-page summary of your research that is not commercial in confidence, and that can be published on the internet. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.*

**Statistical associations identified in historical datasets**

A geospatial database was developed and three years of field assessed cotton pathology data and management practices were collected. Multivariate analyses of survey data were performed to test for any effect of previous crop and cotton trash present on early and late season diseases. Further correlation network analyses were performed on the data to identify relationships between diseases and yield and diseases themselves in the whole data set and in each state.

Key findings include the impact of previous cropping on disease in the subsequent cotton crop. For example, Verticillium wilt was significantly higher following winter cereal crops than cotton. Seed rot was significantly lower following a fallow or winter cereals, boll rot was significantly higher following summer grains, and tight-lock was significantly lower following fallow and winter cereals, than cotton. Tight-lock had the strongest negative relationship with yield in the data set, providing statistical support of anecdotal findings. The amount of cotton trash present in the field did not have any significantly detectable effect on disease in the analyses performed on these data. Given the impact of previous crop on disease incidence, an estimation of crop residues other than cotton may provide insight to how other crops influence disease, such as maintaining inoculum levels through asymptomatic colonisation or providing a suitable carbon source for saprophytic growth. Hence, these findings provide direction for research to investigate cropping rotations that potentially will decrease/increase disease incidence of important diseases of cotton. The groundwork achieved in this project has provided the foundation knowledge and critical directions to improve the collection and storage of data, and to build on the analyses already conducted.

**Disease suppression potential of cotton soils from different cotton growing regions**

Composition and abundance of microbial communities were analysed for soils collected from different regions with different cropping histories and varying disease incidences. Results from the long-term experiments indicate that (i) the diversity and abundance of soil fungal communities varied significantly by crop management history and (ii) fungal communities in suppressive cotton soils were characterized by higher diversity and higher connectedness. The high level of organization along with higher diversity in the soil fungal community in the suppressive soils would provide the cotton plant with a stable microbial reservoir across varied seasonal environmental conditions.

Changes in the microbial diversity and activity in the short-term rotation experiment clearly indicated the significant and important contribution of soil microbiome (bacteria and fungi) for the suppression of Verticillium disease in cotton. The influence of rotation crops such as sorghum and corn could be attributed to (i) increased microbial catabolic diversity and activity (ii) higher diversity of bacteria and fungi, (iii) increased abundances of specific groups of microorganisms involved in antibiosis, antifungal (cell-wall degradation) and plant growth promoting capabilities, and (iv) lower pathogen levels. These changes would have contributed to the suppression of the pathogen, disease incidence and impact. Whereas the fallow treatment caused a significant decline in the total microbial activity and catabolic diversity, genetic diversity of bacteria and fungi resulting in lower pathogen suppression capacity. Although the lower pathogen levels would help in the reduction of disease incidence, long-term adoption of such management practices would not benefit in maintaining or improving the overall soil biological health. The traditional continuous cotton system seems to promote the growth of pathogenic fungi such as *V. dahliae* and result in lower microbial diversity and abundances of beneficial microorganisms.

A laboratory based pathogen suppression potential assay was developed which provides a quantitative measure of a cotton soils ability to support or inhibit soil-borne fungal pathogens such as *V. dahliae*.

Results from this study clearly indicate the presence of a genetically diverse fungal community in cotton soils and distinct variation in the community composition and diversity between fields in different cotton regions. Actinobacteria were the most dominant bacteria in cotton soils and bacterial community composition was significantly different in fields from some regions e.g. Darling Downs vs. Lochlan and Namoi vs. Theodore.

**Verticillium wilt research**

The management of Verticillium wilt requires an integrated approach that ultimately reduces soil inoculum levels with deleterious effects on overall soil biological health. The field trials conducted in this project have shown that rotation can reduce disease but needs to be longer than one year out of cotton where Verticillium levels are high. Two years of rotation to either non-hosts (sorghum and corn) or a bare fallow significantly reduced Verticillium levels compared to growing three years of continuous cotton. One year of rotation (corn, sorghum or fallow) on the other hand was not long enough to significantly lower disease levels. The assessment of microbial changes in the soil under the different rotations sequences suggest that management of this disease through cropping to other non-host crops that may also promote disease suppressive microorganisms may be a better option than fallow as they reduce disease incidence but also maintain overall soil biological health. A decline in overall microbial populations in the long term could potentially make soils more conducive to soil borne diseases.

*V. dahliae* has one of the widest host ranges of any fungal pathogen, including over 400 susceptible crop and weed hosts. It may cause classic characteristic symptoms but also has the ability to develop asymptomatic, endophytic infections. The susceptibility of some rotation crops commonly grown in the Australian cotton farming system has been largely unknown to date. Our studies have shown that grain sorghum is a non-host (previous study) and that faba bean (previous study) and cultivars of chickpea, mungbean, wheat and barley are all susceptible symptomatic hosts with some differential cultivar and strain reactions observed in some of these crops. While the susceptibility of these crops has not been proven under natural field conditions there is clearly the potential for infection to occur and close monitoring of field plants of these alternative hosts should be carefully monitored and assessed when grown in fields known to have a history of Verticillium.

To conclude, these analyses of laboratory and field based research, have provided a wealth of knowledge to address systems questions on disease management. Research to understand management strategies that promote microbial diversity, increase specific groups of beneficial microorganisms, and reduce pathogen capability to cause disease, is required.