Australian Government
Cotton Research and Development Corporation

FINAL REPORT 2016

For Public Release

Part 1 - Summary Details

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

CRDC Project Number: DAN1403

Project Title: Diseases of Cotton XI

Project Commencement Date: 01/07/2013 **Project Completion Date:** 30/06/2016

CRDC Research Program: 1 Farmers

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Part 3 – Final Report

Background

1. Outline the background to the project.

Diseases of Cotton XI project aimed to increase Australia's biosecurity preparedness through early detection by completing annual disease surveillance on commercial cotton farms, recording the presence/absence of exotic cotton diseases and establishing Australia's capacity to screen for exotic strains of bacterial blight.

Seedling diseases continue to threaten the productivity and sustainability of cotton production in Australia. Seedling disease occurs when cotton is invaded by a number of soil-borne fungi including *Pythium* and *Rhizoctonia* spp. Continued independent evaluation of effectiveness of seed treatment fungicides and combinations against seedling disease is needed. These trials provide the most up to date information on new and existing seed treatments available. The cotton industry spends millions of dollars on seed treatments each season. It is important that the effectiveness of current fungicides (including the industry standard) and potentially new treatments are continually evaluated. Including different chemicals and noting the results also indicates the dominant pathogens present during each season. For example when stand counts have been high under Apron (Metalaxyl) treatments, this can indicate that the dominant pathogen was likely *Pythium* spp. Alternatively when stand counts are high under PCNB treatments, this can indicate that *Rhizoctonia* was the likely dominant pathogen.

Biofumigation is the term used to describe the natural suppression of soil borne pests and diseases using plants containing high levels of glucosinolates (GSLs) in their tissue such as Brassica crops. Glucosinolates are naturally occurring sulphur compounds that provide plants with protection. When these plants are incorporated into the soil, tissue is disrupted and the glucosinolates are broken down by the plant enzyme Myrosinase to produce phytochemicals called isothiocynates (ITCs). The ITCs are biocidal to a range of organisms and have the potential to suppress pest and disease organisms. In previous biofumigation experiments at Australian Cotton Research Institute (ACRI), treatments included canola, vetch, chickpea and fallow. The biofumigation trial in this project continued the research from Diseases of Cotton X. The biofumigation crops included vetch, Doublet fodder radish, biofum blend (40% Doublet Fodder Radish, 50% Carinata Brassica and 10% Achilles white mustard) and a fallow treatment. Different Brassicas release different quantities of ITCs, therefore it is important to include more than one variety when assessing crops for biofumigation potential.

The long term disease survey data has shown an increase in Verticillium wilt in NSW over recent years. Cotton varieties resistance to the pathogen that causes Verticillium wilt is temperature sensitive. Consequently varieties that are resistant at 25-27^{o}C are susceptible at 20-22^{o}C. Preliminary examination of some fields in the long term data had shown that fields with a high incidence of black root rot early in the season also had high incidence of Verticillium wilt later in the season. Discussions with leading pathologist Dr Stephen Allen about his experiments and observations also indicated there may be an interaction. The long term data provides an excellent opportunity to look for potential interactions between pathogens and incidence/severity of disease.

The NSW pathology team worked in close collaboration with QDAFF pathologist Dr Linda Smith and also UNE researcher and senior lecturer Dr Lily Pureg studying the pathogens that cause black root rot and Verticillium wilt in cotton. NSW DPI assisted with collecting and supplying pathogen cultures to both Linda and Lilly. NSW DPI continued to work closely with CSIRO (planting off site trials), CSD Dr Stephen Allen with disease surveys and disease enquiries and Dr Nilantha Hulugalle in assessing his field trials for disease.

Objectives

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

The project consisted of 6 objectives, each with milestones attached to it to evaluate the performance and achievement against each objective. The objectives and milestones were:

- Objective 1Respond to industry issues as they arise annually
    - Milestone 1.1 Maintain PathWAY communication tool

- Objective 2 Continue long term disease surveys and surveillance for exotic diseases in NSW and investigate potential interactions between the pathogens *Verticillium dahliae* and *Thielaviopsis basicola*
    - Milestone 2.1 Early and late season surveys
    - Milestone 2.2 Investigate long term data for potential interaction between incidence of black root rot and Verticillium wilt
    - Milestone 2.3 Increase/maintain long term storage of culture collection
    - Milestone 2.4 Improve knowledge of diversity within black root rot isolates
    - Milestone 2.5 Improve knowledge of diversity within Verticillium dahliae isolates

- Objective 3 Continue to provide an independent evaluation of the effectiveness of existing and novel seed treatments for seedling disease and black root rot
    - Milestone 3.1 Independent and impartial evaluation of existing and novel seed treatments for seedling disease and black root rot

- Objective 4 Assess the potential of winter biofumigation crops for their potential to suppress black root rot
    - Milestone 4.1 Assess winter biofumigation crops for the potential to suppress black root rot and Verticillium wilt

- Objective 5 Improve Australia's preparedness for incursions of exotic bacterial blight strains
    - Milestone 5.1 Blight differential lines screened to confirm varietal reaction to blight inoculum
    - Milestone 5.2 Develop framework and commence draft contingency plan for hyper virulent bacterial blight

- Objective 6 Build human capacity through professional development of staff and collaboration between organisations
    - Milestone 6.1 Professional development of pathology staff

Methods

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

Objective 1. Respond to industry issues as they arise annually.

1.1.1 The PathWAY communication tool that was established in 2012 by Dr Karen Kirkby (Figure 1) was used to quantify disease enquiries throughout this project. A monthly summary was sent to all PathWAY points of contact.

Figure 1. Logo developed for PathWay in collaboration with CRDC

Objective 2. Continue long term disease surveys and surveillance for exotic diseases in NSW and investigate potential interactions between the pathogens *Verticillium dahliae* and *Thielaviopsis basicola*

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Commercial cotton crops in up to 100 fields, representing all cotton growing areas of NSW were surveyed during November/December and March/April of each season of the project from 2013 to 2016 (Figure 2). The incidence of disease was assessed at points determined using a step-point method across two transects (each 100 m long) in each field. Disease incidence was based on symptoms, with confirmation of the presence of some pathogens (e.g. the black root rot fungus, using a field microscope and laboratory isolations), and other pathogens with laboratory methods (e.g. Verticillium and Fusarium wilt) (Figures 3to 18). Populations of pathogens in the soil were measured using bioassays either (i) in the laboratory, or (ii) in the glasshouse. Samples suspected to be infected with Fusarium were sent to Queensland DAFF for confirmation and vegetative compatibility group analysis.

Figure 2.Dr Karen Kirkby examining seedlings during early season surveys.

The long term disease survey data was analysed for any potential interaction between high incidence of black root rot and Verticillium wilt (Figures 19 and 20). The effect of dual infection on black root rot severity was also examined (Figure 21). The long term culture collection was added to and maintained during this project (Figure 22). Black root rot isolates were used in many experiments to increase our understanding of the pathogen that causes this disease in cotton (Figures 23 to 25). Many field and laboratory experiments were undertaken to gain a better understanding of the pathogen *Verticillium dahliae*. The location

of inoculum across permanent beds and soil depth (Figures 26 and 27) was studied as well as case studies on particular farms. The effect of irrigation sources (Figure 28), depth of inoculum (Figure 29) and irrigation efficiency (Figure 30) on inoculum levels all provided important information. The effect of different management strategies such as composting gin trash (Figure 31), rotation crops Figure (32 to 39, Table 4 and 5), Raking and burning (Figure 40, Table 6) provided information at field levels on inoculum levels.
The pot experiment set up to determine the minimum level of inoculum needed to cause disease symptoms found very low levels of VCG2A were needed (Figures 41 to 43).

Objective 3. Continue to provide an independent evaluation of the effectiveness of existing and novel seed treatments for seedling disease and black root rot. Seedling diseases of cotton (*Gossypium hirsutum* L.) cause major losses in Australian cotton production each year. Pre and post emergence seedling diseases are caused by fungal pathogens that are favoured by environmental conditions that delay germination, emergence and seedling growth. The most common pathogens include *Pythium* spp., *Rhizoctonia solani* and *Thielaviopsis basicola*. Strong expansion of the cotton industry southwards into the cooler and shorter growing season areas of the Lachlan and Murrumbidgee valleys emphasises the need for effective plant establishment.

Cotton Seed Distributors (CSD) provides customers with the option of fungicide treated seed. Seed treatments offer one means of integrated disease management early in the season. Seed treatments are applied to the seed before the customer takes delivery so it is an efficient and safe means of guarding against early season pests and diseases. The current industry standard fungicide seed treatment for cotton planted in Australia is Dynasty which is a combination of Azoxystrobin, Metalaxyl-M and Fludioxonil. Most seed is also treated with Acibenzolar-S-methyl to assist with the control of Fusarium wilt and black root rot.

Each season NSW DPI, with the assistance of CSD and CSIRO conducted independent screening of novel products and impartial evaluation of efficacy of current standard fungicide seed treatments. Each fungicide was evaluated alone and in combination with other treatments. The seed treatment trials were geographically spread from Mungindi in the Macintyre valley, Narrabri in the Namoi valley, Warren in the Macquarie valley and Hillston in the Lachlan valeey (Figures 44 to 65, Tables 7 to 9). Up to 24 replicated treatments were evaluated in randomised plot trials approximately six weeks after planting at these four sites during the 2013/2014, 2014/2015 and 2015/2016 cotton seasons. Seeds per kilogram reported by CSD (http://www.csd.net.au/seedsperkilogram) for Sicot 71BRF and Sicot 730 was 10,305 and 11,965 respectively. Fungicides and other seed treatments were applied to cotton seed in combination with the standard Dynasty Complete + Cruiser and its components and deletions thereof. Seed was sown in single row-plots using a cone seeder, or in larger plots using box planters.

Statistical analysis
All glasshouse, growth room and field experiments used completely randomised block designs. Analysis of variance with spatial analysis (ASREML) was applied to field experiments with planned comparisons of treatments. Linear and non-linear regression models were fitted to ‘dose response’ experiments.

Objective 4. Assess the potential of winter biofumigation crops for their potential to suppress black root rot
A long-term field experiment assessed the potential of winter biofumigation crops to suppress diseases. In a completely randomized plot design, four treatments were replicated six times

in a field at Australian Cotton Research Institute (ACRI), Narrabri. The trial covered 96 rows, consisting of 24 plots of 8 rows. The treatments included biofum blend (40% Doublet fodder radish, 50% Carinata Brassica and 10% Achilles White Mustard), Doublet fodder radish, Vetch and plots of bare fallow.

The field was laser levelled in March 2013 to improve soil drainage. Beds were pulled up in April and the field irrigated. The rain in April saw many weeds germinate and were controlled using both cultivation and chemical. The planting of the biofumigation crops was delayed due to late seed delivery into Australia, longer than anticipated delays in quarantine and rain during June. In 2013/14 season the biofumigation crops were planted into moisture on the 18th June at a rate of 14kg/ha. Fallow plots were cultivated on the same day. The vetch treatment failed to germinate. Nitrogen was applied 8th August at a rate of 100 kg/ha. Biomass cuts were taken from the biofum blend and fodder radish treatment plots on the 13th September. Biofumigation plots were incorporated and watered immediately after slashing on the 24th August. Six weeks after the biofumigation crops were incorporated; soil cores from each plot in each treatment (fallow, vetch, biofum blend and fodder radish) were taken. The soil was split, sieved and 400g of the biofum and fodder radish treatment were sent to Dr Gupta Vadakattu. On the 4th November cotton (74BRF) was sown into moisture across all treatment plots. Stand counts, average disease severity of black root rot and biomass cuts were taken on the 10th December.

In 2014/2015 season the biofumigation crops were planted into moisture on the 23th June at a rate of 14kg/ha. Fallow plots were cultivated on the same day. The vetch treatment failed to germinate sufficiently despite new seed being purchased. Biomass cuts were taken from the biofum blend and fodder radish plots on the 15th September. Biofumigation plots were then incorporated and watered immediately after slashing on the 16th September. Six weeks after the biofumigation crops were incorporated; soil cores from each plot in each treatment (fallow, vetch, biofum blend and fodder radish) were taken (Figure 66 and 67). The soil was split, sieved and 400g of the biofum and fodder radish treatment were sent to Gupta Vadakattu. On the 28th October, cotton (74BRF) was sown into moisture across all treatment plots. Stand counts, average disease severity of black root rot and biomass cuts were taken in early December (Table 10).

Statistical Analysis: GenStat (11th Edition) (Payne, Murray, Harding, Baird, & Soutar, 2008) Regular Grid spatial modelling (REML) was used to analyse data separately for average disease severity (ADS), shoot and root dry weight (where applicable). Statistical significance was assessed and reported at the 5% probability level.

Objective 5. Improve Australia's preparedness for incursions of exotic bacterial blight strains.

The differential cotton lines imported from USA to differentiate strains of bacterial blight were grown out to increase the seed. The seed from each differential line were separated into paired samples with one lot stored at ACRI and the other lot with CSIRO. Working in collaboration with CSIRO (Dr Ian Wilson), the expected reaction of each differential line is continuing to be tested to ensure integrity in each of the lines. A draft contingency plan for hyper virulent strains of bacterial blight has commenced.

Objective 6. Build human capacity through professional development of staff and collaboration between organisations.

Investing in pathology team members to increase their understanding and expertise training and laboratory exchanges has built capacity for NSW DPI to take leading roles in recognising and diagnosing endemic and exotic diseases. Keeping staff engaged decreased staff turnover.

Results

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

Objective 1: OBJECTIVE 1: RESPOND TO INDUSTRY ISSUES AS THEY ARISE ANNUALLY

Milestone 1.1 Maintain PathWAY communication tool

1.1.2 A total of 229 disease enquiries were reported through PathWay from 2013 to 2016.

During 2013/2014, 2014/2015 and 2015/2016 cotton seasons, 59, 53 and 117 enquiries respectively were responded to using phone, email and face- to-face meetings. Enquiries varied from requests for information about pathogens, disease diagnosis, product screening and requests to review publication information.

1.1.2 Email requests from key points of contact within PathWAY were made via email twice per month and summaries distributed to PathWAY participants at the end of each month.

1.1.3 Where possible, relevant information and/or images were provided to the editors of Spotlight magazine for publication. Information provided related to free disease diagnosis, Come Clean - Go Clean message and updates on PathWAY enquiries and a human capacity story.

1.1.4 Pathology has liaised with CottonInfo Development and Delivery team on relevant fact sheets and field days. A few examples from each season included:

- Information on black root rot provided to Kirrily Bloomfield and used in her issue of the UNCGA & Agvance Weekly Newsletter 26th July 2013
- The participation of Dr Karen Kirkby in a farm tour to two properties in November 2013 where growers were able to speak one on one, ask questions and hear the latest information on disease research and management options
- Karen reviewed the IDM chapter for the Cotton Production Manual for Susan Maas.
- Updates were provided at the 2013, 2014 and 2016 FUSCOM meeting in Toowoomba. The presentations were Verticillium wilt, black root rot and seedling disease
- Presentation at the Lower Namoi Cotton Growers Association meeting 26th November 2015 at CSD, Wee Waa
- NSW DPI coordinated a survey to growers about volunteer and ratoon cotton on behalf of Ngaire Roughley
- Verticillium updates provided at Moree, Goondiwindi and Gunnedah, presentation on Biosecurity at Griffith and Hillston in August 2015
- Presentations on Biosecurity update on *Verticillium dahliae* VCG1A at the AACS Conference
- Verticillium and black root rot fact sheets
- PathWAY has run its natural course for more than three years. The intent when it commenced was to report disease issues as they arose. The jointly funded role of CottonInfo now provides the same service as PathWAY. It is for this reason that PathWAY will cease to be reported on going forward.

OBJECTIVE 2: CONTINUE ANNUAL SURVEILLANCE OF ENDEMIC AND EXOTIC PLANT PATHOGENS ON COMMERCIAL COTTON FARMS IN NSW PRODUCTION AREAS, MONITORING INCIDENCE AND SEVERITY OF DISEASES OF COTTON AND RECORDING THE ABSENCE OF EXOTIC DISEASES

Milestone 2.1 Early and late season surveys

2.1.1 Plant samples were brought back to the laboratory to isolate and confirm causative pathogen of diseases detected during disease surveys.

A total of 200 plants per field from at least two fields per farm were surveyed during both early and late season cotton disease surveys.

SURVEILLANCE OF ENDEMIC AND EXOTIC PLANT PATHOGENS ON COMMERCIAL COTTON FARMS IN NSW PRODUCTION AREAS

Biosecurity Alert – Exotic strain VCG1A *Verticillium dahliae* diagnosed and secondary confirmation established from an independent laboratory in Spain.

15th April 2015 - Industry mail out containing co-branded information sheet with instructions on how to take and send samples of suspected infected plants was sent to all cotton growers. VCG1A was recorded in a 30 year old sample from the NSW DPI long term culture collection as well as in several 2014/2015 season samples. An attempt to eradicate this strain was therefore deemed unfeasible. No other exotic diseases have been recorded.

2013-2016 disease survey results:

Seedling Mortality

Seasonal weather conditions play an important role in the incidence and severity of diseases of cotton in Australia. As part of the disease surveys the number of seeds planted is compared to the number of plants established per metre. This comparison produces an estimate of seedling mortality caused by *Pythium* and *Rhizoctonia* spp, viability, activity of soil insects and physical problems (fertiliser or herbicide burn) and adverse environmental conditions such as subbing at or following planting. In 2013/2014 there were particular problems with crop establishment due to seedling disease pathogens, allelopathy from residues of sorghum and rice as well as scheduling irrigations. Warm dry spring weather conditions dried out the seed bed as well as subsurface sodic layers and plough-pans became an issue. The mean seedling mortality for NSW ranged from 25.2% in the Bourke/Walgett valley in the 2014/2015 season to 51.7% in the Bourke/Walgett valley in 2015/2016 season (Figure 3). There was no cotton planted at Menindee during the 2015/2016 season due to lack of water.

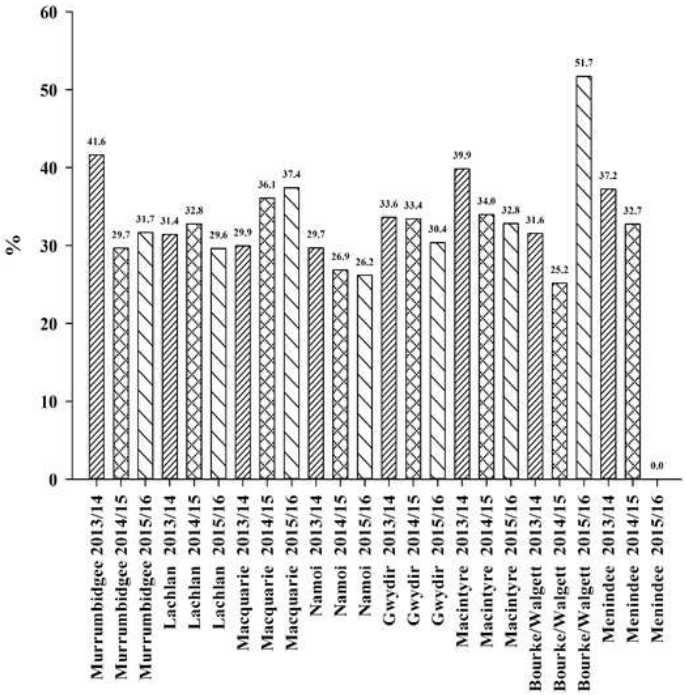

Figure 3. Seedling mortality rates for 2013/2014, 2014/2015 and 2015/2016 seasons.

Verticillium Wilt - Incidence

Verticillium wilt was recorded in all NSW cotton producing areas except in the Lachlan and Menindee valleys during the 2013 to 2016 cotton seasons. Incidence ranged from 0.03% in Murrumbidgee and Macquarie valleys in 2015/2016 to 24.91% in the Namoi in 2013/2014 season. There has been little increase in the incidence in each region over the three seasons, however it should be noted that the severity of the disease has been reported by growers and consultants to increase during this time. These figures represent the survey fields only and not all farms in each valley. The Namoi valley has historically had a higher incidence of disease when compared to the other NSW growing regions (Figure 4).

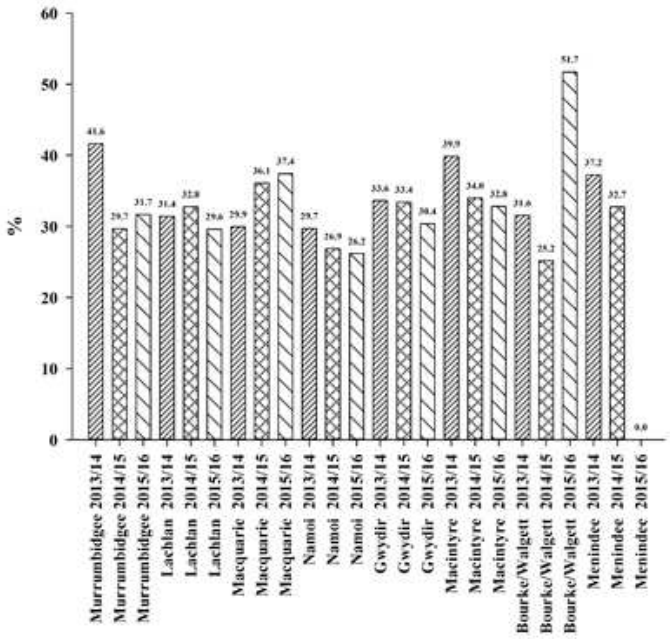

Figure 4. Incidence of Verticillium wilt for 2013/2014, 2014/2015 and 2015/2016 seasons.

Verticillium wilt - Percentage of field infected

The percentage of fields with Verticillium wilt recorded over the 2013 to 2016 seasons ranged from 6.3% in the Murrumbidgee valley in 2015/2016 season to 95.5% in the Namoi valley in 2015/2016 season (Figure 5). The pathogen that causes Verticillium wilt continues to be spread throughout regions and remains an issue for the industry. Emphasis must be placed on the Come Clean - Go Clean farm hygiene strategy to minimise the spread of this disease. Southern growing areas run a higher risk with the shorter growing season and generally cooler climate.

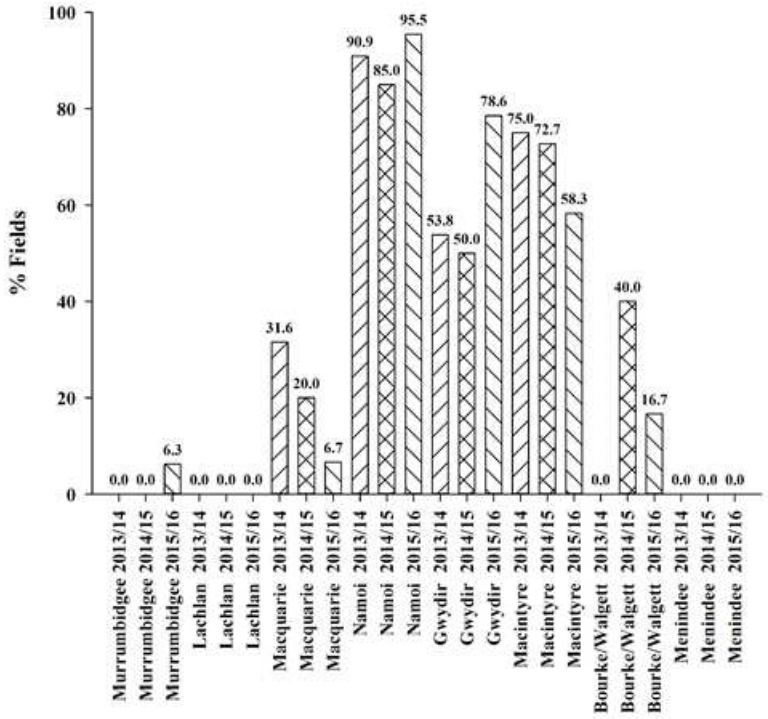

Figure 5. Percentage of fields with Verticillium wilt during 2013/2014, 2014/2015 and 2015/2016 cotton seasons.

Boll Rot - Incidence

The most common boll rot in NSW production areas was Phytophthora boll rot, which develops when soil is splashed up onto low opening bolls. Boll rot is most severe when opening bolls are subjected to extended periods of wet and cloudy weather and harvest is delayed. Boll rot was significantly higher during the 2013/2014 season due to seasonal conditions. During the 2013 to 2016 seasons, boll rot ranged from 0.003% in the Murrumbidgee valley in 2015/2016 season to 0.63% in the Macintyre during the 2013/2014 season (Figure 6). Sclerotinia boll rot was not observed in the 2013/2014 and 2014/2015 seasons. It was recorded on a few fields in the southern growing region during 2015/2016 season.

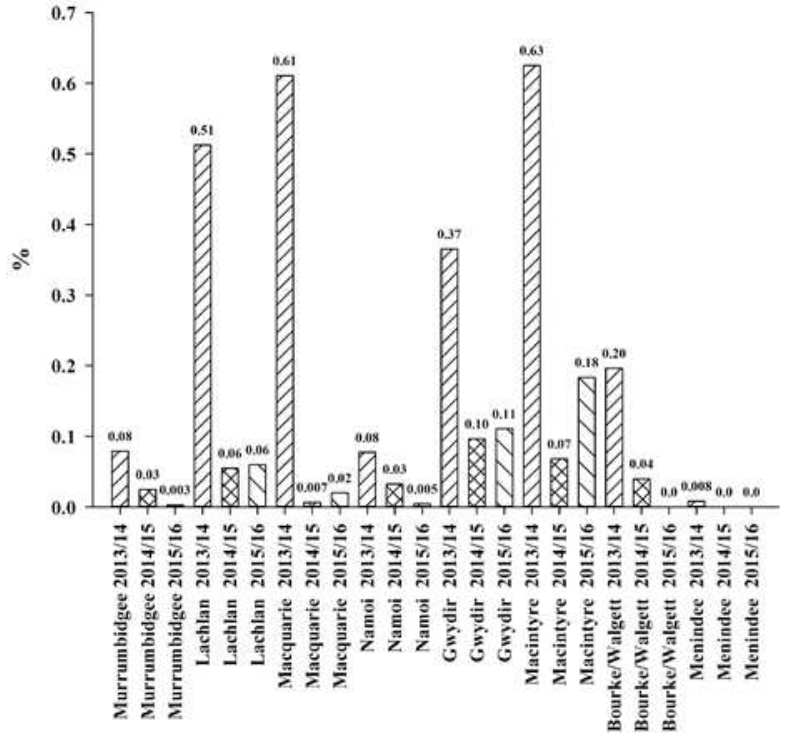

Figure 6. Percentage of boll rots during 2013/2014, 2014/2015 and 2015/2016 cotton seasons.

Bunchy Top - Incidence

Bunchy top was variable during the 2013 to 2016 cotton seasons, ranging from 0 recorded in the 2015/2016 seasons in all regions to 1.18% in the Macquarie valley during the 2013/2014 season (Figure 7).

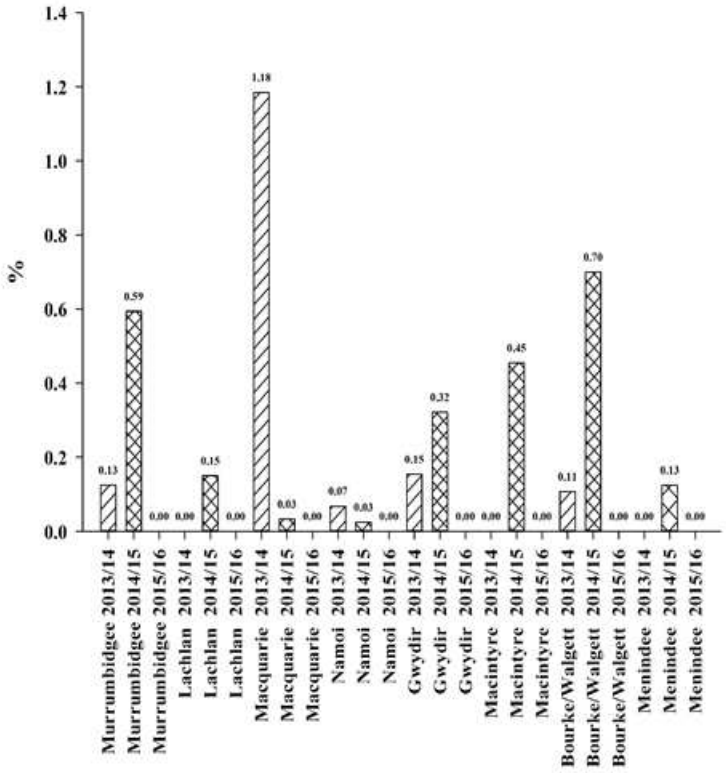

Figure 7. Incidence of Bunchy top during 2013/2014, 2014/2015 and 2015/2016 cotton seasons.

Bunchy Top - Percentage of fields infected

The percentage of fields with Bunchy top recorded ranged from 0 during 2015/2016 season to 40% in the Bourke/Walgett valley region in 2014/2015 season (Figure 8).

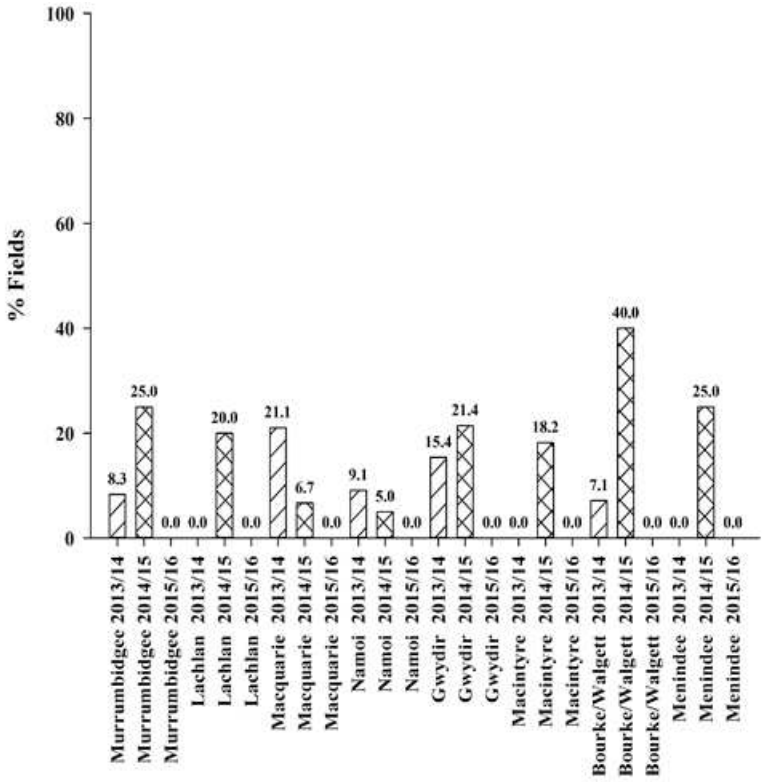

Figure 8. Percentage of fields with Bunchy top during 2013/2014, 2014/2015 and 2015/2016 cotton seasons.

Black root rot - Incidence

Black root rot, caused by the pathogen *Thielaviopsis basicola* is favoured by cool weather conditions early in the season. The pathogen colonises the surface of young cotton roots leading to stunted plant growth. As temperatures warm up, the tap root expands and the blackened root surface is sloughed off and disappears.

The incidence of black root rot was highly variable during the 2013 to 2016 cotton seasons (Figure 9). No cotton was planted in the Menindee valley during 2015/2016, hence 0% recorded. Incidence ranged from 5.5% in the Bourke/Walgett valley in 2015/2016 to 81.3% in the Namoi valley during 2013/2014 season. Warmer weather in the Murrumbidgee valley during 2015/2016 resulted in much lower black root rot being recorded.

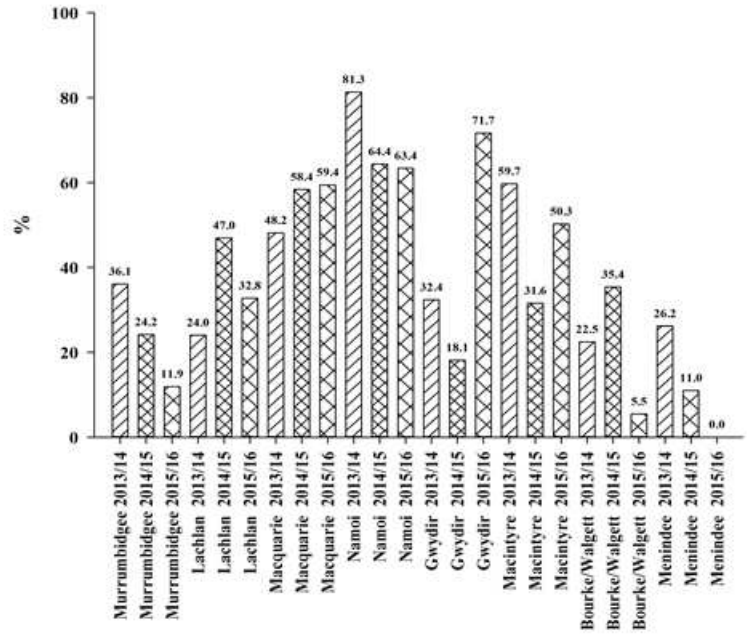

Figure 9. Incidence of black root rot during 2013/2014, 2014/2015 and 2015/2016 cotton seasons.

Black root rot - Severity

Assessment of black root rot severity is based on the proportion of each tap root that is blackened where 0 indicates healthy and 10 indicates 100% of the tap root blackened. The severity of black root rot recorded was variable during 2013 to 2016 seasons (Figure 10). No cotton was planted in the Menindee valley during the 2015/2016 season, hence no record of black root rot. Severity ranged from 1.5% in the Bourke/Walgett valley during 2015/2016 to 35.9% in the Namoi valley during the 2013/2014 season.

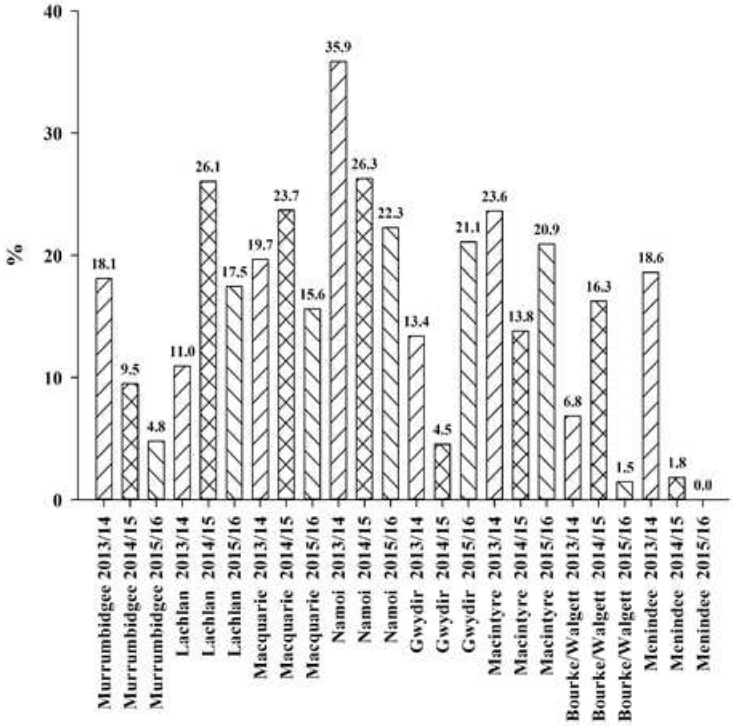

Figure 10. Severity of black root rot during 2013/2014, 2014/2015 and 2015/2016 cotton seasons.

Black root rot - Percentage of fields infected

The percentage of fields with black root rot recorded during 2013 to 2016 seasons was generally high (Figure 11). No cotton was planted the in Menindee valley during 2015/2016 season, hence no fields recorded. Percentage of fields with black root rot recorded ranged from 16.7% in the Bourke/Walgett valley during the 2015/2016 season to 100% in the Macquarie valley during 2013/2014 and 2015/2016 season, 100% in the Namoi valley during 2013/2014 season, 100% in the Gwydir valley during 2013/2014 and 2015/2016 seasons and 100% in the Menindee valley during 2014/2015 season.

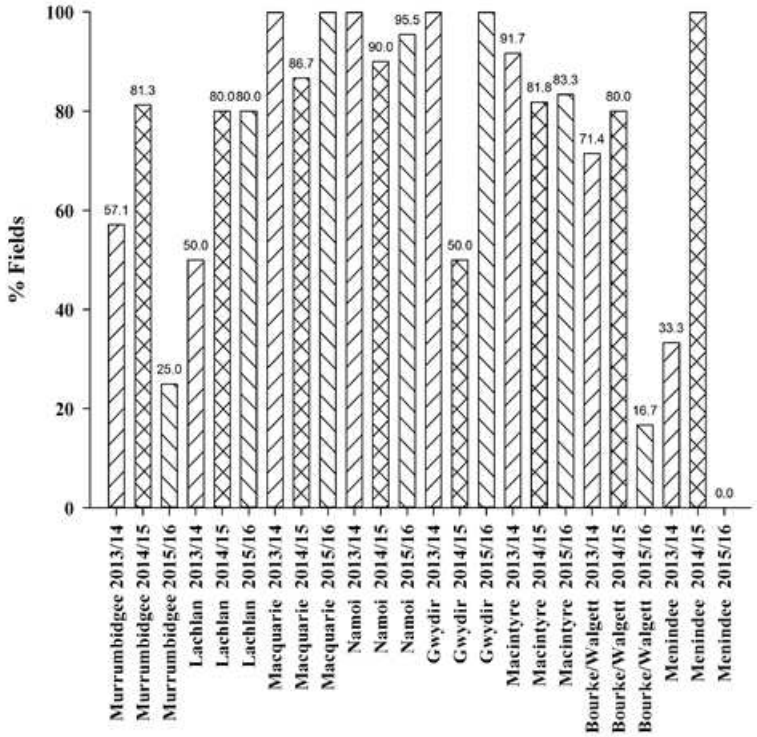

Figure 11. Percentage of fields with black root rot during 2013/2014, 2014/2015 and 2015/2016 cotton seasons.

Fusarium wilt - Incidence

The incidence of Fusarium wilt across the NSW growing regions remains relatively low (Figure 12). No cotton was planted in the Menindee valley during 2015/2016 season, hence 0% recorded. Incidence ranged from 0.03% in Namoi during 2014/2015 to 10.5% in the Gwydir valley during 2014/2015 season. New records of Fusarium wilt on new fields or new farms continue to be raised. Even though some varieties of cotton have high F-ranks and have reduced the losses associated with this disease, it is important that growers and consultants continue to use the Come Clean - Go Clean farm hygiene strategy to minimise the spread of this disease from field to field, farm to farm and region to region.

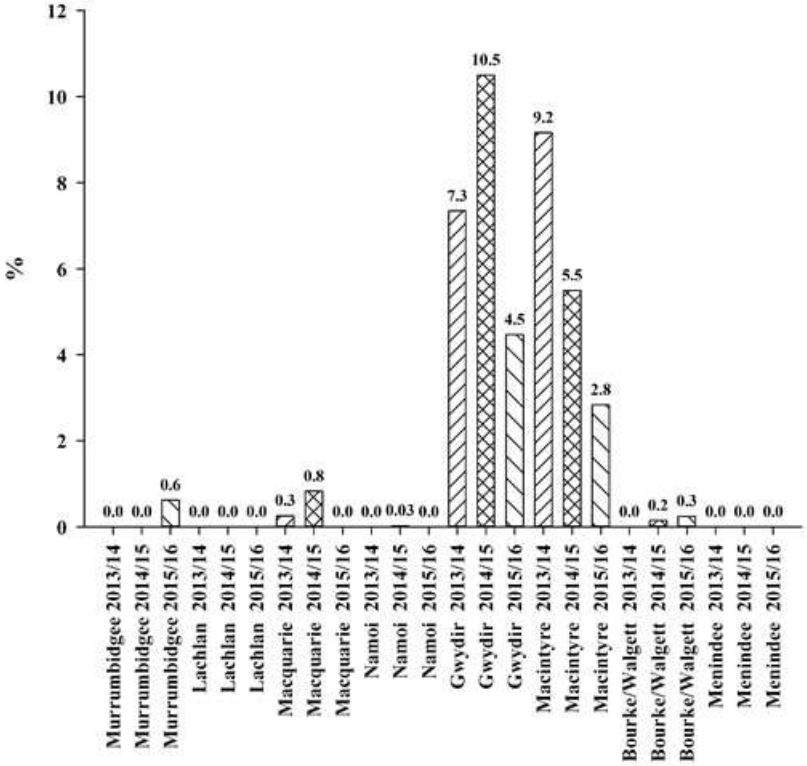

Figure 12. Incidence of Fusarium wilt during 2013/2014, 2014/2015 and 2015/2016 cotton seasons.

Fusarium wilt - Percent of fields infected

Fusarium wilt caused by the pathogen *Fusarium oxysporum* f.sp *vasinfectum* is most severe when October/November rainfall is above averagel and when temperatures are below season averages. The disease is least severe when weather conditions are hot and dry in the spring.

The percentage of fields with Fusarium wilt recorded during the 2013 to 2016 surveys increased in the Murrumbidgee, Gwydir and Bourke/Walgett valleys (Figure 13). Percentage of fields ranged from 5% in the Namoi valley during 2014/2015 to 75% in the Macintyre valley during 2013/2014 season.

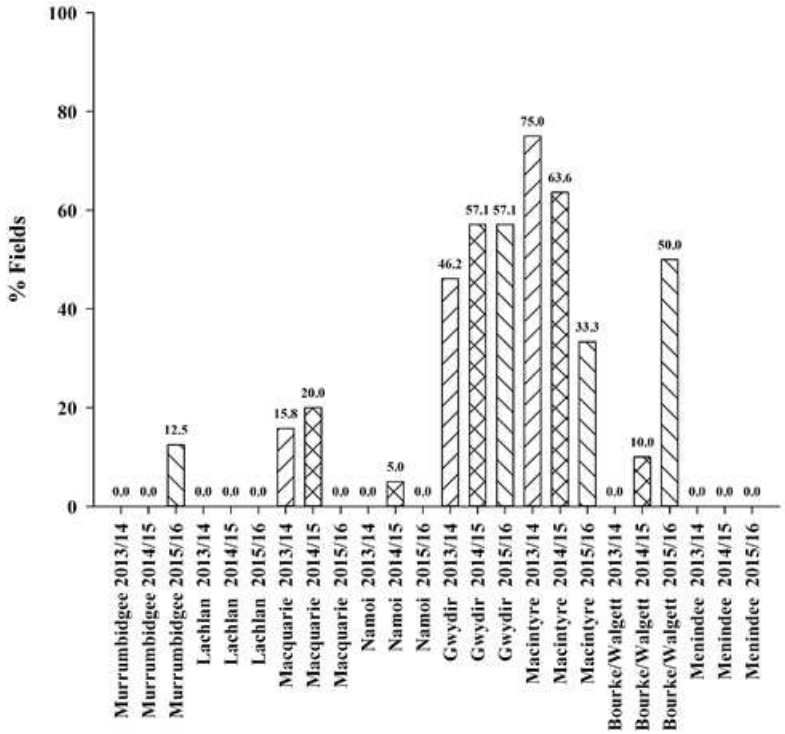

Figure 13. Percentage of fields with Fusarium wilt during 2013/2014, 2014/2015 and 2015/2016 cotton seasons.

Alternaria Leaf Spot

Alternaria leaf spot was present at low levels in almost all crops and was generally of minor significance. The incidence of Alternaria leaf spot has decreased over the years in all regions during 2013 to 2016 seasons. Incidence ranged from 0.001% in the Bourke/Walgett valley during 2015/2016 to 0.65% in the Macquarie valley in 2013/2014 season (Figure 14).

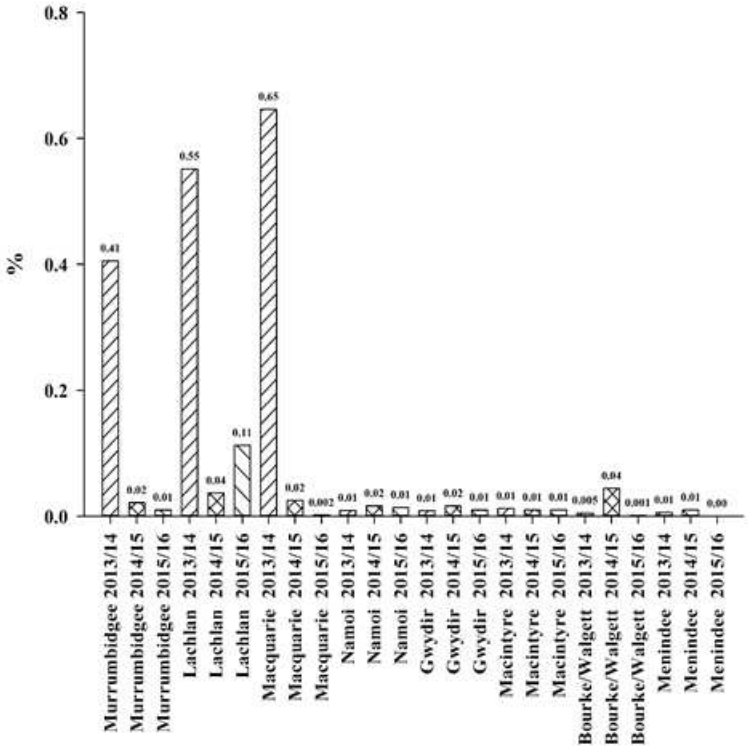

Figure 14. Incidence of Alternaria leaf spot during 2013/2014, 2014/2015 and 2015/2016 cotton seasons.

Hormone Damage

During 2013 to 2015 seasons, hormone damage was generally been low. During the 2015/2016 season there was an increase in the incidence of hormone damage (Figure 15) in crops in NSW. Incidence of hormone damage ranged from 0.02% in the Namoi valley in 2013/2014 to 28.3% in the Gwydir valley during the 2015/2016 season.

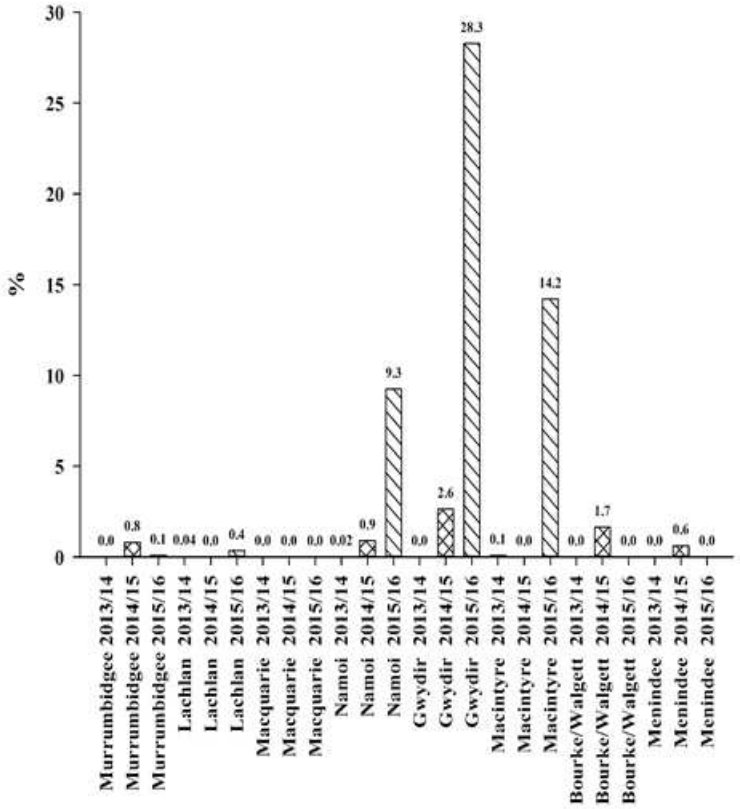

Figure 15. Incidence of hormone damage during 2013/2014, 2014/2015 and 2015/2016 cotton seasons.

Long Term Fusarium Transects

Long term Fusarium transects have been established in fields in the Macintyre, Gwydir and Namoi valleys. The incidence of Fusarium wilt is assessed along these transects in seasons when cotton is planted in these fields. Assessments in the Macintyre valley transects showed an increase in incidence from 2013/2014 season to 2015/2016 season (Figure 16). There was no cotton planted in the Gwydir valley during 2013/2014 and 2015/2016 seasons (Figure 17). There was a marginal increase from 2013/2014 to 2015/2016 season in the incidence of Fusarium wilt recorded in the transect field in the Namoi valley (Figure 18).

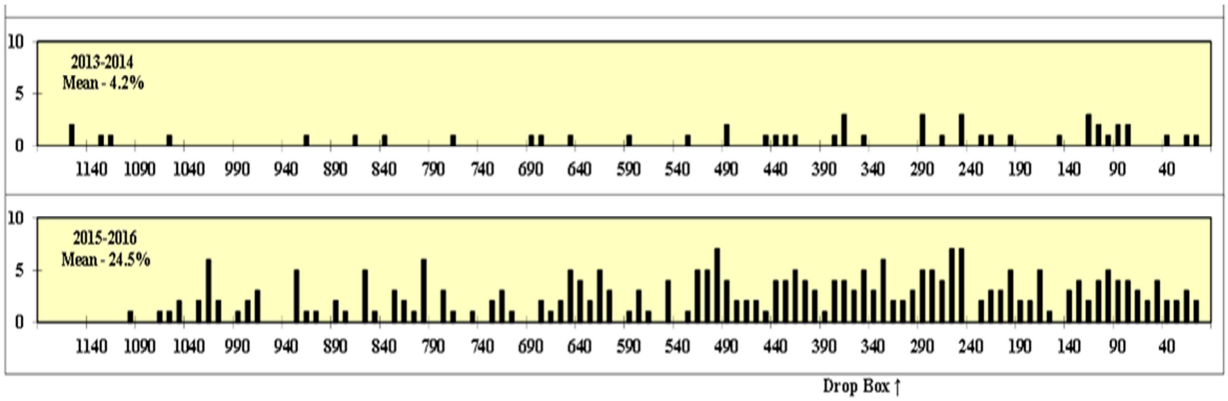

Figure 16. Macintyre Fusarium wilt transect recorded in 2013/2014 and 2015/2016.

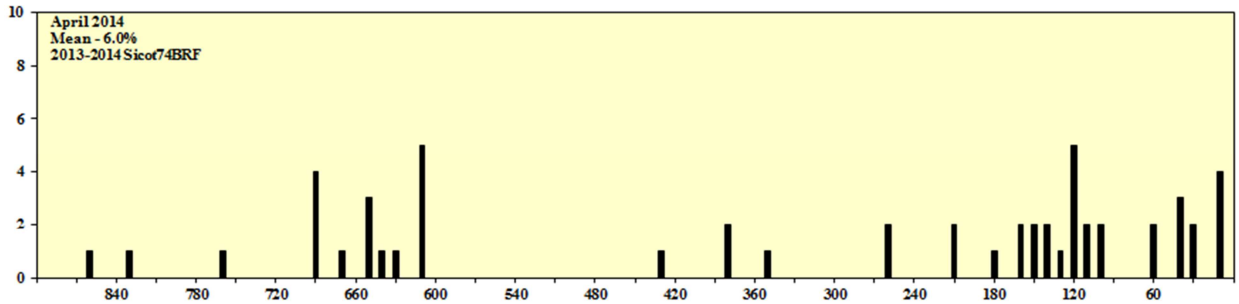

Figure 17. Gwydir Fusarium wilt transect recorded in 2013/2014 season.

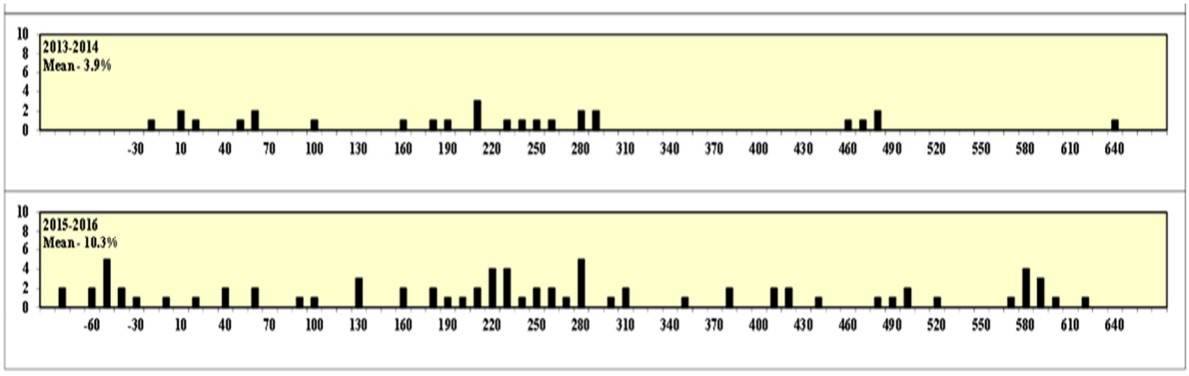

Figure 18. Namoi Fusarium wilt transect recorded in 2013/2014 and 2015/2016.

2.1.2 Early and late season survey results were updated into farm reports and sent to individual farms following each season.

2.1.3 The long term data base has been updated onto one software program (Filemaker).

Milestone 2.2 Investigate long term data for potential interaction between incidence of black root rot and Verticillium wilt

Data was collated into excel sheets and statistically analysed for potential interaction. High incidence was defined as 30% or greater for both diseases. Since 2006/2007, the average incidence of both black root rot and Verticillium wilt has been on the increase. The long term data of both diseases together (Figure 19) and other anecdotal evidence suggests that there may be a link between high incidences of black root rot early in the season with high incidence of Verticillium wilt later in the season. In order to address this question we looked closer at the long term data.

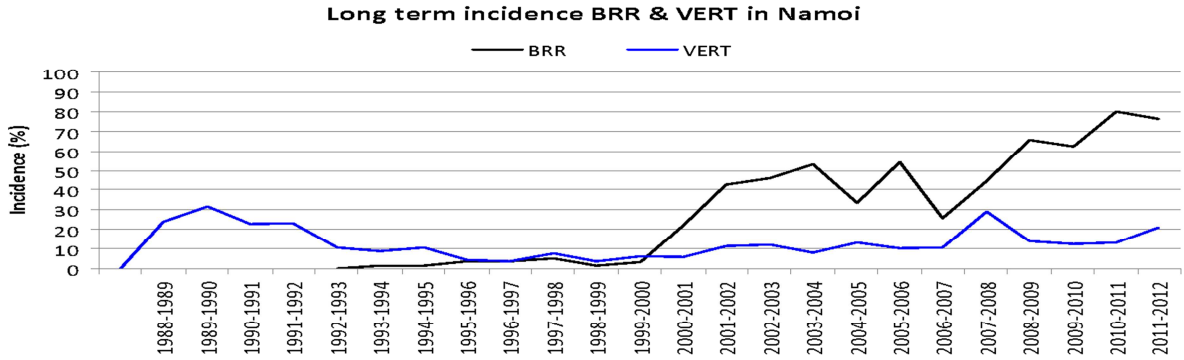

Figure 19. Long term data recorded for incidence of Verticillium wilt and black root rot in the Namoi valley.

Black root rot surveys commenced in 1989/1990 and Fusarium wilt in 1993/1994. Since then the Lachlan, Murrumbidgee and Tandou valleys have recorded no fields with a high incidence of Verticillium wilt, however the other cotton growing valleys did. Black root rot has been recorded in 2021 surveys since 1989/1990 and Verticillium wilt recorded in 2383 surveys since 1984/1985. Black root rot was recorded in 693 fields (34.3%) since 1989/1990. Of these, only 15.6% of the fields had incidence greater than or equal to 30%. Verticillium wilt was recorded in 1299 fields (54.5%) since 1984/1985. Of these, only 5% of the fields had incidence greater than or equal to 30% (Table 1).

Table 1. Fields with black root rot and Verticillium wilt

	Black root rot (since 1989/90)	Verticillium wilt (since 1984/85)
Number of surveys that disease was recorded in	2021	2383
Number of fields surveyed	693	1299
% fields surveyed	34.3%	54.5%
Number fields with ≥ 30%	315	120
% fields with incidence ≥ 30%	15.6%	5%

Table 2. Number of fields in each valley with high incidence of black root rot and Verticillium wilt

Valley	≥ 30% black root rot	≥ 30% Verticillium wilt
Bourke/Walgett	18	12
Macintyre	38	10
Gwydir	27	9
Namoi	160	80
Macquarie	56	9
Lachlan	10	0
Murrumbidgee	4	0
Tandou	2	0
Total	315	120

Overall, there was 19 out of 160 fields (11.8%) in the Namoi valley that had ≥ 30% incidence of both black root rot and Verticillium wilt; 1 field out of 38 (2.6%) in the Macintyre and 2 out of 27 fields (7%) in the Gwydir valley were recorded with high incidences of both diseases (Table 2). These results suggest that just because some fields have a high incidence of black root rot, it does not necessarily mean those same fields have a high incidence of Verticillium wilt. Of course there are examples of fields with high incidence of both diseases (11.8% in the Namoi valley, 2.6% in the Macintyre valley and 7% in the Gwydir valley) however there are many more fields with high levels of one disease and low levels of the other.

During 2013/2014, NSW DPI worked in collaboration with Cotton Grower Services on a large field experiment on a commercial cotton farm in the Namoi valley to examine the effects of three treatments on the incidence of both black root rot and Verticillium wilt. Neither treatment had significantly lower incidence of disease compared to the control. The data was examined for potential interaction between the two diseases, however none was found. The treatment FlowPhos 13Z had the lowest incidence of black root rot (68.1%) however it did not have the lowest incidence of Verticillium wilt (58.1%). There were significant differences between treatments for incidence of black root rot but no significant differences in the incidence of Verticillium wilt.

May 2016, the NSW DPI statistician Dr Steven Harden analysed the historical survey data to look for potential interaction between high incidence of black root rot and high incidence of Verticillium wilt for each NSW cotton valley with high incidence of both diseases recorded. There was no strong correlation in any year from 2001 to 2016 (Figure 20) between high incidence of black root rot and high incidence of Verticillium wilt. This analysis did not include factors such as variety, seed rates, number of cotton crops planted etc.

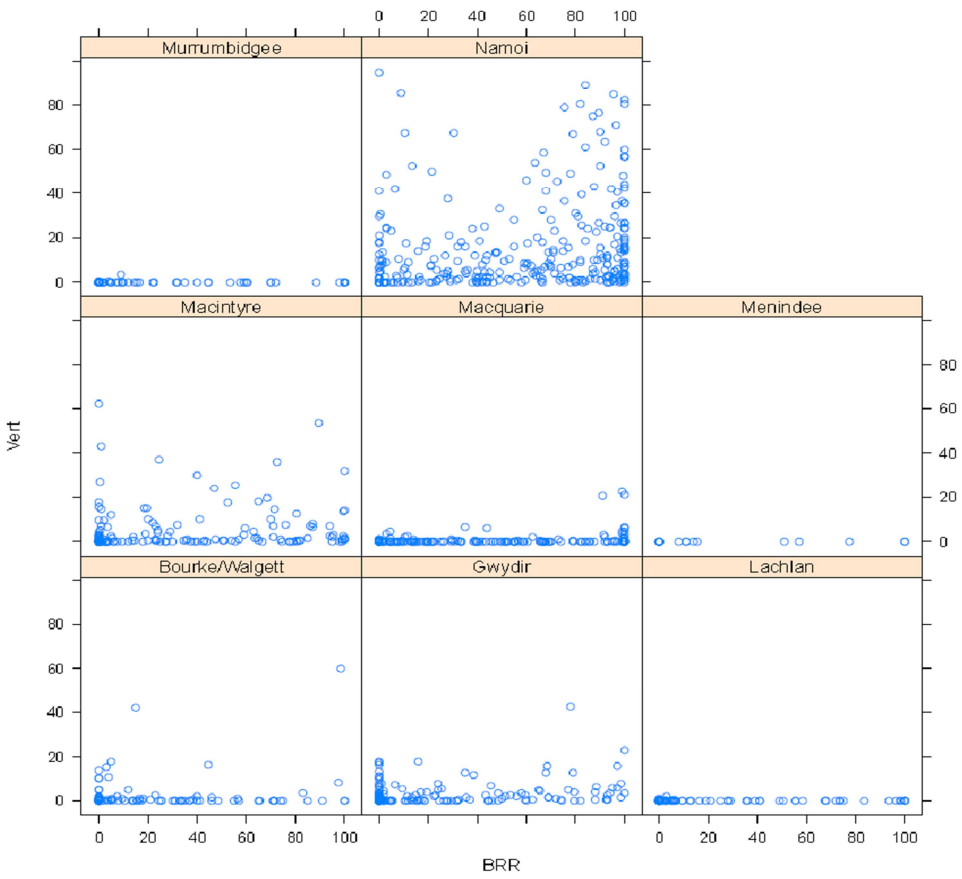

Figure 20. No correlation between high incidence of black root rot and Verticillium wilt found in historical survey data.

Given there was no correlation found between high incidence of black root rot and Verticillium wilt, a controlled pot experiment was used to examine potential effects of dual infection on the severity of black root rot.

A completely randomized and replicated pot experiment was set up in the growth room to assess a potential interaction between the two diseases black root rot and Verticillium wilt caused by *Thielaviopsis basicola* and *Verticillium dahliae* respectively and any effect on disease severity. Sicot 71 BRF seeds (supplied by Cotton Seed Distributors) were germinated in root training books filled with each treatment. Soil known to be naturally infected with *V. dahliae* was collected from a farm in the Namoi valley. A subsample of the soil was collected and kept for future isolations to quantify inoculum levels. The experiment had 6 replications and 4 treatments.

Treatments: (VERT): Soil collected from a farm in the Namoi valley known to be infected with high levels of *V. dahliae*. (BRR): Soil from the same field was pasteurized twice before being inoculated with around 100 ccf/g soil with *T. basicola*. (BRR X VERT): Soil from Treatment 1 and 2 was combined in equal quantities in a cement mixer. (CONTROL): Soil from the same field was pasteurized twice. Inoculum was replaced with equal amounts of distilled water.

Seedlings were grown in controlled conditions within the growth room with minimum and maximum temperatures set at 14^{o}C and 23^{o}C suitable for development of black root rot. After four weeks seedlings from each treatment were gently removed from root trainer books and roots washed to remove soil and debris before being assessed for severity of

black root rot using the scale of 0-10 where 0 indicates healthy and no blackening of the tap root and 10 indicates 100% of the tap root blackened. Twelve seedlings from each treatment were randomly selected and transplanted into 3L pots containing each of the 4 soil treatments. Pots were watered individually from the base to avoid potential movement of one treatment to the next. Plants were assessed weekly for symptoms of disease as well as plant height.

Black root rot was significantly higher ($p<0.001$) with dual infection of both pathogens compared to the control (no pathogens) and Verticillium alone treatments. Average severity of black root rot based on the scale of 0-10 was: 6.2 in the dual *T. basicola* and *V. dahliae* infected treatment, 4.6 in *T. basicola* alone, 2.3 in *V. dahliae* alone and zero in the control treatment (Figure 21).

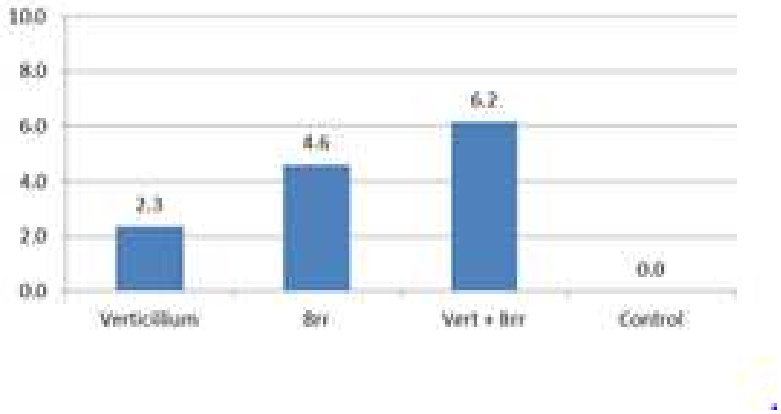

Figure 21**.** Significant difference in severity of black root rot in dual infected seedlings compared to control and Verticillium infected treatments. There was no significant difference between dual infected seedlings and black root rot infected treatments.

Black root rot was higher in the dual infected seedlings compared with the seedlings infected with *T. basicola* alone. This may be due to fact that the *V. dahliae* pathogen that causes Verticillium wilt is capable of penetrating young, uninjured or injured cotton seedlings anywhere from the root cap (protective sheath covering the root tip) to the hypocotyl, the stem portion from the top of the root to the cotyledons (seed leaves). Roots that are extensively invaded through the root cap can cease growing; however lateral roots can develop back off the root tip. Penetration is directly through the epidermal cell layer in the area of most rapid root growth and root hair zone about 1cm from the root tip. Research has shown under low inoculum levels little penetration occurs beyond the first few cortical layers, however under high inoculum levels, some of the invading hyphae grow rapidly through the epidermal cells and penetrate deeper into the cortical layers compared to low levels of inoculum. Under high inoculum levels and mass invasions, defence mechanisms of plants are overcome. Hyphal development is towards the vascular tissues of the stele (central portion of the root).

Relevance of these findings to the industry - where fields have a history of both black root rot and Verticillium wilt it is important for the cotton to get out of the ground as quickly as possible. Black root rot is a disease expressed early in the season, usually in the first four weeks following planting. Planting seed into cool soil increases the risk of black root rot disease. Black root rot does not kill the seedlings but does slow the growth and extends crops maturity into the cooler conditions later in the season. This also increases the risk of Verticillium wilt.

Milestone 2.3 Increase/maintain long term storage of culture collection.

Over the years fungal isolates have been stored in the NSW DPI historical culture collection. These were clean cultures, however they were not single spore cultures. There are 522 isolates in the culture collection and not all have been single spored. The number of each isolate causing disease in the collection are as follows: Alternaria - 123, *Rhizoctonia* sp -2, Sclerotinia - 11, *T. basicola* - 88, *V. dahliae* - 202, F. *oxysporum* fsp. *vasinfectum* - 92, Sudden wilt - 8, *Pythium* sp - 16, Dreshslera - 4, *Bacillus subtilis* - 1, Curvularia - 1, Molds - 1 and 4 unknown.

During 2015/2016 the pathology team commenced single sporing of *T. basicola* and *V. dahliae* isolates (Figure 22). Fungal pathogens were re-isolated, subcultured and single spored prior to being stored in the culture collection. The total number of single spore isolates for *T. basicola* and *V. dahliae* was 41 and 202 respectively now in the NSW DPI culture collection at the completion of this project. From these, 41 *T. basicola* isolates and 119 *V. dahliae* isolates collected from symptomatic plants from a wide geographical area were submitted to NSW DPI Herbarium as a reference library and assigned DAR numbers.

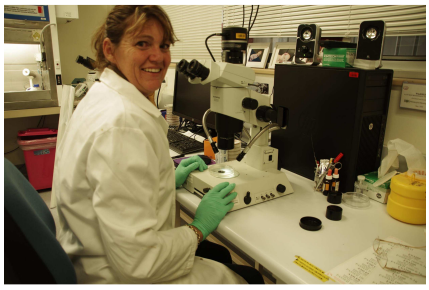

Figure **22.** Microscopic examination of culture plates prior to subculturing

Molecular analysis of *Verticillium dahliae* isolates

Historical culture collection: 62 isolates were analysed with 25 defoliating and 37 non-defoliating. Of the 25 defoliating isolates, 21 were predicted to be VCG1A by IGS analysis, the other four were inconclusive. Of the 37 non-defoliating isolates, 25 were predicted to be VCG2A by IGS analysis, nine were predicted to be VCG4B and three were inconclusive.

2014/2015 samples: 42 samples were analysed with 16 defoliating and 26 non-defoliating. A further 11 isolates were both defoliating and non-defoliating. Of these, nine isolates were predicted to be VCG1A and VCG2A and two were VCG1A and VCG4B by IGS analysis.

2015/2016 samples: 54 samples were pathotyped with 22 defoliating VCG1A and 31 non-defoliating and one isolate being both defoliating and non-defoliating.

Fusarium isolates

Since 2013 to June 2016 there were 64 isolates suspected as Fusarium that were sent to Dr Linda Smith for confirmation and VCG analysis.

2013/2014 season - two isolates sent and two confirmed.

2014/2015 season - 17 isolates sent and 8 confirmed. No results received for other nine isolates.

2015/2016 season - 45 isolates sent and 9 confirmed. No results received for other 36 isolates.

Milestone 2.4 Improve knowledge of diversity within black root rot isolates

2.4.1 Laboratory culture collection experiments

Morphology and growth rates of *Thielaviopsis basicola* isolates from different valleys.

The morphology and growth rates of black root rot cultures from different geographical regions were assessed using laboratory assays. *T. basicola* cultures in the NSW DPI culture collection were subcultured and single spored before morphology and growth rates were assessed. Single spore cultures were incubated at 24^{o}C. For ten days the colour of each isolate was assessed and radial measurements recorded to establish growth rate per day. There was no significant difference in growth rate between isolates from various geographic valleys. There was a significant difference recorded in growth rates for colony colour (Figure 23) but no significant difference of growth rate between isolates from different valleys (Figure 24).

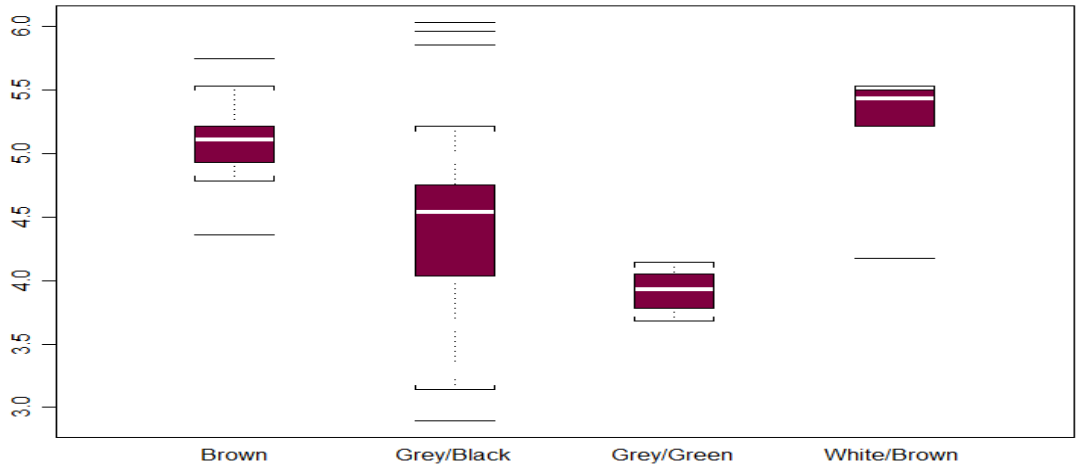

Figure 23. Significant difference in growth rates between isolate colour

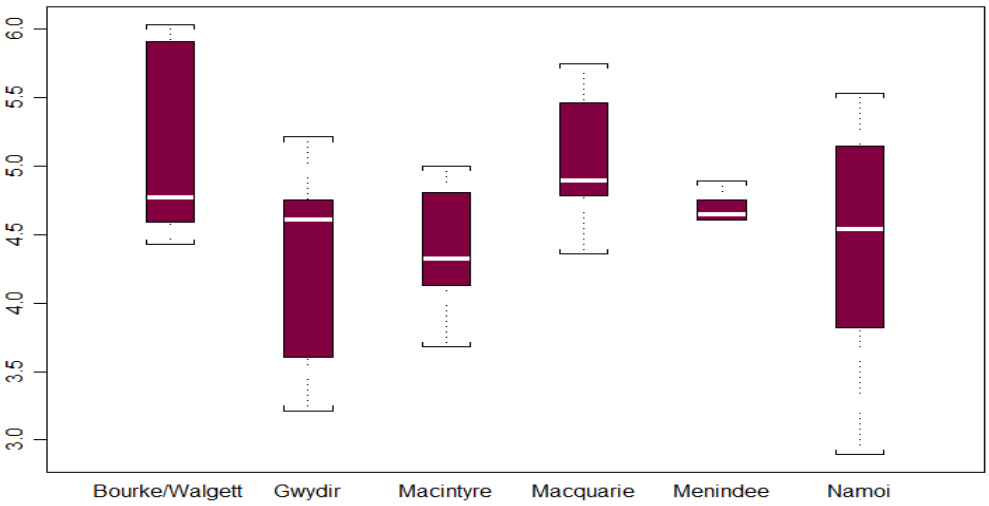

Figure 24. No significant difference in growth rates between isolates from different valleys.

Pathogenicity of *Thielaviopsis basicola* isolates from different valleys.
NSW DPI hosted summer scholarship recipient Johanna Nielsen. During the scholarship Johanna's project DAN1509 assessed the pathogenicity of selected *T. basicola* isolates. Johanna submitted her project report to CRDC (Figure 25).

Australian Government
Cotton Research and Development Corporation

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Tel: 02 6792 4088
Fax: 02 6792 4400

27 July 2015

Johanna Nielsen
c/o Karen Kirkby
Cotton Pathologist
ACRI Locked Bag 1000
Narrabri NSW 2390

Dear Karen,

CRDC ID: DAN1509
Project Title: Pathogenicity and morphology assays of selected *Thielaviopsis basicola isolates- Johanna Nielsen*

Thank you for the submission of your Final Report for the above project. We wish to advise that your report has been reviewed and accepted by the Program Manager.

Please find the following feedback –

"Johanna
Thank you for your well written report detailing work conducted as part of your summer scholarship. It is obvious from your report that you really enjoyed working with the pathology team at ACRI, and the work undertaken has given you a greater appreciation of the impact that the black root rot pathogen has on cotton as well as their epidemiology. We look forward to seeing more of you in coming years."

Yours Sincerely,

Dr Ian Taylor
General Manager
R&D Investments

Figure 25**.** Acceptance letter for Johanna Nielsen project report

Genetic diversity of *Thielaviopsis basicola* isolates examined by Sarah Cooper (UNE PhD student).
Sarah's results were reported to CRDC in the 2014 May Progress Report ID CRDC1305. In short, Sarah found no differences in the ITS sequences from 24 different *T. basicola* isolates collected from different geographical locations. On the 11th July 2016, Sarah submitted her thesis titled "Microbial Tools for Advancing the Management of Soil and Seedling Health in Cotton Production Systems".

Efficacy of various concentration rates of Farmcleanse, Ethanol, Cavicide and Bleach on the germination of *Thielaviopsis basicola.*
Aim: to assess the effects of various concentration rates of bleach, ethanol, Farmcleanse and cavicide on the germination of *Thielaviopsis basicola* on TbCEN media plates. Results of this experiment showed ethanol did not inhibit the growth of *T. basicola*. Consequently, when working with samples suspected to be infected with *T. basicola*, laboratory policy was amended to use 20% Farmcleanse to wipe down benches, equipment and utensils.

2.4.2 Growth room experiments

Pathogen spatial variation (transmission efficiency) of *Thielaviopsis basicola*.

Soil was collected from a nearby commercial cotton farm and twice pasteurised before potted into large plastic containers (35L) with holes drilled in the base for drainage. There were three replicates. A total of 35 seeds were planted per plastic container with one seed in the middle of each container inoculated with 1ml *T. basicola* inoculum at a concentration of 100 ccf/g (colony chain fragments per gram of soil) by pipetting underneath the seed in the center. 1 mL of distilled water was pipetted under every other seed in the container. Seeds were planted 3cm deep and 5cm apart in seven rows across and five rows down. Seedlings were assessed after 28 days for infection and severity. This experiment failed to show any seedlings with blackened roots, even under the inoculated seeds. The experiment was replicated with a higher inoculum level (300 ccf/g). The only seedlings with symptoms of black root rot were those that germinated from the inoculated seeds at planting. No other seedlings showed symptoms of black root rot despite there only being 5cm between seeds.

Black root rot inoculum across permanent bed and effect on disease severity and growth

A total of 31 pots containing soil cores taken across the permanent bed line from a commercial cotton farm were maintained in the growth room from 23/01/2014. Ten Sicot 74 BRF seeds were planted per pot. Seedlings were assessed for average disease severity and plant height, 28 days after planting. There was no significant difference in the average disease severity of black root rot across the plant line. There was also no significant difference in plant height when cotton was grown in soil collected from across the permanent bed line.

Milestone 2.5 Improve knowledge of diversity within *Verticillium dahliae* isolates
***2.5.1 Verticillium dahliae* isolated from soil collected from within the in-furrow and across permanent beds.**

Pilot study results:
For the in-furrow soil cores, position across the furrow was not significant (P=0.238), LSD = 22.25 with no difference between left of centre (LOC), centre (C) and right of centre (ROC). The number of propagules per gram (ppg) of inoculum in soil was significantly lower within soil samples from the in-furrow than that of the plant bed line samples (Figure 26). For the plant bed soil cores, position was significant (P=0.019), LSD = 111.3. LOC was greater than C and ROC. Differences in inoculum between 0-10cm and 11-20cm were also assessed no significant difference recorded (P=0.837), LSD 73.9 between depth at each position.

	LOC	C	ROC
Bed	3241[a]	1704[b]	1831[b]
In-furrow	379[a]	215[a]	305[a]

Figure 26. Significant difference in inoculum levels across permanent beds

Following on from the pilot study a large scale field trial was set up with the assistance of a grower in the Namoi valley. A total of 72 soil cores were collected from a commercial cotton field (Farm W). Soil samples were placed in paper bags and left to air dry for two to three weeks. Soil cores were taken from three positions across permanent beds: left of centre (LOC), centre of plant line (C), right of centre (ROC) at 0-10cm and 11-20cm depths. Soil cores were also taken from three positions within the in-furrows (LOC, C, ROC).

The results of the larger field trial did not match the results of the pilot study. Soil isolations from the field trial showed no significant difference in inoculum levels between LOC, C and ROC across permanent beds. There were significantly (59%) more propagules per gram (ppg) recorded in the permanent beds compared to in-furrows. Soil depth was a significant factor with 71% more ppg in the top 10cm of soil profile compared to 11-20cm. There was no significant difference in ppg at either depth taken from in-furrow (Figure 27).

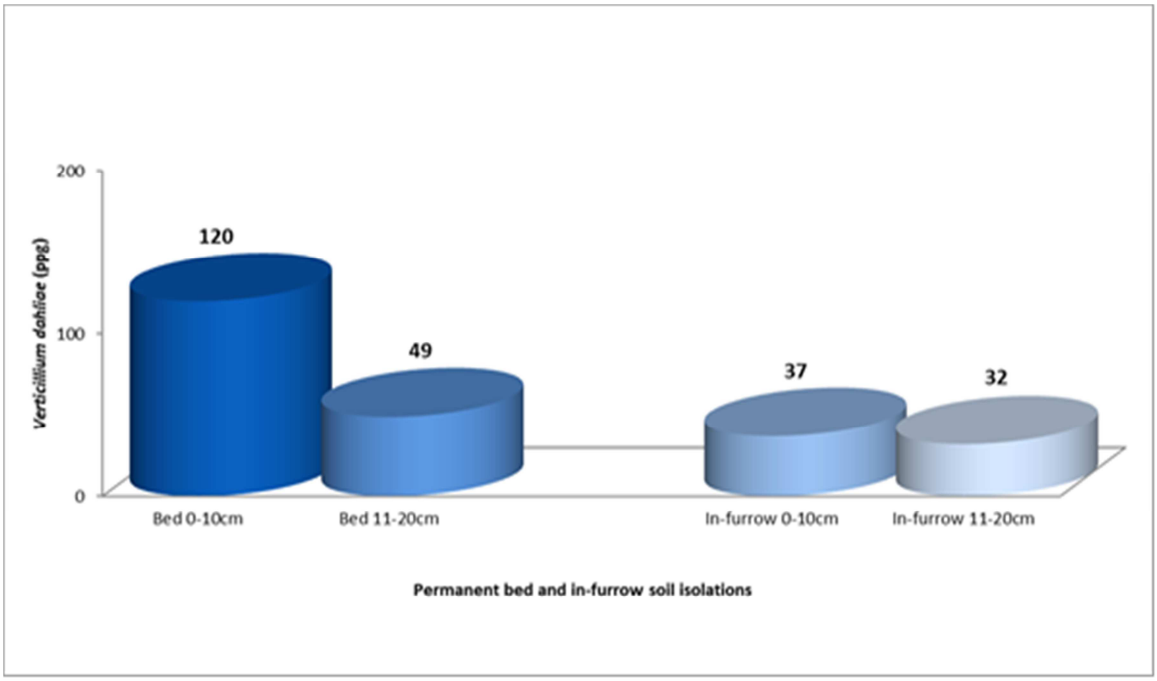


Figure 27. Significantly more ppg of *Verticillium dahliae* in soil collected from across permanent beds than in-furrow

These findings have particular relevance to the cotton industry. Precision farming places the germinating seedlings directly above the inoculum in the soil. Good bed preparation is vital. It is important to take care to avoid any mechanical damage to the roots as damage will provide an entry point for pathogens. Management of nutrition is also important given the majority of the inoculum is in the top 10cm of the soil profile. Nutrition should be managed to encourage deep root development. Given inoculum was recorded in-furrow it is important to reduce irrigation runoff to minimise moving the pathogen around the farm in tail water.

2.5.2 Verticillium farm case studies

Farm F – Effect of irrigation source on ppg

Verticillium wilt was particularly severe at Farm F. This farm has a long history of using bore water to irrigate cotton crops. A randomised, replicated pot experiment was prepared to examine the effect of two different watering sources on *Verticillium dahliae* propagules per gram (ppg). There was no significant difference in ppg between the two water source treatments (Figure 28).

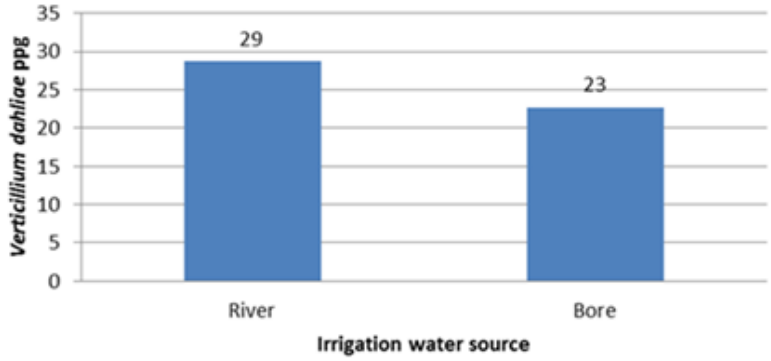


Figure 28. There was no significant difference in ppg when watering from river or bore water.

Farm F – Effect of depth on *Verticillium dahliae* ppg

Soil cores were collected from the permanent beds from a commercial cotton farm near Breeza (Farm F) and Verticillium propagules per gram were assessed using the dilution plate technique. There were 77% more ppg in the top 0-10cm compared to 11-20 cm in the permanent beds (Figure 29) which is very similar to the 71% reported in the top 10cm for Farm W field study.

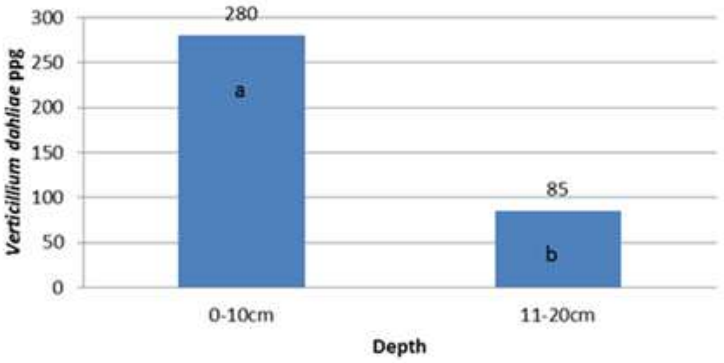


Figure 29. **S**ignificant difference in ppg at 0-10cm compared to 11-20cm in soil collected from Farm F.

FARM F – Irrigation efficiency testing carried out in collaboration with Janelle Montgomery and Peter Smith
Outcomes: as reported by Janelle Montgomery

The field test data was obtained for a preliminary examination of variation in flow rate across the machine.

- The variation in flow was 20%. Although this is well above the conventional limit of variation of 5 per cent, given the age of the machine and sprinkler pack (32 years old, original sprinklers) and various leaks at couplings on each tower, it is to be expected. This amount of variation would normally be expected to have a significant impact on the evenness of application (distribution uniformity). In self-mulching black clay soil such as in the field tested, the high level of transverse movement of water in the soil (subbing) would tend to even out the variation across the field, reducing any areas of overwatering or under-watering.
- A distribution uniformity test using catch cans would be necessary to accurately determine how even the application of water was across the field, however this was not possible on the day as the cotton plants were too large. Plastic buckets were held under the nozzles for designated time (Figure 30).
- The machine has no pressure regulators installed above the emitters. It has become common practice to install pressure regulators on all emitters. They are fitted on the droppers above the sprinklers and limit the maximum pressure at the nozzles to aid in control of 1). Flow rate variation across the system length 2). Desired droplet size 3). Distribution uniformity 4). stream radii.
- The relatively poor variation compared to industry standards of this system is probably due to a lack of pressure regulators, worn and damaged emitters, and leaks. In light of these, the flow variation of ±20% can be considered reasonable. In itself, and because the variation was observed along the entire system, this would be unlikely on its own to cause excessively moist conditions conducive to disease in the centre of the field.
- The pattern in variation does not match the pattern of disease occurrence.
- One observation was that water was running down the wheel tracks way ahead of the towers, along with significant mud attached to the tyres of each tower, this could certainly add to the movement of soil down the field.
- Field drainage under a lateral move irrigator is very important. A field survey will identify low lying areas and reveal if further laser leveling is necessary.

Janelle Montgomery & Peter Smith

NSW DPI

Figure 30. Irrigation efficiency testing.

Compost - the use of compost gin trash on fields

Verticillium inoculum was isolated from gin compost in 1998 by Dr Stephen Allen and in 2015 by Dr Karen Kirkby and the results compared. Stephen Allen reported his findings in:

Allen, S, J. (1998)Diseases of Cotton (V). Cotton Research & Development Corporation Final Report. DAN 95C

"One of the major concerns of using gin trash is the potential for carryover of pathogens such as *Verticillium dahliae*. Previous work has shown that this pathogen is very common in gin trash. According to the results obtained from studies in the USA the temperatures achieved during the composting process are sufficient to kill the pathogen".

Compost samples from Warren and Goondiwindi were submitted for testing for the presence of *V. dahliae* using Ethanol Streptomycin Agar as a selective medium. "Test results indicated that the majority of the compost was free of the pathogen. However the pathogen could still be recovered from the small amounts of incompletely composted material from the edge of the piles".

"Samples from Goondiwindi were taken from an undisturbed pile of gin trash. Samples from well within the pile were relatively free of the pathogen but samples from the surface of the pile contained up to 30 propagules of *V. dahliae* per gram of material".

In January 2015, NSW DPI took samples from Narrabri for testing for the presence of *V. dahliae* using Ethanol Streptomycin Agar (ESA) as a selective medium. Samples were taken from undisturbed trash piles, within composted trash piles and from edges of composted trash piles. Results indicated that samples from within the piles were relatively free of the pathogen, however samples from the undisturbed piles and from the edge of composted piles averaged 20 and four propagules per gram of material respectively (Figure 31).

Allen, S. J (1989) Diseases of Cotton. Cotton Research Council – Final Report. DAN 26L p33-34

Stephen Allen reported "The average number of propagules/gram soil ranged from 246 in autumn to 70 in spring. Up to 2000 microsclerotia per gram were recorded in some soil samples".

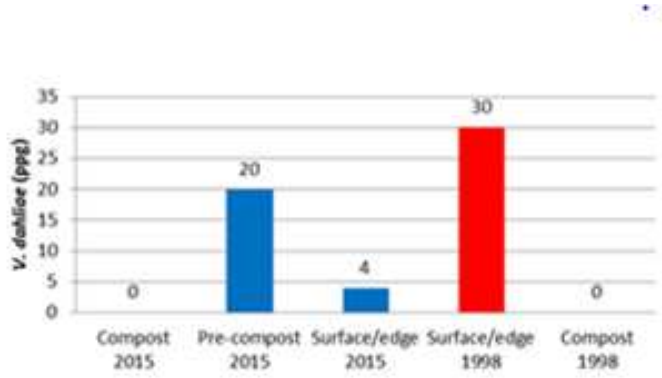


Figure 31. *Verticillium dahliae* ppg were isolated from compost collected from surface edge.

The results of both studies in 1998 and 2015 confirm the need to compost the gin trash thoroughly in order to eliminate the carryover of the pathogen causing Verticillium wilt.

Farm M – Effect of rotation crops on *Verticillium dahliae* inoculum levels in field

A long term field study was conducted on a commercial cotton farm in the Namoi valley that had three severe patches of Verticillium wilt in the field. NSW DPI pathology team worked closely with the grower to set up a replicated rotation of non-host crop trial where each year soil cores were taken from the same GPS locations (six in total) and used to quantify the inoculum levels using the dilution plate technique. The following is the report provided to the grower.

Patches of severe Verticillium wilt were observed and significant yield losses recorded on Farm M Field 4 during 2011/2012 cotton season. A yield map following the harvest in 2012 (Figure 32) and historical data was supplied from the grower. Historical data including ground water quality testing results from 2002, soil analysis from 2004 and a deep nitrogen report from 2011 were provided by the grower. Soil analysis results are summarised in Table 3A and 3B and shown as Figures 34 to 38. Following completion of all soil isolations (Table 4), a request was made to the grower for a summary of field planting history, nutrients and land preparations which were provided (Table 5).

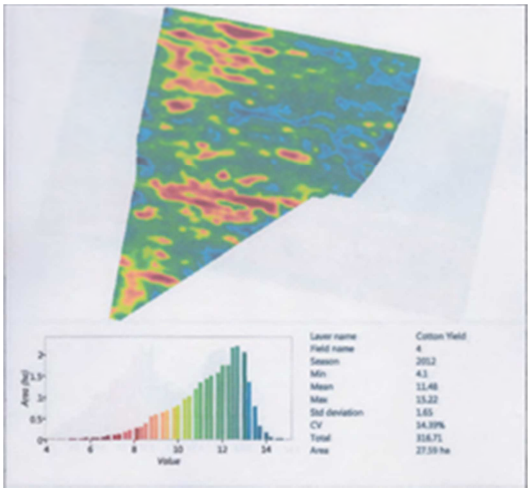


Figure 32. Yield map for Field 4 2011/2012 season

April 2012: A late season survey of Verticillium wilt was carried out in Field 4 prior to the cotton harvest to establish the incidence of disease. Field 4 had three severe patches of wilt affected plants that were evident. Field observations showed infected plants with and without external symptoms of wilting, leaf mottling/necrosis and dying/dead plants. Stem cuts revealed vascular discolouration in plants with and without external symptoms. A total of six GPS points were recorded with three from within the severe patches and three outside the patches.

May 2012: Following the cotton harvest in April, soil was collected from each GPS and the soil cores from within patches pooled. The soil cores from outside patches were also pooled and sent to the Nematode Diagnostic Laboratory of Agri-Science Queensland, DAFF. No plant parasitic nematodes were detected in either of the samples (Figure 33).

Queensland Government

Department of Agriculture, Fisheries and Forestry

22 May 2012

Dr Karen Kirkby
Australian Cotton Research Institute
21888 Kamilaroi Highway
NARRABRI NSW 2390

Dear Karen,

Subject: Nematode results

Two soil samples were received at the Nematology Diagnostic Laboratory of Agri-Science Queensland, DAFF, at Dutton Park on 9/05/2012. The soil was placed in Whitehead trays for 3 days to extract plant-parasitic nematodes.

The samples were marked as follows:
Mirrabooka – Verticillum wilt QLD Laboratory number NO2371 and
Mirrabooka – Non-Verticillum wilt QLD Laboratory number NO2372

No plant-parasitic nematodes were recovered from either sample.

If you require any further information regarding this matter, please do not hesitate to contact me on 07 3225 4342 or email Jennifer.Cobon@daff.qld.gov.au

Yours sincerely

Jennifer Cobon
Senior Experimentalist

Figure 33. Soil test results for presence or absence of Nematodes. No plant parasitic nematodes were recovered in soil from the severe patches or outside the patches.

July 2012: Soil cores were taken to a depth of 120cm on the 17th July 2012 and sent to Nutrient Advantage laboratory for A12 analysis. The advice and recommendations from NSW DPI soil specialist Dr Nilantha Hulugalle was sought. Factors that were apparent from the soil analysis results (Table 3A/B and individually summarized in Figures 30-34) included: the soil being sodic (Sodium Ammacet Meq/100g), as it should be half of what it is. The soil was very alkaline with poor structural stability making it prone to water-logging. The Chloride levels are moderate to quite high. Electrical conductivity should be less than 0.5; however six out of the eight samples (30-120cm) are more than 0.5. Nitrates are reasonably high, with lots of nitrates in the soil. Nitrate levels should be less than three following a crop.

Recommendations included: fixing the drainage in the field, improve the structure of soil using gypsum 9-10t/ha however Nilantha cautioned this may not be economical. Discontinue using bore water if possible as continued use of this water may negate other changes made and will only bring temporary benefits. Nilantha also recommended growing cereal crops for three years, such as wheat or barley followed by sorghum followed by a cereal crop before going back into cotton. Growing cereal crops will help build permanent porous drainage in the field as well as minimising the build-up of Verticillium inoculum in the soil.

Table 3A. Soil analysis for Field 4 in soil collected within and outside the patches of severe wilt.

Sample Name	Sample Depth From	Sample Depth To	pH (1:5 Water)	pH (1:5 CaCl2)	Elect. Conductivity	Chloride	Nitrate Nitrogen (NO3)	Phosphorus (Colwell)	Phosphorus Buffer Index (PBI-Col)	Available Potassium	Calcium (Amm-acet.)	Potassium (Amm-acet.)
					dS/m	mg/kg	mg/kg	mg/kg		mg/kg	Meq/100g	Meq/100g
High Vert	0	30	8.7	7.9	0.38	25	8.2	12	140	550	27	1.4
High Vert	30	60	9	8.2	0.51	130	7.8	19	130	400	23	1
High Vert	60	90	9.1	8.3	0.67	330	6.8	36	120	450	21	1.2
High Vert	90	120	9.1	8.4	0.85	570	5.6	46	120	510	19	1.3
Low No	0	30	8.9	8.1	0.4	29	8.7	8	140	480	28	1.2
Low No	30	60	9.1	8.3	0.56	110	18	10	140	400	24	1
Low No	60	90	9.2	8.4	0.63	200	13	27	130	430	20	1.1
Low No	90	120	9.1	8.4	0.67	300	8.1	32	120	530	23	1.4

Table 3B. Soil analysis for Field 4 in soil collected within and outside the patches of severe wilt.

Sample Name	Sample Depth From	Sample Depth To	Magnesium (Amm-acet.)	Sodium (Amm-acet.)	Cation Exch. Cap.	Calcium/Magnesium Ratio	Sodium (Amm-acet.)	Sulfate Sulfur (KCl40)	Calcium (Amm-acet.)	Magnesium (Amm-acet.)	Potassium (Amm-acet.)	Potassium to Magnesium Ratio
			Meq/100g	Meq/100g	Meq/100g		%	mg/kg	%	%	%	
High Vert	0	30	19	4.8	52.2	1.4	9.2	14	52	36	2.7	0.1
High Vert	30	60	21	8.3	53.3	1.1	16	38	43	39	1.9	0
High Vert	60	90	21	10	53.2	1	19	40	39	39	2.3	0.1
High Vert	90	120	22	12	54.3	0.9	22	41	35	41	2.4	0.1
Low No	0	30	20	5.2	54.4	1.4	9.6	14	51	37	2.2	0.1
Low No	30	60	23	9.1	57.1	1	16	28	42	40	1.8	0
Low No	60	90	23	10	54.1	0.9	18	36	37	43	2	0
Low No	90	120	22	10	56.4	1	18	30	41	39	2.5	0.1

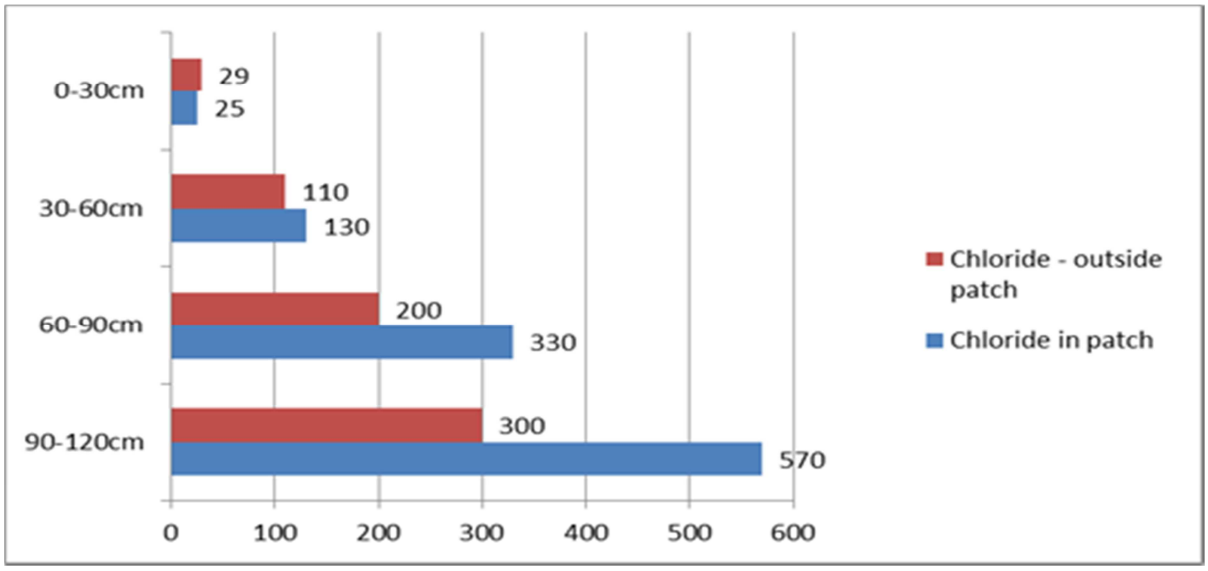


Figure 34. Chloride levels from within and outside patches taken from 120cm soil cores

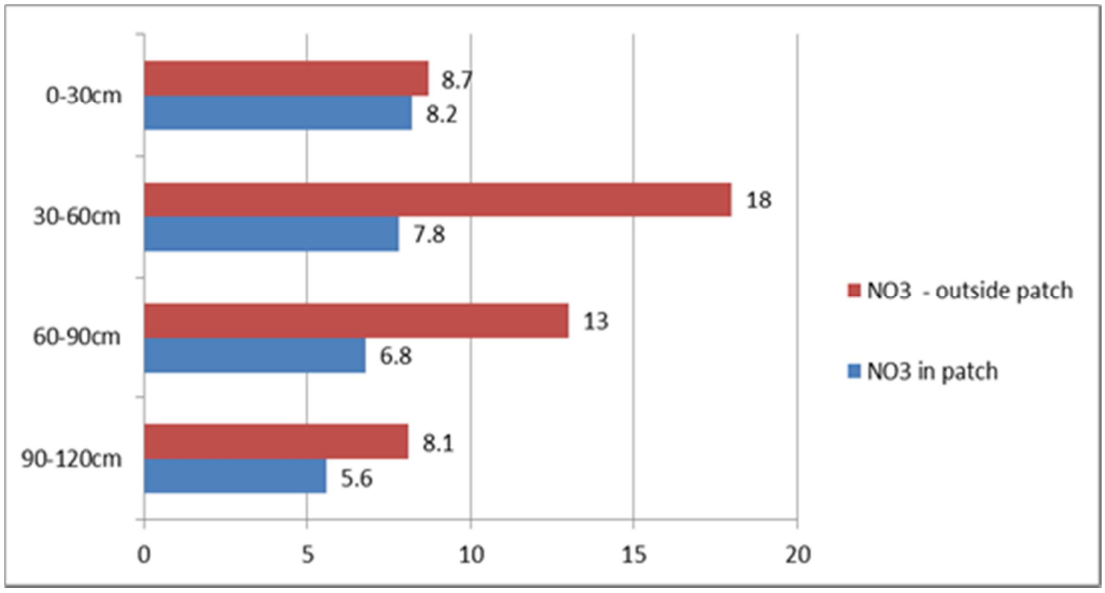


Figure 35. Nitrate levels from within and outside patches taken from 120cm soil cores

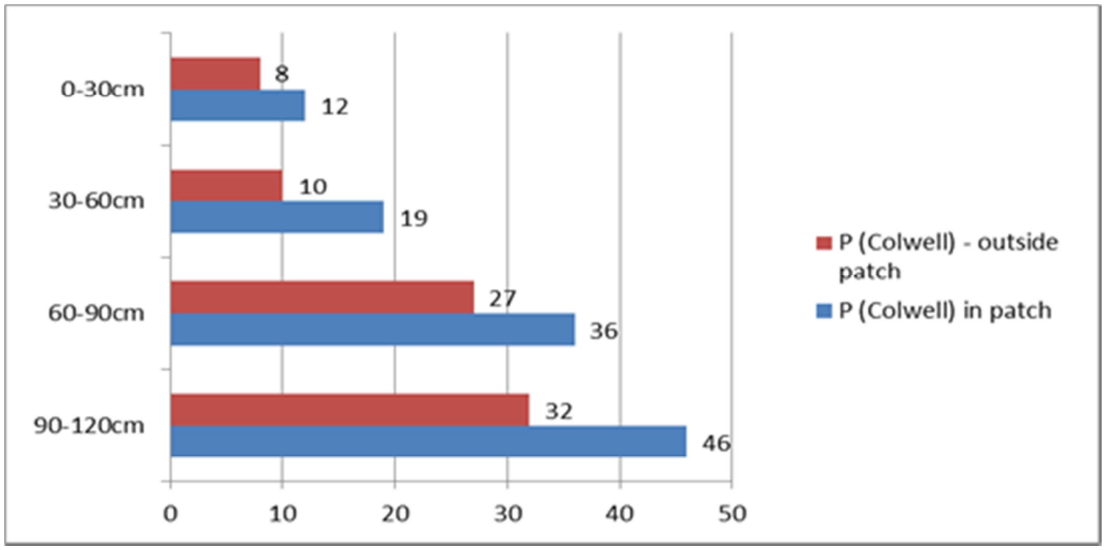


Figure 36. Phosphorus levels from within and outside patches taken from 120cm soil cores

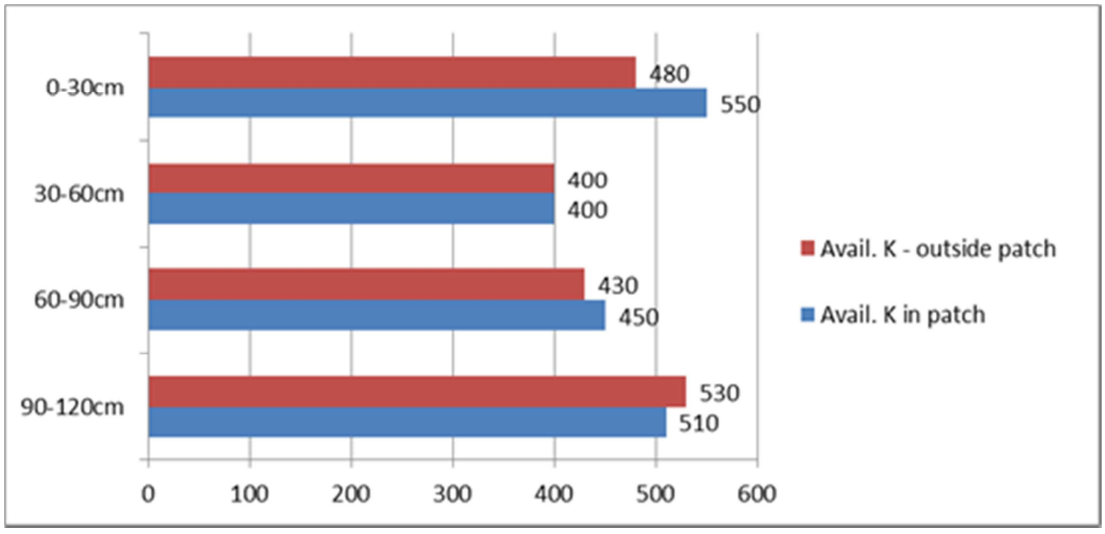


Figure 37. Potassium levels from within and outside patches taken from 120cm soil cores

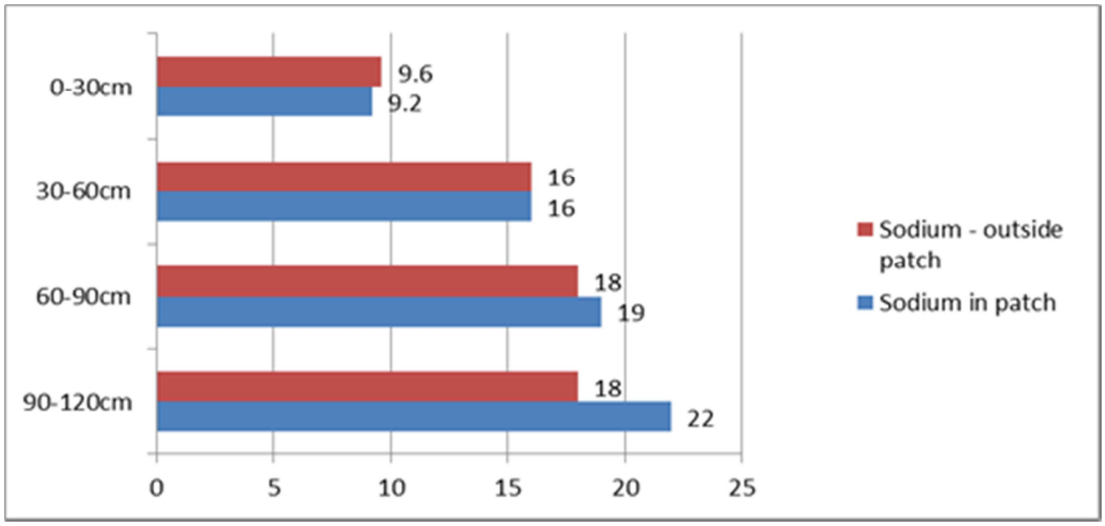


Figure 38. Sodium levels from within and outside patches taken from 120cm soil cores

May 2013: The average number of *Verticillium dahliae* propagules per gram (ppg) of dry soil was determined for each of the six GPS positions (Table 4). Soil cores were air dried for two to four weeks to allow the hyphae and conidia to die before being plated onto selective agar media to determine the average ppg in soil. The average ppg for each GPS point was very high, ranging from 736-936 within the severe wilt patches and 608-736 outside these patches. Overall the average of the six points was 745 ppg.

September 2014: During the Durum wheat crop, plant samples were collected from the same six GPS points. Plants taken from the GPS points within the patches were larger, had higher dry weight biomass and generally had more grain compared to those collected outside the GPS points (where cotton plants had severe external symptoms). This was attributed to more nutrients being left in the soil in the areas where cotton was severely affected by Verticillium wilt, with some plants dying early in the season as a result of infection.

December 2014: Following the wheat, sorghum, wheat rotation, there was a significant decline in the ppg at each of the six GPS points (Table 4). The ppg within the severe patches ranged from 110-206 ppg and outside these patches ranged from 88-106 ppg. The average ppg for all GPS points was 124 ppg.

July 2016: Following an additional sorghum crop in 2015/2016, there was a significant decline in the ppg at each of the six GPS points (Table 4). The ppg within the severe patches ranged from zero to four and outside these patches ranged from zero to four ppg. The average ppg for all GPS points was two ppg. The overall reduction inoculum levels from the first soil isolations after the cotton crop in 2012 to the soil isolations in July 2016 demonstrates the beneficial effect of rotating with non-host crops such as wheat. The addition of sorghum resulted in improved soil stability. The significant reduction in overall inoculum levels is represented in Figure 39.

Table 4. Incidence of Verticillium wilt (%) and inoculum levels (ppg) in soil collected May 2013, Dec 2014 and June 2016.

% Incidence Nov 2012	Patch	GPS	Average ppg May 2013	Average ppg Dec 2014	Average ppg Jun 2016
100%	In	GPS 173	936	138	0
94%	Out	GPS 174	672	106	4
100%	In	GPS 175	784	110	4
88%	Out	GPS 176	608	94	4
100%	In	GPS 177	736	206	0
60%	Out	GPS 178	736	88	0

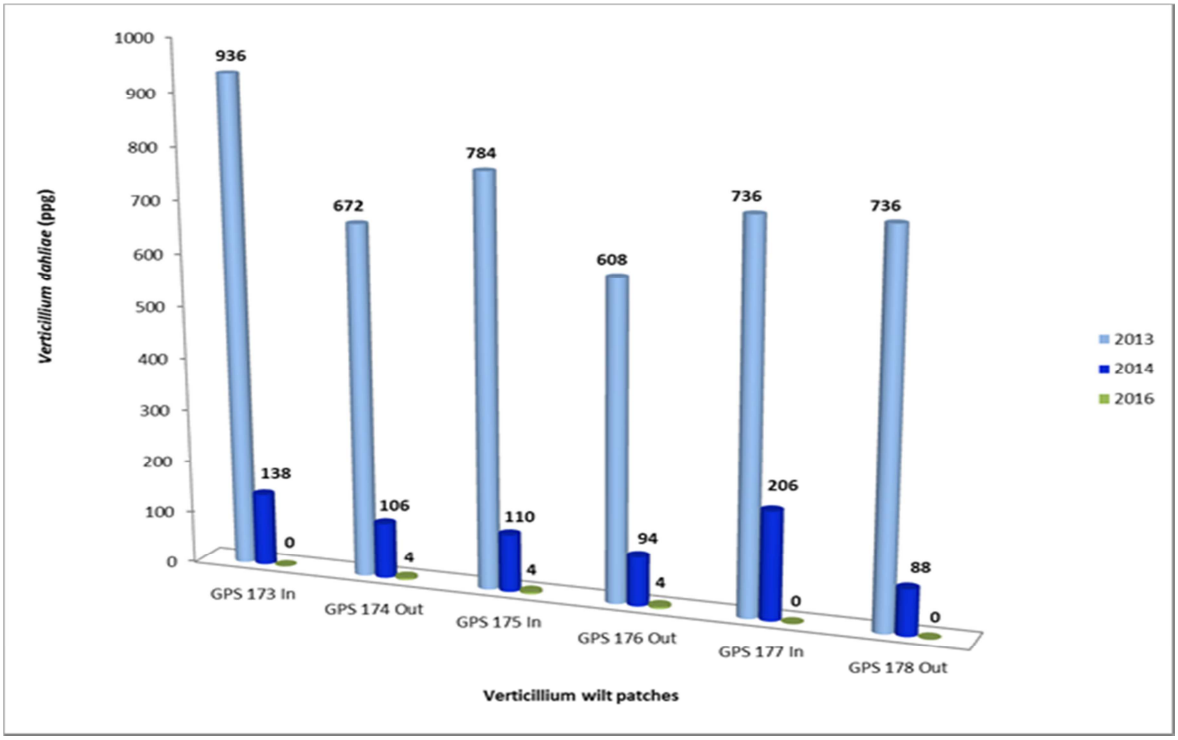


Figure 39. A significant reduction in Verticillium inoculum (ppg) following wheat, sorghum, wheat, sorghum rotations

Table 5. Filed 4 planting history throughout 2013, 2014 and 2016

30th June 2012 - 1st July 2013 **Field 4**	**30th June 2013 - 1st July 2014** **Field 4**	**30th June 2014 - 1st July 2015** **Field 4**	**30th June 2015 - 1st July 2016** **Field 4**
Crops April 2012 Picked cotton June 2012 Planted Durum Wheat Harvested November 2012 **Disease incidence Apr 2012**	**Crops** Sorghum planted September 2013. Harvested February 2014 **Soil isolations May 2013**	**Crops** Durum wheat Planted 1st June 2014. Harvested November 2014 **Soil isolations Dec 2014**	**Crops** Sorghum planted September 2015, Harvested January 2016 **Soil isolations July 2016**
Nutrition Cotton crop 8 1/2 kg zinc 70 kg N. In soil applied 210 kg N to the Hectare, 25LPH Clear start to hectare (Phosphorous) 40 KG N for Durum. 58 kg N late application.	**Nutrition** 82 kg N in soil plus 190 kg N, plus 30 kg N, 15LPH Clearstart	**Nutrition** 142 kg N 60 kg N 60 kg MAP At planting time	**Nutrition** 17 kg N in soil 221 kg N 15 LPH Clearstart
142 kg N for Durham 58 kg N late application at planting 60k MAP May 2012 disced and hilled up, planted with narrow tyne planter	Disced Scarified, hilled, crop cultivated.	Disced Hilled Planted Narrow tyne planter	Disced twice, scarified, hilled up After sorghum harvest, slashed ready to sow Durum.

The inclusion of rotation crops in this field had agronomic and economic benefits. Long term rotations with non-host crops such as wheat and sorghum assisted in the significant reduction of *V. dahliae* inoculum in the soil. The addition of sorghum into the rotation contributed to improved soil stability and another means of income.

Farm F – Effect of management practices on inoculum levels

Working in collaboration with Dr Stephen Allen, two field sites (Farm F and Farm LM) were set up to establish if raking and burning cotton trash/stubble would reduce Verticillium inoculum in the soil (Figure 40). The completely randomised and replicated experimental design included three treatments: Control, Blow, and Burn (Table 6). Plots were 12m long, and four rows wide. Three soil cores were taken from each of the two middle rows in each plot and combined. Plots started 14m from the head ditch. Soil cores were air dried in the glasshouse for three to four weeks, spilt then sieved. 10g sub samples with five replications were used for soil isolations using the dilution plate technique.

Figure 40. Field trial at Farm M set up to assess the effect of raking and burning on Verticillium inoculum levels in soil

Table 6. Propagules per gram (ppg) of Verticillium following three treatments at two farm trials.

Treatment	**Farm F ppg**	**Farm LM ppg**
Burn	512[a]	49[a]
Blow	400[a]	31[a]
Control	437[a]	41[a]

No significant difference in ppg between the three treatments at each of the two farm sites. The *V. dahliae* pathogen is able to survive in finer residue such as petioles, leaves and bracts. Raking the trash only served to spread the pathogen further around the field.

Farm F - Quantitative assessment of Verticillium wilt symptoms in plants growing in soil of different inoculum levels

Naturally infected soil was collected from a commercial cotton farm (Farm F) and ppg determined using the dilution plate technique (Figure 41). The 25% and 50% soil treatments were made by adding the required amount of twice pasteurised soil to the naturally infected soil. The twice pasteurised soil was used as the control treatment. There was no significant effect of ppg on days to emergence. All seedlings had emerged before or five days after planting in all treatments (Figure 42). There was no difference in symptom expression at 355 ppg, 204 ppg or 141 ppg. Further studies using lower ppg of VCG1A, VCG2A and VCG4B strains have been included in the milestones of the new project.

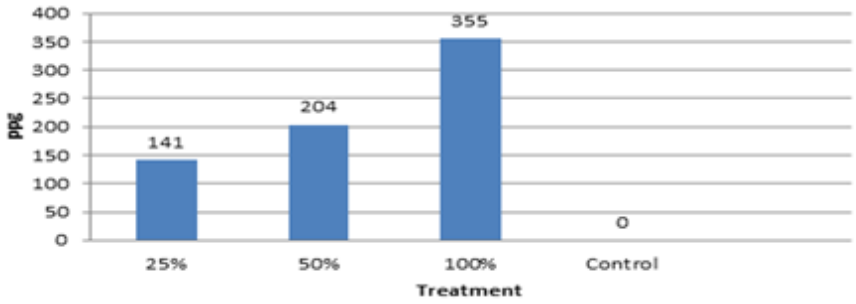


Figure 41. Verticillium inoculum levels in soil mixed to approximately 25%, 50% and 100% infected soil.

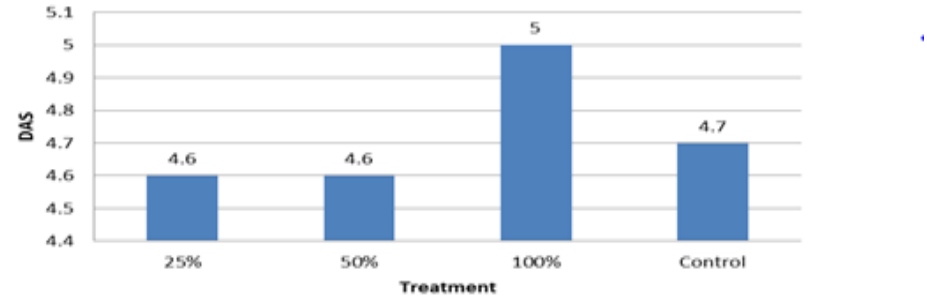


Figure 42. No significant effect of ppg on days to emergence between treatments.

There was no significant effect of ppg on plant height, however you can see infection slows growth approximately 42 days after sowing (Figure 43).

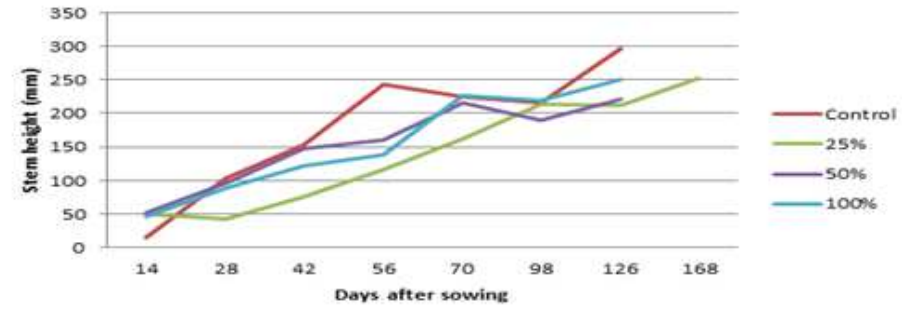


Figure 43. No significant effect of ppg on days to emergence.

OBJECTIVE 3: INDEPENDENT AND IMPARTIAL EVALUATION OF EXISTING AND NOVEL SEED TREATMENTS FOR SEEDLING DISEASE AND BLACK ROOT ROT

Milestone 3.1 Independent and impartial evaluation of existing and novel seed treatments for seedling disease and black root rot

2103/2014 Namoi valley early planting results

Stand counts were extremely low ranging from as low as 0.04% to 5.25% (Figure 44). Caution is advised when interpreting the significance of any of the fungicide treatments given such low germination/establishment. Consequently, no stand count was recorded after six weeks.

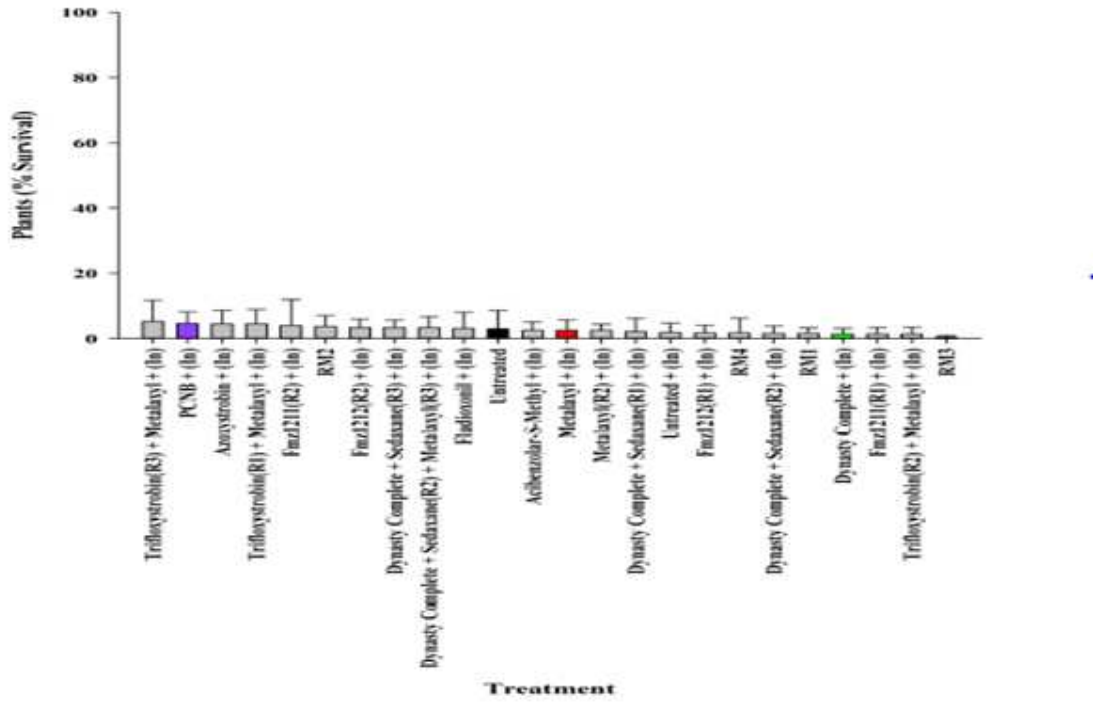


Figure 44. Percentage survival three weeks after planting at ACRI 15th September

2013/2014 Namoi valley normal planting results

Stand counts remained low regardless of the seed treatment. Stand counts ranged from 14.75% to 36.20% (Figure 45). Seedlings from PCNB treatment were significantly higher than those treated with Apron suggests *Rhizoctonia* may have been the dominant pathogen. The lack of effect of Apron also suggests that *Pythium* spp. were also present. Both symptoms of *Rhizoctonia* and *Pythium* spp. were observed on seedlings throughout this trial. Stand counts were significantly higher in plots treated with the three rates of DC094 + Apron mixes and Azoxystrobin treatments. There was no significant difference between untreated seed and the industry standard.

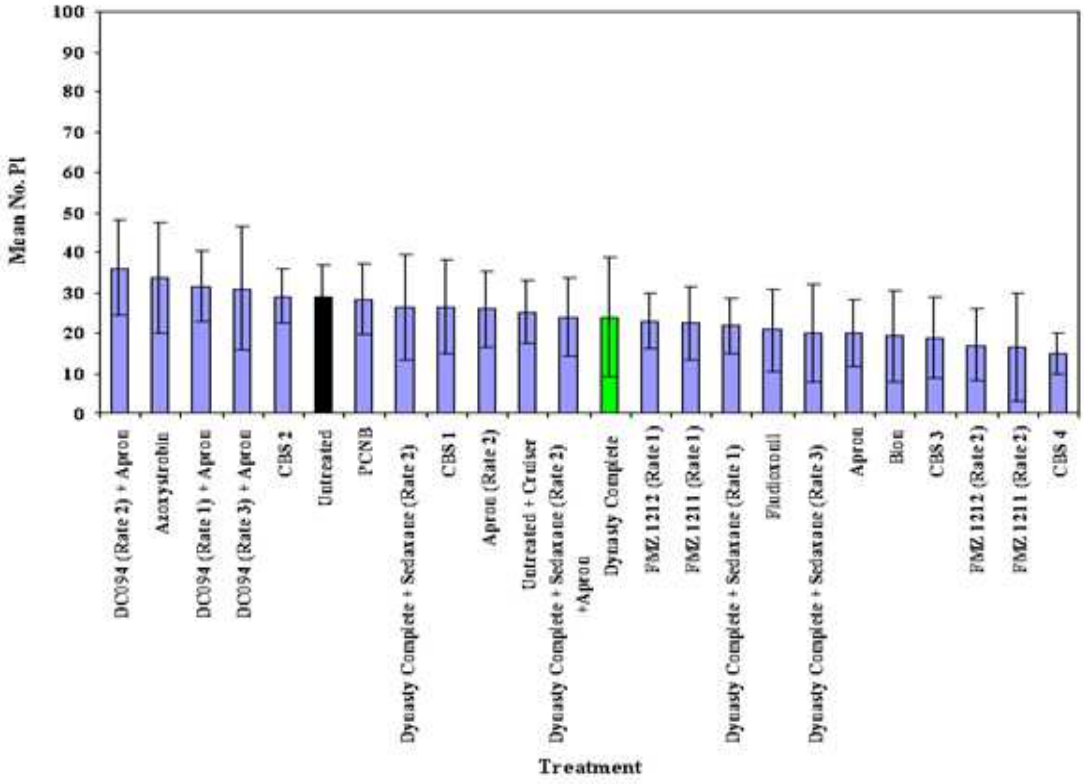


Figure 45. Mean stand counts three weeks after normal planting (16/10/13) following seed treatment with various fungicides at ACRI Narrabri site. Significant differences between seed treatments ($P<0.001$). The industry standard is highlighted in green.

Results were similarly low after six weeks (Figure 46), stand counts ranging from 13.56% to 35.78%. The three rates of DC094 + Apron mixes and Azoxystrobin treatments still performed best. There was no significant difference between untreated seed and the industry standard. PCNB was not significantly higher than Apron suggesting both *Rhizoctonia* and *Pythium* spp. were present.

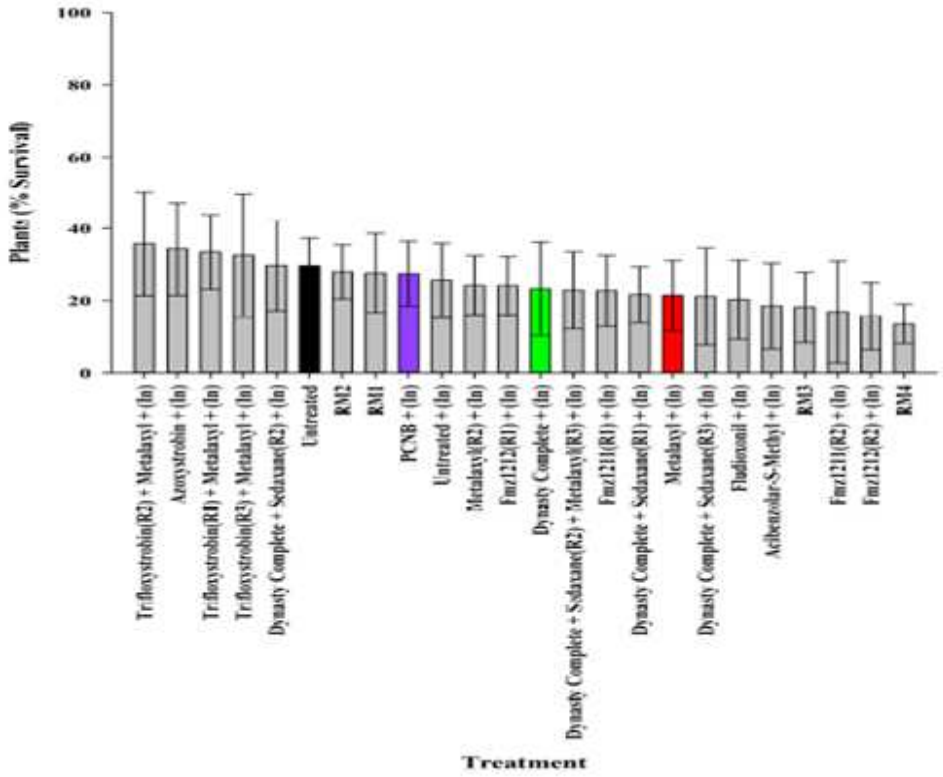


Figure 46. Mean stand counts six weeks after normal planting (16/10/13) following seed treatment with various fungicides at ACRI Narrabri site. Significant differences between seed treatments ($P<0.001$). The industry standard is highlighted in green.

2013/2014 Macintyre valley results

At the Mungindi valley site, seed treatments had no significant (P=0.993) effect on reducing seedling mortality in comparison to untreated plots (Figure 47). Seedling mortality averaged 52% across the trial. Apron was not significantly different from PCNB which suggests a mixture of disease pathogens were present. The lack of effect of any treatment also suggests there were factors other than disease causing high mortality this season.

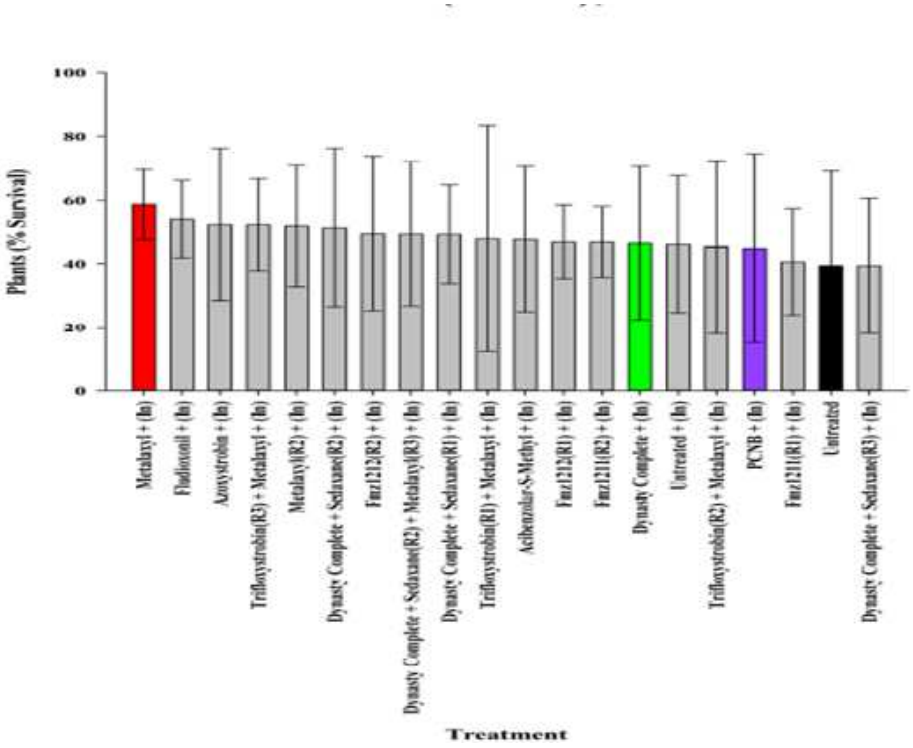


Figure 47. Mean stand counts six weeks after planting (05/10/13) following seed treatment with various fungicides at a Mungindi valley site showing significant differences between seed treatments (P<0.001). The industry standard is highlighted in green.

2013/2014 Lachlan valley results

Stand counts were significantly higher in plots treated with the three rates of DC094 + Apron and FMZ1212 (Rate 2) compared to the industry standard (Figure 48). There was no significant difference between untreated seed and the industry standard. At Hillston, in the Lachlan valley the seedling mortality averaged 44%.

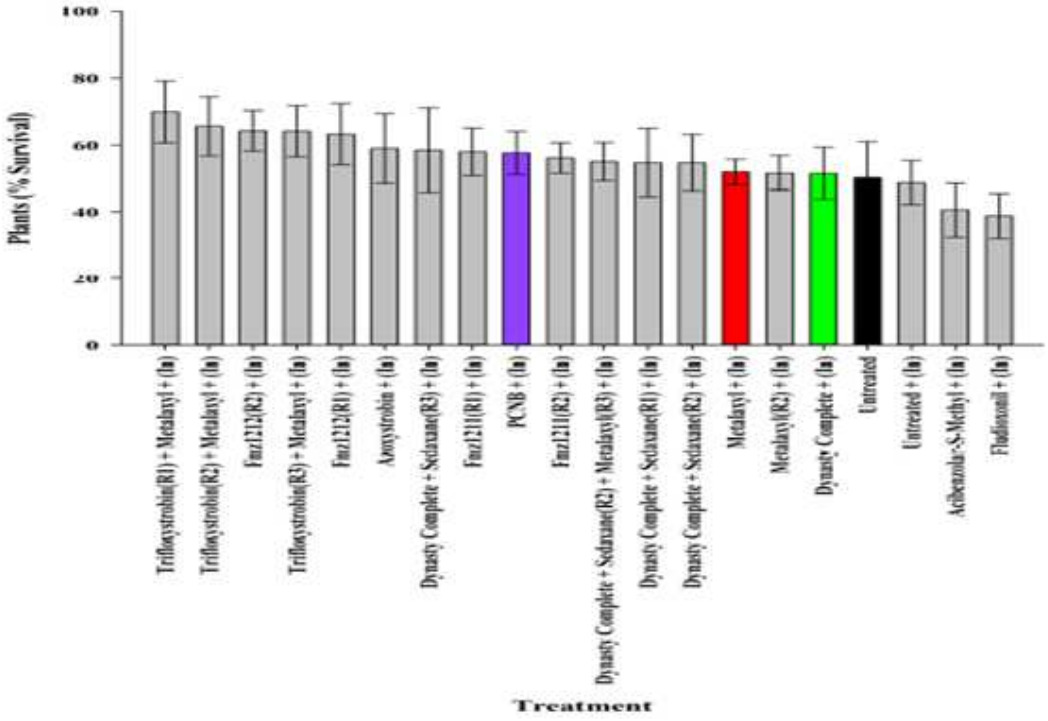


Figure 48. Mean stand counts six weeks after planting (30/09/13) following seed treatment with various fungicides at the Lachlan valley site. Significant differences between seed treatments ($P<0.001$). The industry standard is highlighted in green.

2013/2014 Macquarie valley results

Stand counts were significantly higher in plots treated with DC094 (Rate 2) + Apron, FMZ 1212 (Rate 2), DC094 (Rate 3) + Apron, FMZ 1212 (Rate 1), FMZ 1211 (Rate 2) and Dynasty Complete + Sedaxane (Rate 2) + Apron compared to the industry standard (Figure 49). There was no significant difference between untreated seed and the industry standard. At the Warren site in the Macquarie valley, seedling mortality averaged 47%.

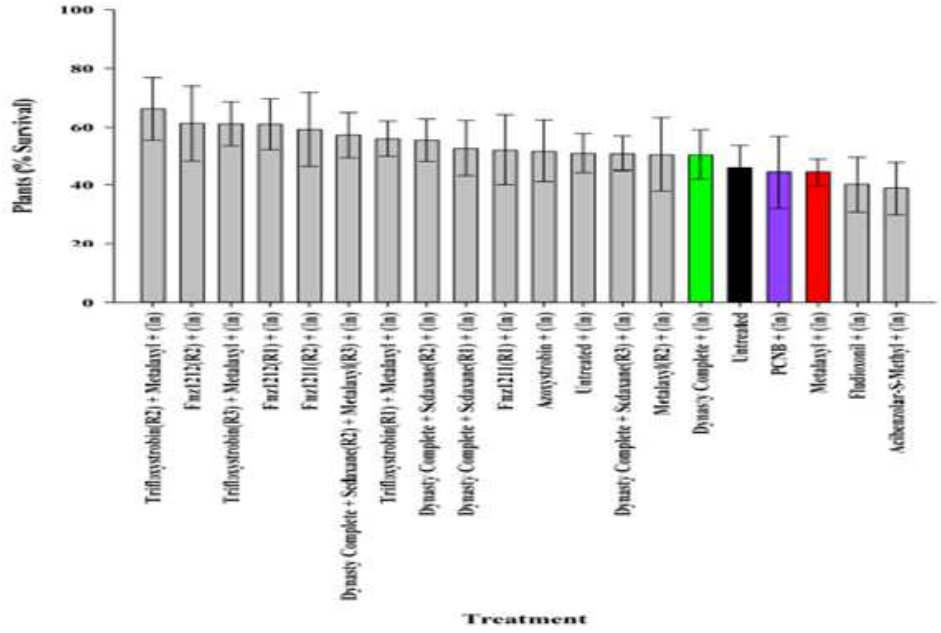


Figure 49. Mean stand counts six weeks after planting (01/10/13) following seed treatment with various fungicides at a Warren site. Significant differences between seed treatments ($P<0.001$). The industry standard is highlighted in green.

2014/2015 Namoi valley early planting results

The early plant trial was planted dry on the 15th September 2014 when soil temperature was low (9°C) and watered up a day later. Stand counts were made six weeks after planting. The percentage survival was very low ranging from 8% to 36% despite there being a significant difference between seed treatments (Figure 50). The cool conditions at the time of planting provided ideal conditions for seedling disease development evidenced by such low survival percentages. Soil temperature at planting was 14.5°C and did not exceed 16°C for more than 14 days following planting. The relatively poor survival of seedlings from treatments P+T and TM+T (both active for *Rhizoctonia)* and higher survival in treatments M+T and M+I (active against *Pythium*) suggest the dominant pathogen at this trial was most likely *Pythium* spp.

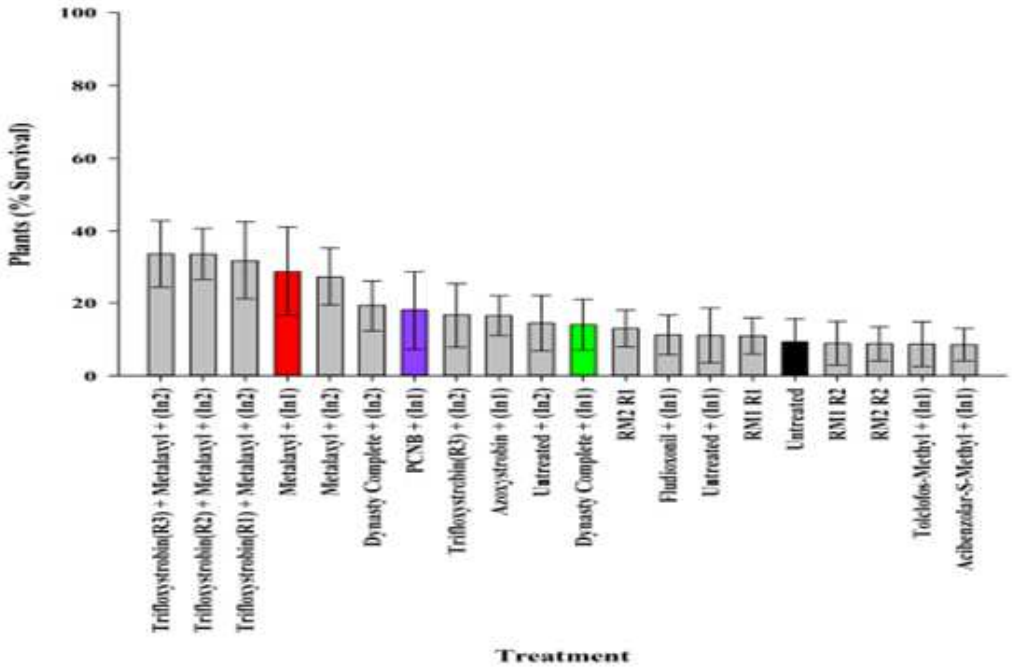


Figure 50. Percentage survival of seedlings six weeks after planting at ACRI planted on the 15th September 2014. Lines indicate 95% confidence interval for the predicted re-transformed values.

2014/2015 Namoi valley normal planting results

The normal planting trial was planted on the 20th October 2014, into moisture when soil temperature was 18°C and rising and flushed the following day. Stand counts were made six weeks after planting. Percentage survival ranged from 53% to 83% (Figure 51). The industry standard Dynasty Complete plus insecticides were amongst the top performers. The warm temperatures following planting, along with the significant difference between untreated and untreated plus both insecticides indicated that a large percentage of mortality could be attributed to insect pressure. There was no significant difference between the combinations of Metalaxyl and insecticide combinations with the standard seed treatment, suggesting that *Pythium* spp. was the dominant pathogen at this trail planted mid-October, 2014. Comparison between September and October planting dates during 2014/2015 season demonstrated the increased survival of plants when planted on the 20th October (Figure 52). Warmer soil temperatures in October meant there was reduced risk of mortality associated with seedling pathogens such as *Pythium* and *Rhizoctonia* spp.

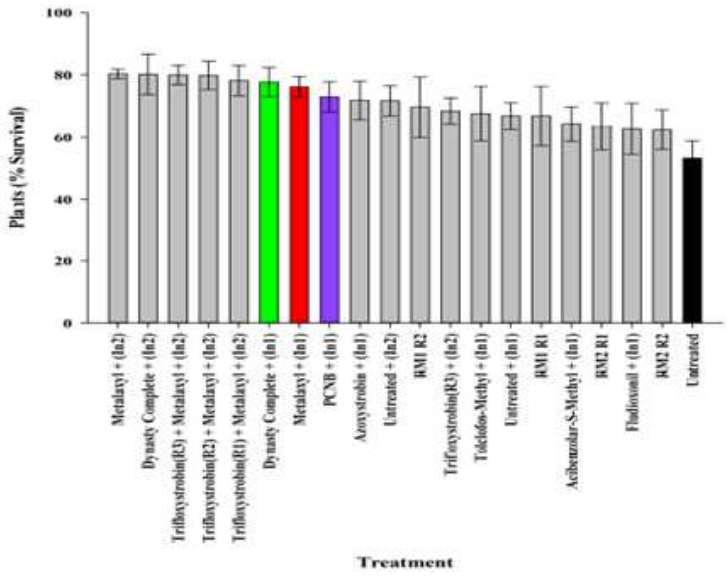


Figure 51. Percentage survival of seedlings six weeks after planting at ACRI planted on the 20th October 2014. Lines indicate 95% confidence interval for the predicted re-transformed values.

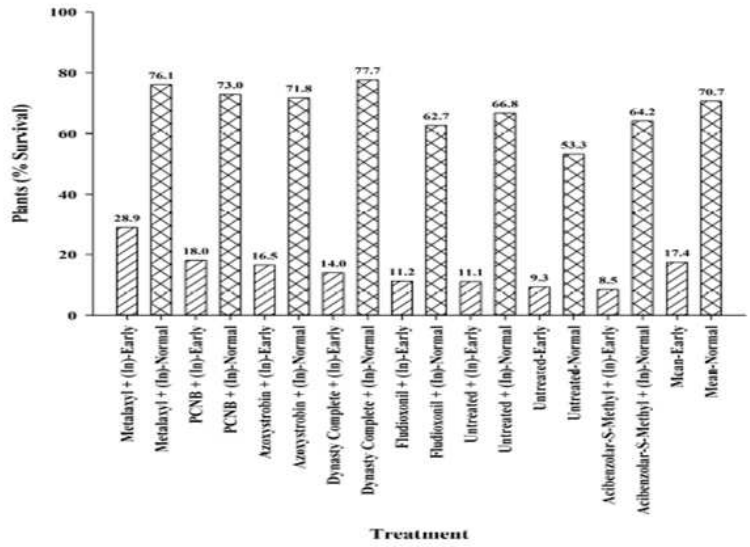


Figure 52. Comparison of seedling survival under various seed treatments when planted in September compared to October.

2014/2015 Macintyre valley results

There was a significant difference between seed treatments at this trial site. The percentage survival ranged from 61% to 71% (Figure 53). Weather conditions were warm and rising at the time of planting this trail. The Cotton Seed Distributors (CSD) Cruiser Fund Soil Temperature Network in Mungindi reported the minimum air temperature did not go below 15^{o}C for the month of October. The maximum temperature reached 34^{o}C around the 30th October. Weather conditions were not conducive to disease development following planting on 17th October, evidenced by 64% survival of seedlings from both untreated treatment (UN) and untreated plus insecticide (UN+T) and 66% in the untreated plus insecticide (UN+I). The highest survival of 71% was recorded for the treatment TM+T (active control against *Rhizoctonia*) indicating the dominant pathogen at this trial may have been *Rhizoctonia.* Survival in treatment TM+T was significantly higher than 8 other treatments (Un, Un+T, T3+M+I, M+I, RM1R1, RM1R2, Rm2R1 and RMR2).

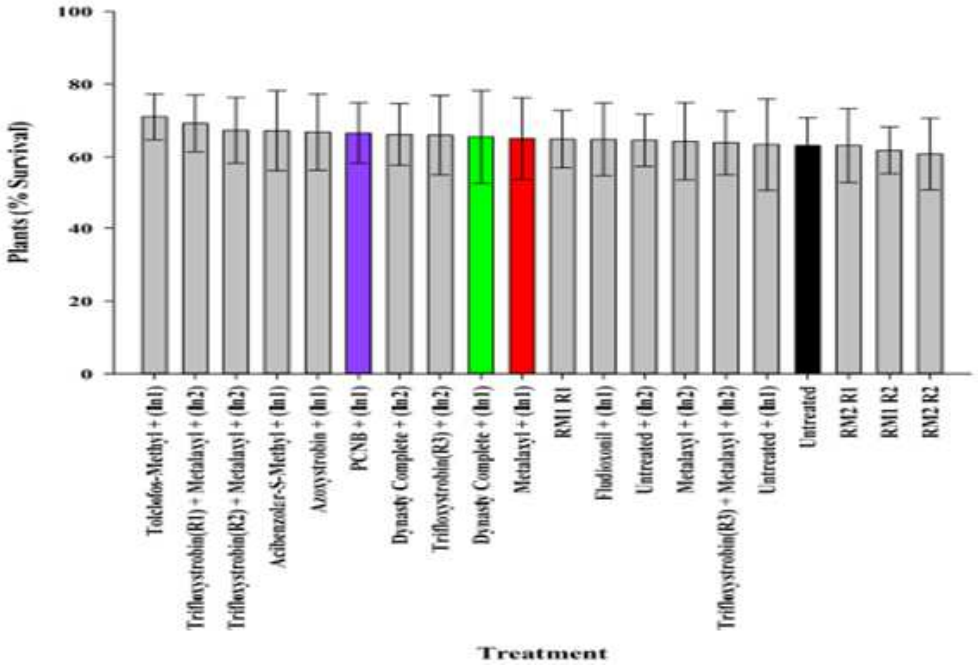


Figure 53. Percentage survival of seedlings six weeks after planting at Mungindi on the 17th October 2014. Lines indicate 95% confidence interval for the predicted re-transformed values.

2014/2015 Lachlan valley results

There was a significant difference between seed treatments at this trial site planted on the 29th September 2014. The percentage survival ranged from 38% to 55% (Figure 54). The dominant pathogen at this trial was most likely *Pythium* spp. given there was no significant difference between the combinations of Metalaxyl and insecticides and the standard seed treatment plus insecticide.

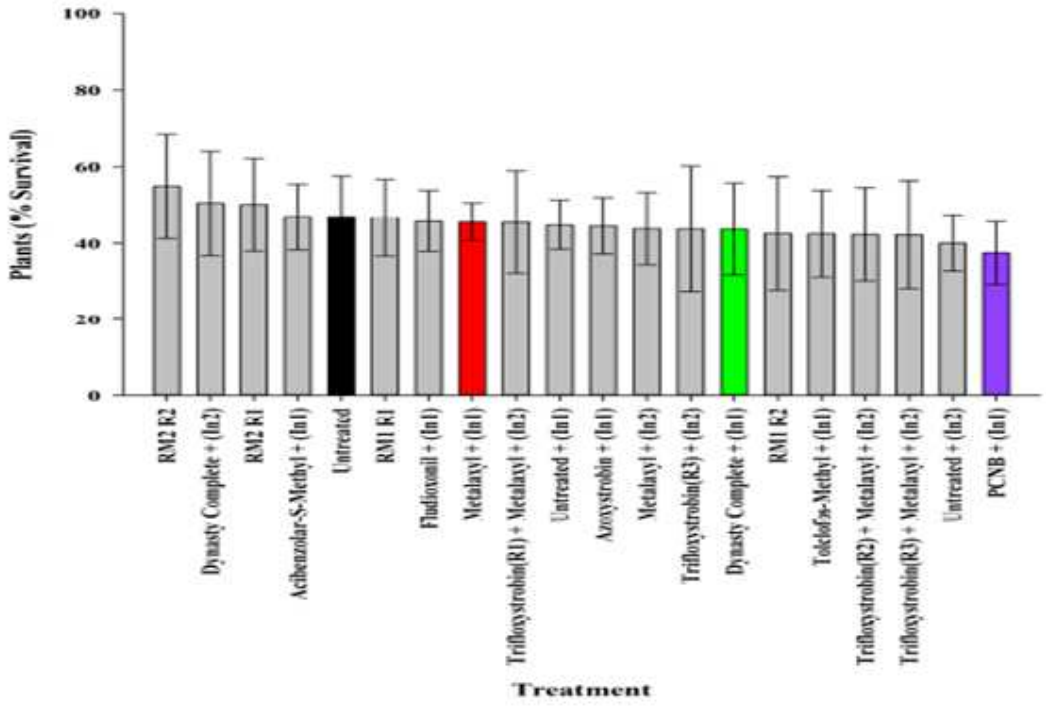


Figure 54. Percentage survival of seedlings six weeks after planting at Hillston planted on the 29th September 2014. Lines indicate 95% confidence interval for the predicted re-transformed values.

2014/2015 Macquarie valley results

There was a significant difference between seed treatments at this trial site. The percentage survival ranged from 63% in the untreated to 80% in the novel RM1R2 treatment (Figure 55). The dominant pathogen at this trial planted on the 10th October 2014 was most likely *Pythium* spp. given there was no significant difference between the combinations of Metalaxyl and insecticides and the standard seed treatment plus insecticide.

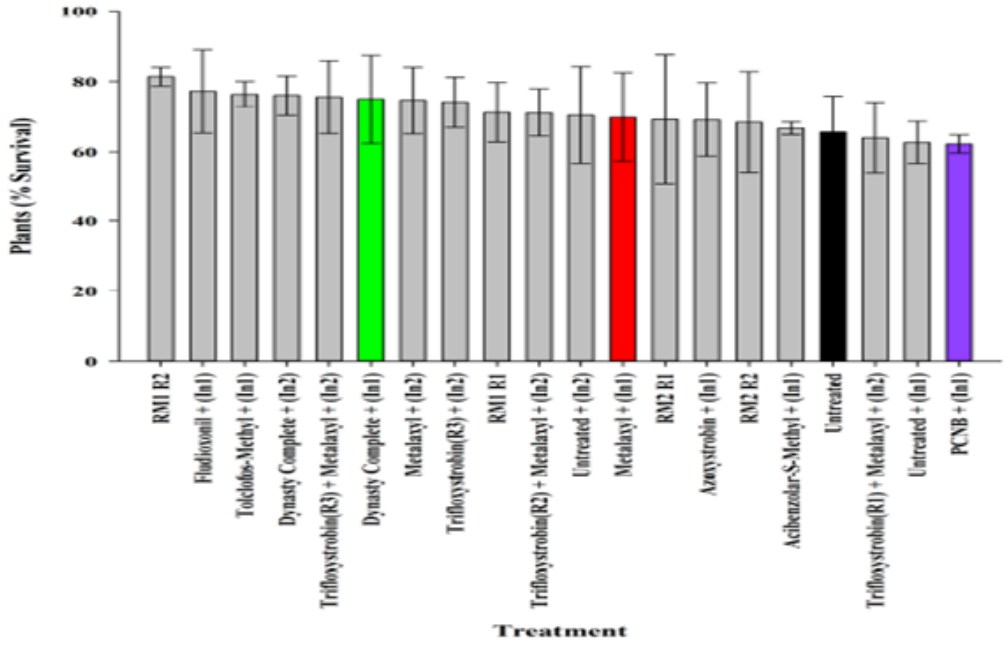


Figure 55. Percentage survival of seedlings six weeks after planting at the Macquarie valley site at Warren planted on the 10th October 2014. Lines indicate 95% confidence interval for the predicted re-transformed values.

2015/2016 Namoi valley seed treatment results

There was no significant difference in stand counts at three (Figure 56) and six weeks (Figure 57) after planting between industry standard and the highest stand counts. The results of the untreated compared to the untreated plus insecticide indicates there was very little insect pressure. Minimum soil temperature ranged from 11°C to 13°C during September rising to 16°C on 2nd October. Dominant pathogen at ACRI site most likely *Pythium* spp. as indicated by a higher stand count in treated with Metalaxyl plus Thiamethoxam.

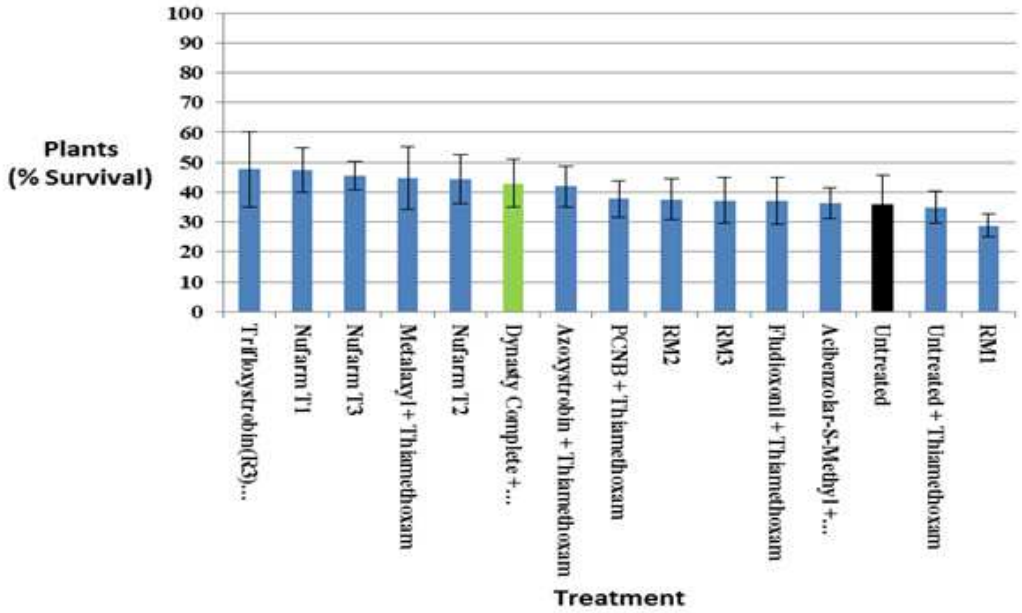


Figure 56. Percentage survival of seedlings six weeks after planting at ACRI planted on the 15th September 2015. Lines indicate 95% confidence interval for the predicted re transformed values.

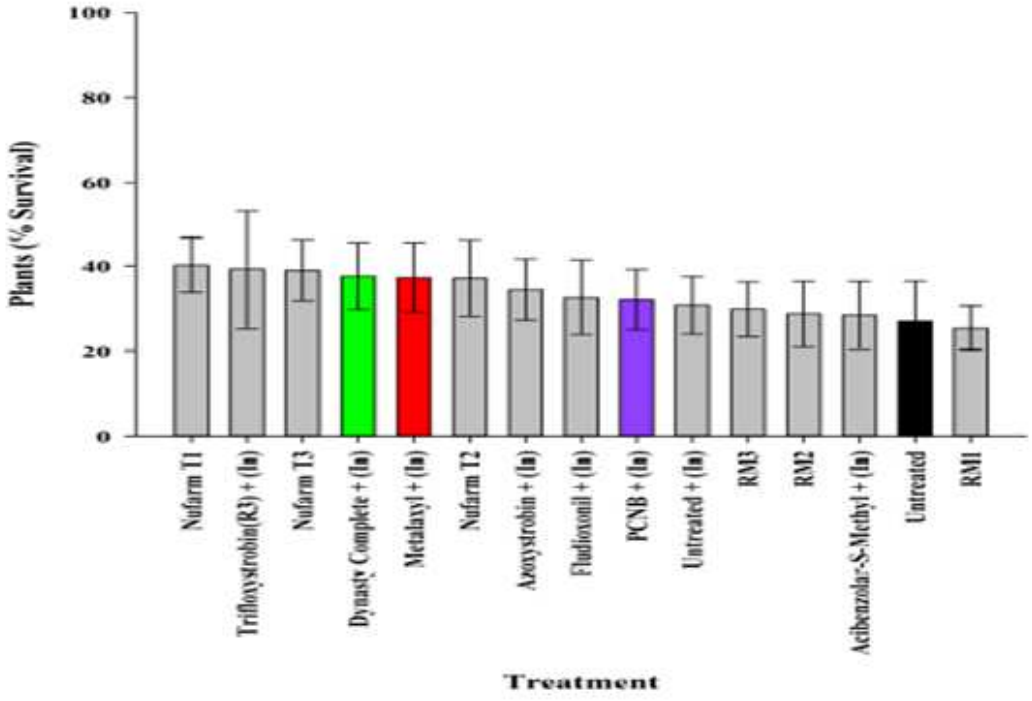


Figure 57. Percentage survival of seedlings six weeks after planting at ACRI planted on the 15th September 2015. Lines indicate 95% confidence interval for the predicted re-transformed values.

2015/2016 Normal valley planting results

No significant difference between the industry standard plus Thiamethoxam and the untreated stand counts (Figure 58). The dominant pathogen at ACRI site was most likely *Pythium* spp. as indicated by a higher stand count in plots treated with Metalaxyl plus Thiamethoxam insecticide. Moisture issues with subbing following planting contributed to lower stand counts across all plots.

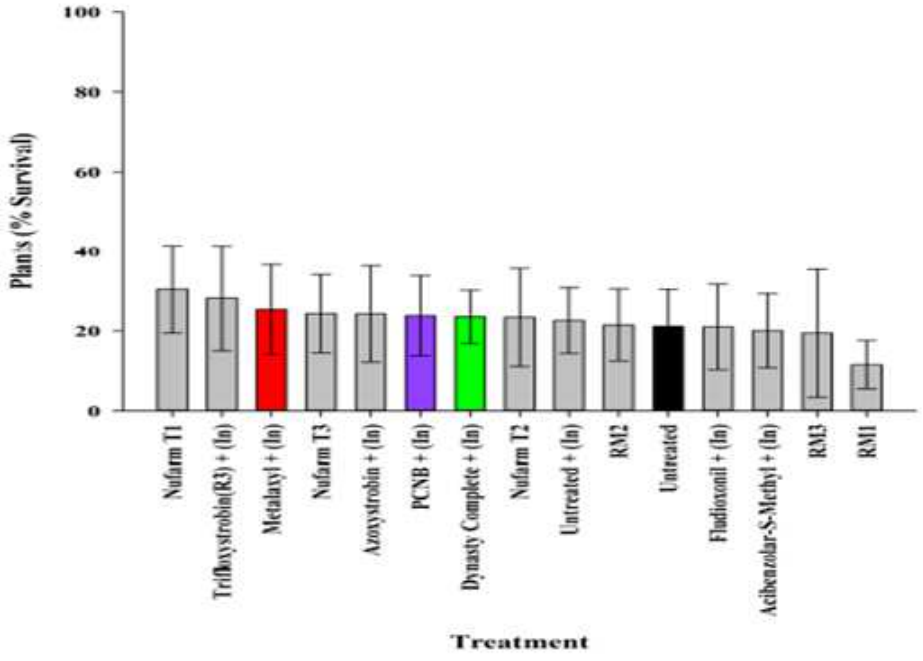


Figure 58. Percentage survival of seedlings six weeks after planting at ACRI planted on the 15th October 2015. Lines indicate 95% confidence interval for the predicted re-transformed values.

2015/2016 Macintyre valley seed treatment results

No significant difference between the industry standard plus Thiamethoxam and the untreated stand counts (Figure 58). The dominant pathogen at this site was most likely *Pythium* spp. as indicated by a higher stand count in plots treated with Metalaxyl plus Thiamethoxam insecticide.

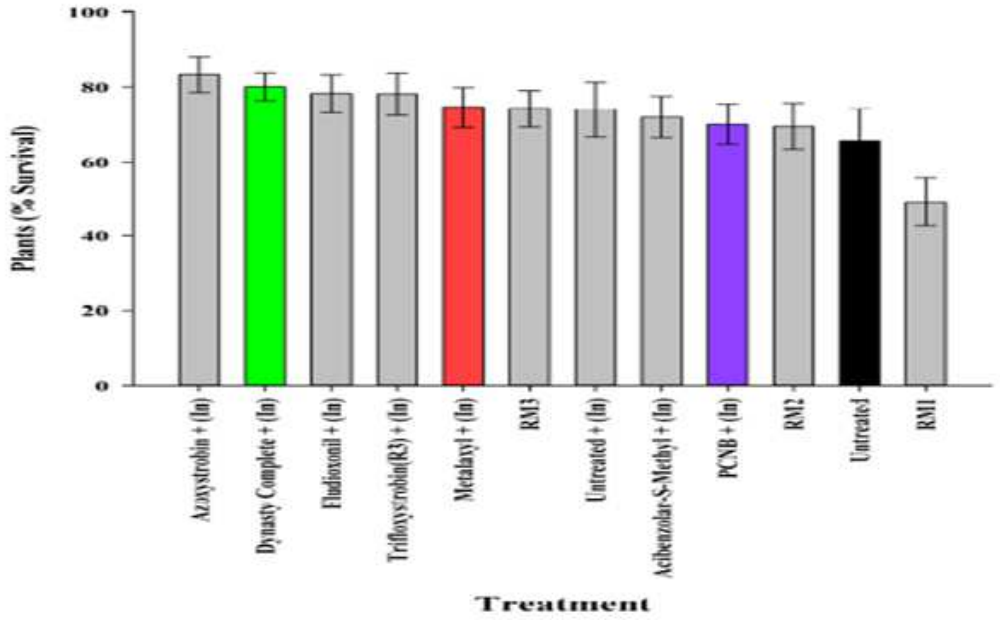


Figure 59. Percentage survival of seedlings six weeks after planting at Macintyre trial site 2015/2016. Lines indicate 95% confidence interval for the predicted re-transformed values.

2015/2016 Lachlan valley seed treatment results

There was a significant difference between the industry standard plus Thiamethoxam insecticide and the untreated stand counts (Figure 60). This trial was dry sown then watered up. Pressure from both *Pythium* and *Rhizoctonia* spp. was evident with similar survival between Metalaxyl plus Thiamethoxam which is active against *Pythium* spp. and PCNB plus Thiamethoxam which is active against *Rhizoctonia* sp.

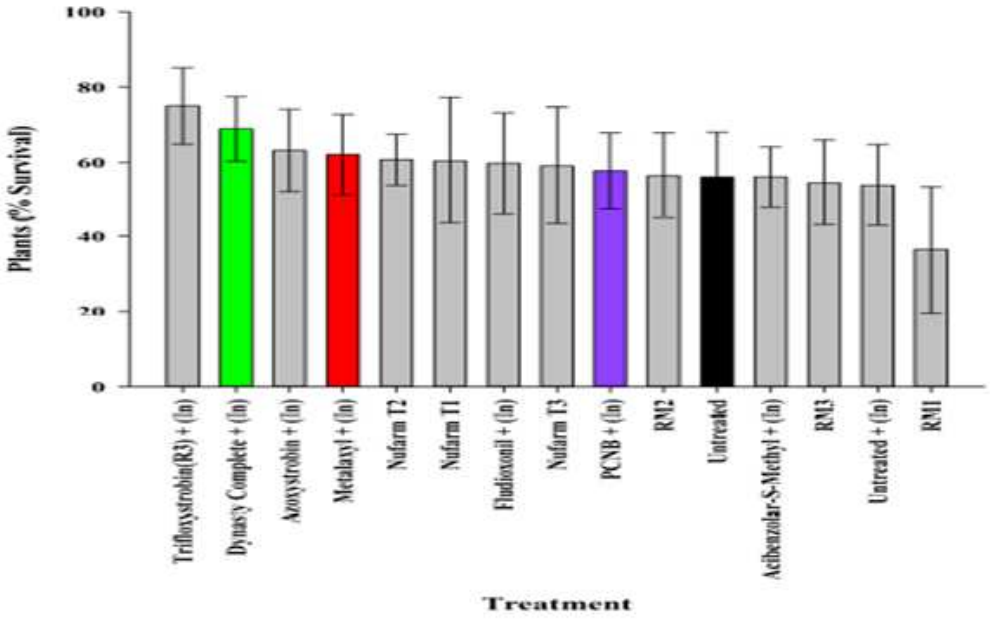


Figure 60. Percentage survival of seedlings six weeks after planting at Hillston planted on the 3rd October 2015. Lines indicate 95% confidence interval for the predicted re transformed values.

2015/2016 Macquarie valley seed treatment results

No significant difference between the industry standard plus Thiamethoxam and the untreated stand counts (Figure 61). The dominant pathogen at the Macquarie site was most likely *Rhizoctonia* spp. due to the higher stand count in plots treated with PCNB plus Thiamethoxam which is active against *Rhizoctonia* spp.

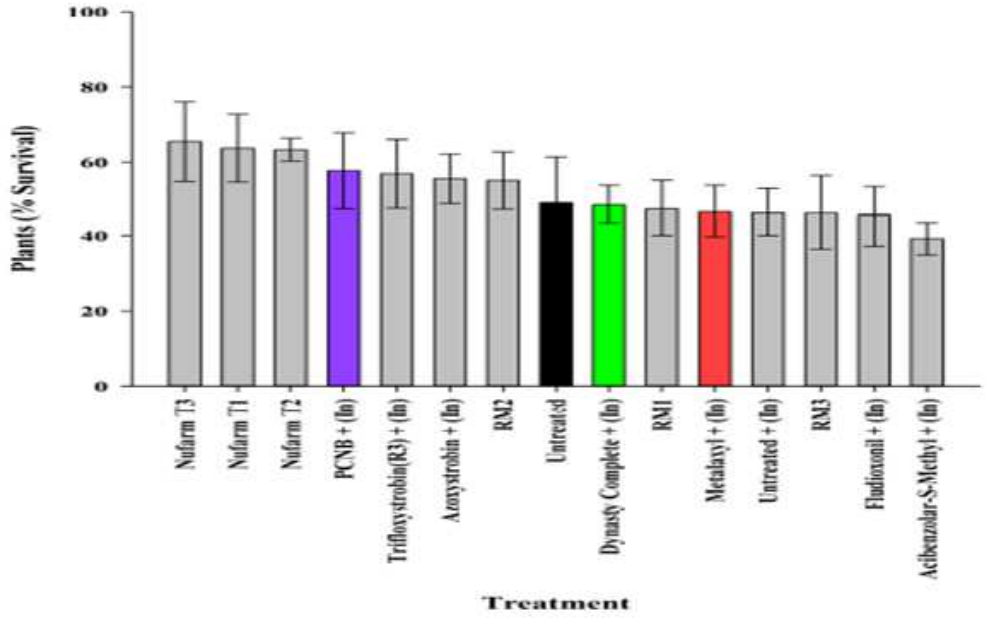


Figure 61. Percentage survival of seedlings six weeks after planting at Warren planted on the 20th September 2015. Lines indicate 95% confidence interval for the predicted re-transformed values.

Seed was treated with individual treatments and sent to the QLD pathology team however there are no results from QLD due to hail damage resulting in complete stand losses.

When seedling disease control is provided by Apron alone or as good as, or better than the control provided by the current standard treatment (Dynasty Complete) this indicates that *Pythium* spp. may have been the most significant pathogen at that site and in that season. Alternatively, if the seedling disease control provided by Apron alone is no better than untreated seed then this indicates that *Rhizoctonia* spp. May be the most significant pathogen at that site and in that season.

Novel seed treatments
CvT64 Pot Experiment: assess efficacy of product on *Verticillium dahliae*

A replicated completely randomised pot trial was set up with two treatments in the growth room with maximum temperature set at 22^{o}C and minimum temperature at 14^{o}C. The two treatments were CvT64 and control (water). There were six replicates of each treatment. The product CvT64 was diluted at 1:50 and shaken vigorously to achieve a milk-like solution before application to the soil. Naturally infected soil (*Verticillium dahliae*) in the pots was saturated to 60% field capacity. Ten cotton seeds were planted per pot two weeks later and pots watered individually from the base of each pot to avoid potential transference of pathogens between pots.

Plants in the control (water) treatment had emerged after one week while emergence took an extra week in the CvT64 treatment. No significant difference in number of plants that emerged. There was a significant difference in the number of ppg in soil between the pots treated with CvT64 and the control (water) treated pots (Figure 62). The positive result in reducing ppg was negated by the phytotoxic effect. Soil isolations (Figure 62) indicated a significant reduction in *V. dahliae* ppg.

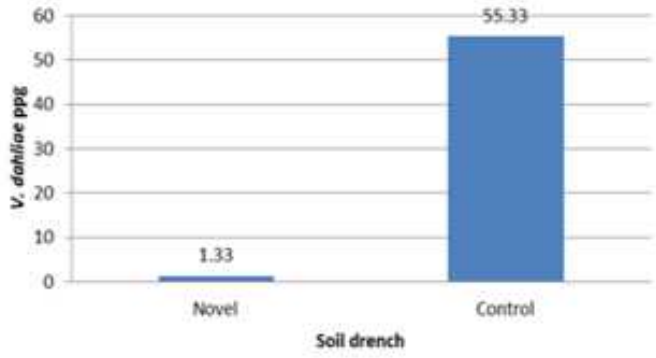


Figure 62. A significant reduction in the number of ppg following soil drenching of CvT64.

This experiment was repeated however in the second experiment no plants germinated in pots treated with CvT64. The product appeared to have a phytotoxic effect, inhibiting (in the first experiment) and completely stopping germination of cotton seeds in the second experiment (Figure 63).

Figure 63. Soil treated with CvT64 in the second experiment failed to germinate.

2015-2016 In-furrow trial at ACRI Field 4

Dynasty at two rates plus Trifloxystobin was compared with Dynasty and water when applied in furrow at planting. There was no significant difference between the two concentrations of DC094 + Dynasty and the industry standard Dynasty treatment (Figure 64).

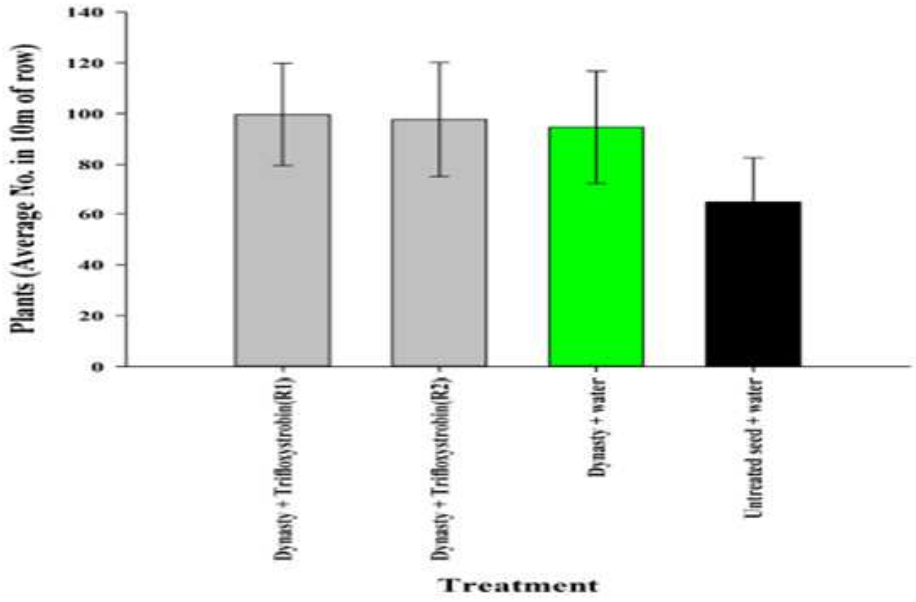


Figure 64. Results of the in-furrow field trial at ACRI.

Evaluation of seed dressings for the control of black root rot in cotton
Trial Objectives:

1. Evaluate the efficacy of FMZ 1211 and FMZ 1212 as a seed dressing targeting *Thielaviopsis basicola* and *Rhizoctonia solani* in cotton as per Table 7.
2. Assess any crop safety from seed dressing

Results: FMZ 1212-626 had significantly lower average disease severity of black root rot compared to the industry standard (Table 8). No phytotoxic effect was observed on seedlings grown in growth room conditions. *Rhizoctonia* was observed on seedlings.

Table 7. Treatment list:

Trt No.	**Product(s)**	**AI**	**Application method**	**Product rate 1L /100kg seed**
1	Untreated	-	-	-
2	Dynasty	Azoxystrobin 75g/L Metalaxyl-m 37.5g/L Fludioxonil 12.5g/L	Seed dressing	200mL
3	FMZ 1211 (Rate 1)	Experimental 400g/kg	Seed dressing	156g
4	FMZ 1211 (Rate 2)	Experimental 400g/kg	Seed dressing	313g
5	FMZ 1212 (Rate 1)	Experimental 200g/L	Seed dressing	313mL
6	FMZ 1212 (Rate 2)	Experimental 200g/L	Seed dressing	626mL

Table 8. Glasshouse novel product screening experiment results

	ADS	**% Survival**	**Plant height (cm)**	**Shoot dry weight (g/plant)**	**Root dry weight (g/plant)**
Dynasty Complete	8.229[a]	74 [a]	59.25 [ab]	0.1171 [b]	0.06549 [c]
FMZ1211-156 (Rate 1)	7.946[a]	77 [a]	59.27 [ab]	0.1377 [ab]	0.10361 [a]
FMZ 1211-313 (Rate 2)	8.032[a]	69 [a]	49.23 [b]	0.1301 [b]	0.09141[ab]
FMZ1212-313 (Rate 1)	7.730[a]	75 [a]	59 [ab]	0.1201[b]	0.08826[abc]
FMZ1212-626 (Rate 2)	5.318[b]	75 [a]	64.37 [a]	0.1597 [a]	0.07762 [bc]
Untreated	7.531[a]	62 [a]	55.75 [ab]	0.1382 [ab]	0.07248 [bc]
LSD	1.393	19.124	12.57	0.02673	0.0243
P value	=0.007	=0.549	=0.259	=0.072	=0.051

TM Field Trial

A field strip trial (Figure 65) was undertaken to assess the efficacy of a novel product (TM) on seedling mortality and black root rot severity. There was no significant difference in stand count or disease severity of black root rot (Table 9). There was a noticeable difference in soil structure with the soil in the TM treated plots significantly more friable.

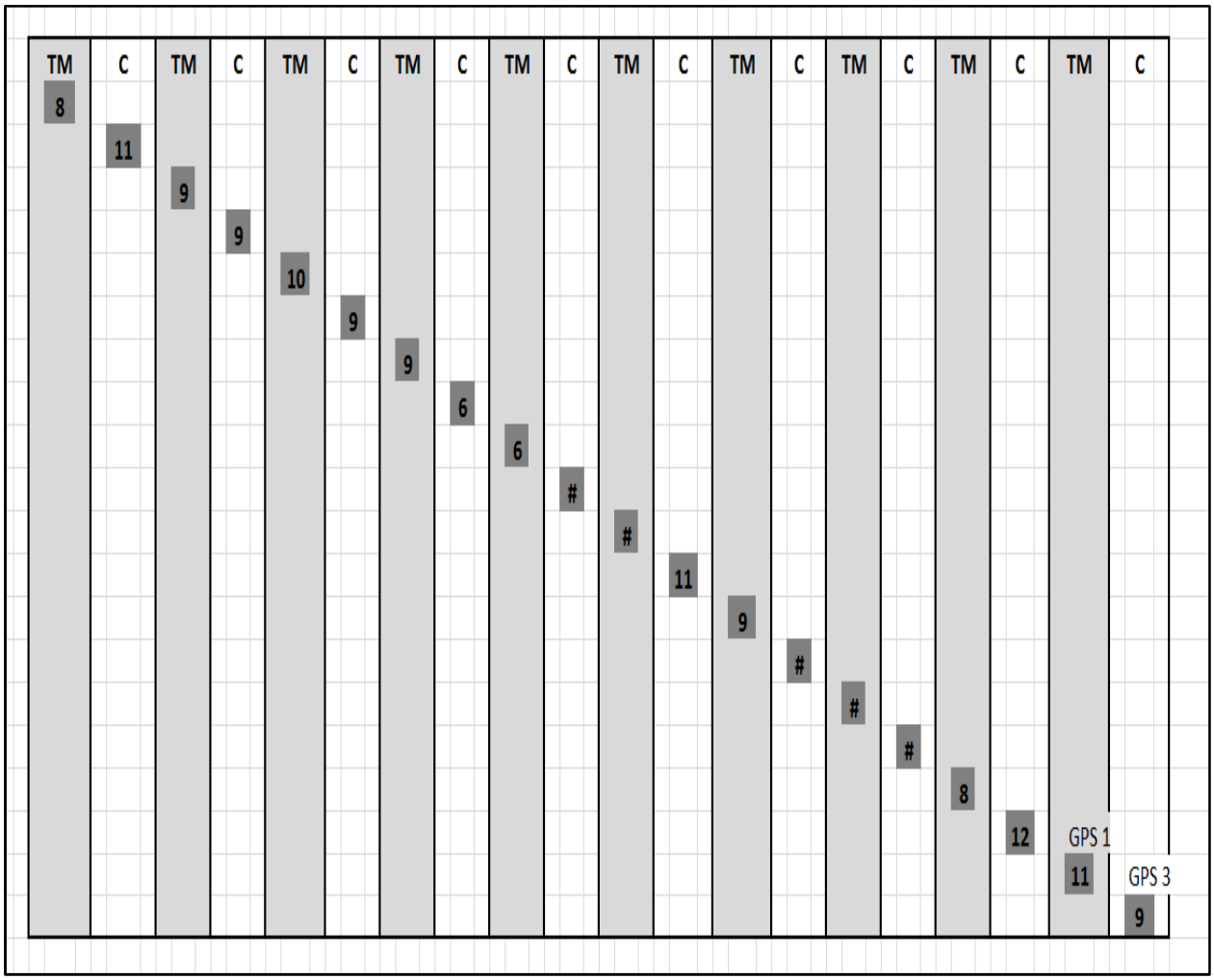


Figure 65. Field strip trial in the Namoi region assessing the efficacy of the TM product.

Table 9. Stand count and average disease severity of black root rot in field strip trial.

	Stand Count	ADS
TM	9.05	2.97
C	9.75	3.11

OBJECTIVE 4: ASSESS WINTER BIOFUMIGATION CROPS FOR THE POTENTIAL TO SUPPRESS BLACK ROOT ROT AND VERTICILLIUM WILT

Milestone 4.1 Assess winter biofumigation crops for the potential to suppress black root rot and Verticillium wilt

2013/2014
Results of the stand counts, average disease severity of black root rot and biomass cuts taken in early December.

Table 10. Effect of biofumigation crops on average disease severity of black root rot and incidence of Verticillium wilt

	Biofumigation crop (t/ha) after 91 days	Cotton (plants/m)	Dry weight cotton biomass (g/plant)	Average disease severity	% Incidence Verticillium Wilt* de-transformed data	Average *V. dahliae* ppg soil **no data
Biofum blend	5.014[a]	5.118[a]	0.7656[a]	4.833[a]	0.404 [a]	153.33
Fodder radish	4.662[a]	4.926[a]	0.7282[a]	4.810[a]	0.054 [a]	226.67
Vetch		5.338[a]	0.6749[b]	5.543[b]	0.816 [a]	213.33
Fallow		5.195[a]	0.6030[b]	5.664[b]	0.321 [a]	**
P value	**P=0.109**	**P=0.630**	**P<0.001**	**P<0.001**	**P=0.402**	

Figure 66. Biofumigation crops being mulched

There was no significant difference in t/ha produced between the two biofumigation treatments. The biomass produced for the biofumigation blend growth was 55kg/day and the Fodder radish growth was 51 kg/day. There was no significant difference in plant stand counts per metre between treatments. There was no detrimental effect on cotton establishment or growth when planted six weeks after incorporation of the biofumigation crops. Cotton biomass (grams per plant) was significantly higher following the two biofumigation treatments compared to the vetch and fallow. The average disease severity of black root rot rated on a scale of 0-10 was significantly lower in cotton seedlings following the two biofumigation treatments compared to the vetch and fallow. NOTE this is statistically significant; however it may not be biologically significant.

Figure 67. Biofumigation crops being mulched/incorporated and pressed before watering

There was very poor growth of the biofumigation crops in winter 2014, a consequence of less rainfall and less water on hand to irrigate the sacrificial crops (Table 11).

Table 11. Biomass results in 2014

	Biofumigation crop (t/ha) after 96 days
Biofum blend	1.1389[a]
Fodder radish	1.3051[a]
Vetch	
Fallow	
P value	**P=0.380**

Although there were promising results last season with a significantly lower (but not biologically significant) difference in average disease incidence of black root rot, this trail did not proceed. In a year when there was little in crop rain and less water available to irrigate there was a lower biomass produced from the biofumigation crops.

The beneficial effect of biofumigation blends in a cotton rotation is determined by the biomass or yield of the crop, concentration of the glucosinolates in the cover crop, the effectiveness of mulching, incorporating and sealing the topsoil and the fumigation process itself. Adequate moisture is needed to grow the biomass in yields high enough to have an effect, as well as timely application of irrigation after incorporation to activate the biofumigation process. Biofumigation crops are not a feasible rotation in times of water shortage or on farms with very long irrigation runs.

Milestone 4.2 Maintain Verticillium wilt nursery in Old 2 Field

4.2.1 Old 2 Field Nursery

This field was redeveloped and laser levelled to rectify drainage problems throughout the field. Cotton was planted early October each season to provide a suitable host for the pathogen. The high weed load in this field was being targeted with discussions/meeting with farm staff on a regular basis to best manage the reduction of each species present. A new drop box and pipe work was purchased and put into place.

Last season the Nutgrass was particularly bad and probably due to the soil disturbance from the laser levelling. Every effort was made to control this weed and others through timely applications of herbicide and cultivations where appropriate.

OBJECTIVE 5: IMPROVE AUSTRALIA'S PREPAREDNESS FOR INCURSIONS OF EXOTIC BACTERIAL BLIGHT STRAINS

Milestone 5.1 Blight differential lines screened to confirm varietal reaction to blight inoculum

The seed obtained from the differential lines imported and grown in quarantine were divided in half and stored in separate locations (one batch with NSW DPI and the other with CSIRO). The division was to reduce the risk of storage failure and loss of differential lines. Vials of bacterial blight that had been stored in the fridge at ACRI for many years were sent to Elizabeth MacArthur Agricultural Institute (EMAI) to assess viability and confirm race. Some of the blight cultures failed to germinate.

EMAI bacteriologist Dr Toni Chapman obtained and had many blight cultures and has been working in collaboration with the NSW pathology team and CSIRO Dr Ian Wilson. CSIRO are currently growing the differential lines and testing methods of inoculating with various blight strains. This is very important as the integrity of the differential lines imported were not guaranteed from USDA as Dr Peggy Thaxton had retired and no-one assumed responsibility for maintaining the blight differential lines.

The validity of the differential cotton lines for screening different races of bacterial blight is currently being tested through the collaborative effort of NSW DPI pathology, EMAI and Dr Ian Wilson in Canberra. The initial results showed discrepancy with expected reactions, in particular from Acala 44 which is supposed to be susceptible to all races but recorded some resistant reactions. Dr Wilson is currently repeating the testing of the differential lines using the toothpick method as outlined in the National Diagnostic Protocol.

Milestone 5.2 Develop framework and commence draft contingency plan for hyper virulent bacterial blight

The draft bacterial blight contingency plan has been supplied to CRDC.

OBJECTIVE 6: BUILD HUMAN CAPACITY IN COTTON PATHOLOGY
Milestone 6.1 Professional development of pathology staff

Dr Karen Kirkby

- Presented at the Association of Australian Cotton Scientist Conference held in Narrabri 2013 and in Toowoomba 2015
- Presentations FUSCOM meetings in Toowoomba in 2013 and 2014
- Presentation to NSW DPI Board of Management 2015
- Presentation to Chinese delegation visit 2016
- Invited judge at the 2013, 2014, 2015 and 2016 Science and Engineering Investigation Awards (PICSE Cotton)
- Presented lectures and ran practical laboratory sessions for the UNE Cotton Production Course 2014 and 2015
- Applied for and was granted a CRDC Sustaining Rural Communities Bursary Project "Careers by Kids for Kids" in collaboration with CSIRO cotton education officer Trudy Staines in 2014
- Presentation to 18 PhD students at ACRI, organised by PICSE in 2016
- Presentation to the Crop Consultants Australia Seminar in 2016
- Presentation to the Lower Namoi Cotton Grower Association in 2016
- Presentation to the Landmark Agronomist meetings in Gunnedah in 2016
- Presentation to the Pursehouse Rural Growers meetings in Gunnedah in 2016
- Participated in Narrabri Public School Science Day, St Xavier's School Science Day as well as hosting students from Wee Waa High School and Calrossy
- Training in Molecular Biology at EMAI in 2015 and 2016
- Presentation at the Murrumbidgee Rural Studies Centre in 2014
- Presented at Biosecurity workshops at Hillston and Griffith 2015
- Presentation to Probus tours 2014 and 2015
- Completed Certificate IV in Training and Assessment (Tocal) 2015
- Reviewed the 3rd Cotton Compendium in collaboration with Dr Jason Woodward Associate Professor, Texas Tech University, Lubbock Texas in 2015
- Personally sponsored and organised Suicide Prevention Skills Workshop (Wincott/Farmlink) in 2016
- Attended and obtained Certificate of Accreditation in Mental Health First Aid Course in 2015
- Attended the NSW DPI Annual Diagnostic Workshop in Canberra in 2016

Peter Lonergan

- Attended the International Fusarium Laboratory Workshop 2013
- Presentations at FUSCOM Meetings in Toowoomba in 2013, 2014, and 2016
- Invited judge at the Science and Engineering Investigation Awards (PICSE) in 2016
- Helped run practical laboratory sessions for the UNE Cotton Production Course in 2014, 2015 and 2016
- Presented at the Association of Australian Cotton Scientist Conference in Toowoomba in 2015
- Presented at the Landmark Agronomist meeting in Gunnedah in 2016
- Participated in the Narrabri Public School Science Day, St Xavier's School Science Day, Small School's Science Day, Gunnedah South School Science Day as well as hosting students from Wee Waa High School and Calrossy
- Training in Molecular Biology at EMAI in 2016
- Supported and participated in "Careers by Kids for Kids" project funded by CRDC Sustaining Rural Communities Bursary in 2014
- Attended and obtained Certificate of Accreditation in Mental Health First Aid Course in 2015

Sharlene Roser

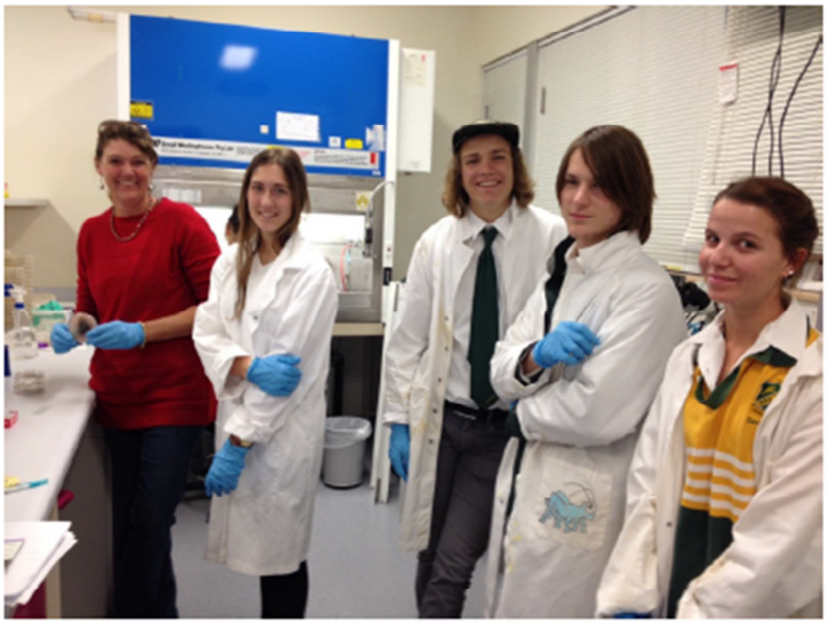
- Presentations at FUSCOM meetings in Toowoomba in 2013, 2014 and 2016
- Helped run practical laboratory sessions for the UNE Cotton Production Course in 2014, 2015 and 2016
- Presented at the Association of Australian Cotton Scientist Conference in Toowoomba in 2015
- Presented at the Landmark Agronomist meeting in Gunnedah in 2016
- Participated in the Narrabri Public School Science Day, St Xavier's School Science Day, Small School's Science Day, Gunnedah South School Science Day as well as hosting students from Wee Waa High School and Calrossy
- Training in Molecular Biology at EMAI in 2015 and 2016
- Training in Fusarium identification at Ecoscience Precinct Brisbane in 2016
- Supported and participated in "Careers by Kids for Kids" project funded by CRDC Sustaining Rural Communities Bursary in 2014
- Successfully completed Certificate III in Laboratory Skills Course in 2014 (TAFE/UNE)
- Successfully completed Off Road 4WD Training in 2014
- Successfully completed AQF III Chemical Application Accreditation in 2014
- Attended and obtained Certificate of Accreditation in Mental Health First Aid Course in 2015
- Attended Suicide Prevention Skills Workshop 2016
- Trained in pathogen isolation techniques, microscopic examination/identification of various pathogens

COURSE participants learn how to identify and distinguish different cotton diseases with the help of NSW Biosecurity DPI cotton pathologist Dr Karen Kirkby.

BIOLOGY EXCURSION

Year 12 Biology students accompanied Mrs Grellman to the CSIRO plant pathology department at the ACRI at Myall Vale to complete a Biology experiment on plant diseases. There were two main aims for the outing: firstly, to complete a case study of a particular plant pathogen and learn industry standard inoculation techniques; and secondly, to experience the plant pathology industry, to meet scientists, talk about careers and use CSIRO's sophisticated and expensive equipment.

Students were fortunate to have Dr Karen Kirkby prepare a case study of the Thielaviopsis basicola pathogen which causes black root rot in cotton and carrots. Students were able to prepare slides to view under microscopes and view the pathogen in a $20,000 dissecting microscope. They were also able to observe culturing techniques in an industry setting. Overall it was a very productive outing, as students were able to observe the pathogen, look at strategies for farm management and the impact of this disease on cotton plants and the industry. We would like to thank Karen for hosting our students and acknowledge the huge advantage our students have when utilizing school and industry links.



In photo, L-R Dr Karen Kirkby (Plant pathologist), Izabela Gligorevic, Ryan O'Neill, Ben Lavis and Georga Cruckshank

Milestone 6.2 Collaboration between organisations

NSW DPI Pathology collaborated with researchers from within DPI, CSIRO, DAFQ, CSD, as well as students, growers and consultants.

Students

- Scholarship students (Horizon/PICSE/CSIRO) - Kate Lumber (2015), Rebekah Watson (2014), Hannah Morris (2014), Johanna Nielsen (2015), Melissa McAllister (2016), Mark Watkins (2016), Candy Taylor (2014), Sarah Hain (2016)
- Work experience students (2013-2016) - Ben Delaney, Joel Samuelson, Katarnie Toomey, Liam Parker, Cassie Hicks
- Primary School Science Days - Narrabri Public School, Narrabri St Xavier's, Gunnedah South Public School, Small School's Science Day
- Hosted School Groups Wee Waa High School (2013, 2014, 2015, 2016), Calrossy Anglican School (2014, 2016) and Farrer Boys School (2016)
- Sarah Cooper UNE - Co-supervisor PhD student - Thielaviopsis basicola genetic diversity
- Trudy Stains - Community action fund for Sustaining Rural Communities Project

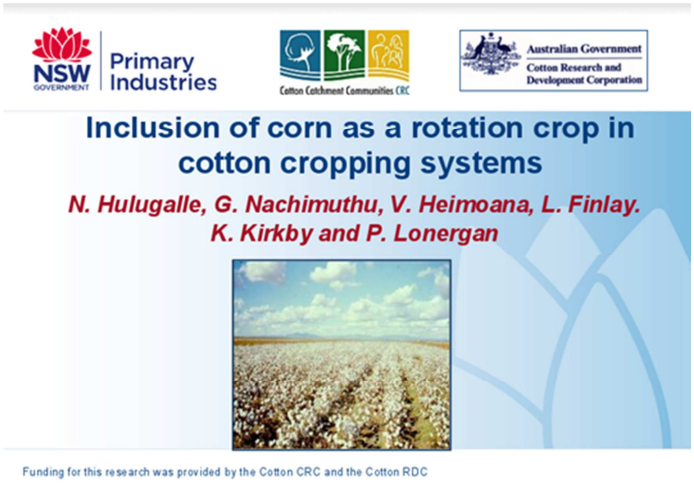
Growers and Consultants

- Mal McFarland
- Paul Swanbra
- Peter Dampney
- Geoff Hunter
- Cotton Grower Services
- Craig Chapman
- Kathy Heirtl
- Andrew Watson Boggabri
- Brendon Warnock
- Luke Findley
- Kaylx and Cotton Info representatives

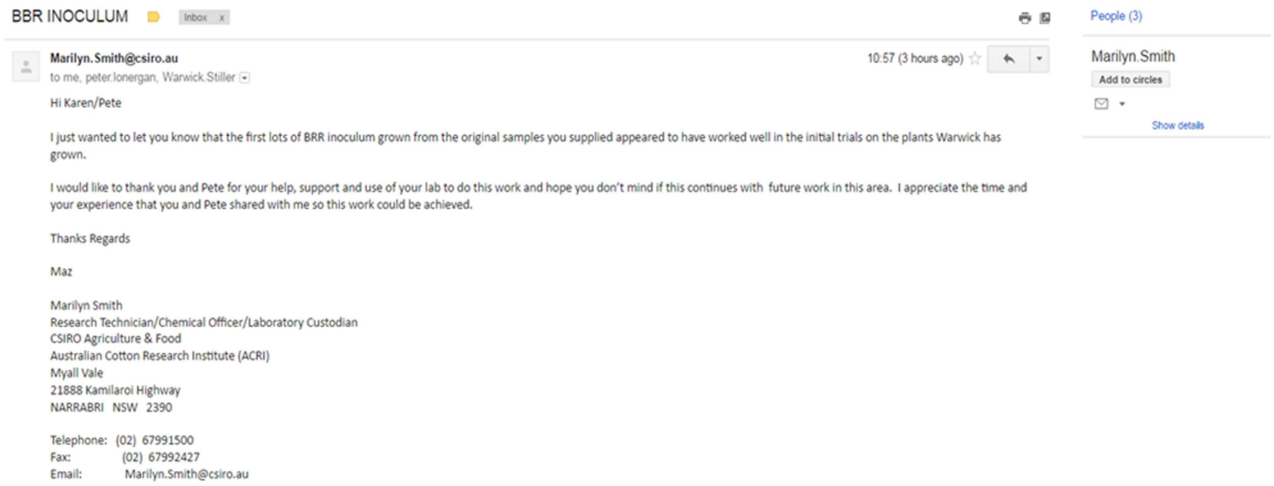
Researchers

- Dr Stephen Allen - Laboratory work including plant isolations, subculturing, help with any laboratory experiment, making media, stock solutions, inoculum as well as microscopic work. Field, glasshouse and growth room experiments and trials including planting, potting up, assessing plants, helping with box trials, travelling with Dr Allen to look at numerous farms and fields. Helping write articles, reviewing papers and collaborating with him on scientific papers.
- Dr Toni Chapman and Grant Chambers - A genuine collaboration commenced in 2014/2015 season with Dr Chapman and Grant Chambers (EMAI) working on Verticillium dahliae resulting in the first report of VCG1A in Australian cotton.
- Dr Gupta Vadakattu - Enquiries about Rhizosphere samples, collected soil and samples from Biofumigation trial site and sent to Gupta Vadakattu and Linda Scheikowski for microbial diversity analysis under biofumigation trial crops) in 2014, 2015 and 2016.
- Dr Robert Mensah - Seed treatment trials which involved treating seed, planting seed, assessing seedlings, entering data, compiling data and writing a report. Laboratory work was undertaken for Robert which involved isolating, subculturing, cleaning up cultures, making media, providing clean cultures to store for future use. Pathology also trained his staff on how to isolate, subculture, make liquid inoculums and how to wash fungal plates. As well as storing all of his cultures in our cool room.

- Dr Nilantha Hulugalle and Dr Guna Nachimuthu - Assessing fields at ACRI for black root rot as well as taking samples and isolating from them for confirmation, determining biomass and providing analysed results.



- Dr Andrew Watson - black root rot and Sclerotinia enquiries in southern cotton growing regions
- CSIRO - Supplying bacterial blight differential cotton lines (seeds) and seed treatment trials in 2014, 2015 and 2016
- Dr Linda Smith - *Fusarium oxysporum* fsp. *vasinfectum* identification and sending plant samples and culture plates for VCG analysis as well as supplying treated seed for seed treatment trials in QLD.
- Dr Warwick Stiller - Providing Thielaviopsis basicola cultures and assisting his staff in identification of BRR using microscope as well as use of the Pathology laboratory to isolate, subculture and incubate.


BBR INOCULUM Inbox x

Marilyn.Smith@csiro.au 10:57 (3 hours ago)
to me, peter.lonergan, Warwick.Stiller

Hi Karen/Pete

I just wanted to let you know that the first lots of BRR inoculum grown from the original samples you supplied appeared to have worked well in the initial trials on the plants Warwick has grown.

I would like to thank you and Pete for your help, support and use of your lab to do this work and hope you don't mind if this continues with future work in this area. I appreciate the time and your experience that you and Pete shared with me so this work could be achieved.

Thanks Regards

Maz

Marilyn Smith
Research Technician/Chemical Officer/Laboratory Custodian
CSIRO Agriculture & Food
Australian Cotton Research Institute (ACRI)
Myall Vale
21888 Kamilaroi Highway
NARRABRI NSW 2390

Telephone: (02) 67991500
Fax: (02) 67992427
Email: Marilyn.Smith@csiro.au

People (3)
Marilyn.Smith
Add to circles
Show details

- Reviewed the 3rd Cotton Compendium in collaboration with Dr Jason Woodward, Associate Professor, Texas Tech University, Lubbock Texas in 2015.

Outcomes

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

The Diseases of Cotton XI project planned outcomes:

The biannual cotton disease surveys quantified the relative importance of disease threats and ensured adequate responses were made by the cotton industry, communities and government agencies. The biannual cotton disease surveys are evidence of industry commitment to its obligations under the Emergency Plant Pest Response Deed. Black root rot and Verticillium wilt are current threats to the sustainable cotton production in NSW. Sclerotinia is an emerging threat to the southern cotton industry.

Annual seed treatment trials in NSW indicated that fungicide efficacy and seedling disease pressure fluctuated between fields and between seasons. Environmental conditions had a substantial impact on the efficacy of fungicides and there is potential for seedling pathogens to become tolerant of commercial fungicides. The independent assessment of seed treatment completed across the cotton growing regions annually provided greater understanding of the factors contributing to cotton seedling mortality.

The industry has improved preparedness for an incursion of exotic diseases through increasing the NSW DPI pathology teams' expertise in identification of many Fusarium species including exotic races, increasing the differential lines imported into Australia to screen for hyper virulent bacterial blight strains.

Expected papers to be published from Sarah Cooper's PhD with results of genetic diversity of black root rot isolates collected from various geographic locations within NSW.

6. 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.

Conclusion

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

Objective 1 Respond to industry issues as they arise annually

Milestone 1.1 Maintain PathWAY communication tool

A total of 229 disease enquiries were reported through PathWay from 2013 to 2016. The Pathology team liaised with CottonInfo Development and Delivery team on relevant fact sheets and field days.

Objective 2: continue annual surveillance of endemic and exotic plant pathogens on commercial cotton farms in NSW production areas, monitoring incidence and severity of diseases of cotton and recording the absence of exotic diseases

Milestone 2.1 Early and late season surveys

As a direct result of the cotton disease surveys, the exotic strain VCG1A *V. dahliae* was diagnosed and secondary confirmation established from Spain. On the 15th April 2015 - Industry mail out containing co-branded information sheet with instructions on how to take and send samples of suspected infected plants. VCG1A was recorded in a 30 year old sample as well as 2014/2015 season samples therefore eradication was deemed unfeasible. No other exotic diseases were recorded.

Milestone 2.2 Investigate long term data for potential interaction between incidence of black root rot and Verticillium wilt

Statistical analysis of the long term data found no strong correlation between high incidence of black root rot and a high incidence of Verticillium wilt in any year from 2001 to 2016.

Milestone 2.3 Increase/maintain long term storage of culture collection.

Over the years fungal isolates have been stored in the NSW DPI historical culture collection. These were clean cultures, however they were not single spore cultures. There are 522 isolates in the culture collection and not all have been single spored.

Milestone 2.4 Improve knowledge of diversity within black root rot isolates

Laboratory culture collection experiments

Morphology and growth rates of *Thielaviopsis basicola* isolates from different valleys.

There was a significant difference recorded in growth rates for colony colour but no significant difference of growth rate between isolates collected from different valleys.

Genetic diversity of *Thielaviopsis basicola* isolates examined by Sarah Cooper (UNE PhD student).

No differences in the ITS sequences from 24 different *T. basicola* isolates collected from different geographical locations.

Growth room experiments

Black root rot inoculum across permanent bed and effect on disease severity and growth

There was no significant difference in the average disease severity of black root rot across the plant line or in plant height when grown in soil collected from across the permanent bed.

Milestone 2.5 Improve knowledge of diversity within *Verticillium dahliae* isolates
No significant difference in inoculum levels across permanent beds. There were significantly (59%) more propagules per gram (ppg) recorded in the permanent beds compared to in-furrows. Soil depth was a significant factor with 71% more ppg in the top 10cm of soil profile compared to 11-20cm. There was no significant difference in ppg at either depth taken from in-furrow.

These findings have particular relevance to the cotton industry. Precision farming places the germinating seedlings directly above the inoculum in the soil. Good bed preparation is vital. It is important to take care to avoid any mechanical damage to the roots as damage will provide an entry point for pathogens. Management of nutrition is also important given the majority of the inoculum is in the top 10cm of the soil profile. Nutrition should be managed to encourage deep root development. Given inoculum was recorded in-furrow it is important to reduce irrigation runoff to minimise moving the pathogen around the farm in tail water.

Verticillium farm case studies
Farm F – Effect of irrigation source on ppg

There was no significant difference in ppg in soil following two water source treatments.

Farm F – Effect of depth on *Verticillium dahliae* ppg

There were 77% more ppg in the top 0-10cm compared to 11-20cm in the permanent beds.

Compost - the use of compost gin trash on fields
The results of both isolation studies in 1998 and 2015 confirm the need to compost the gin trash thoroughly in order to eliminate the carryover of the pathogens causing disease.

Farm M – Effect of rotation crops on *Verticillium dahliae* inoculum levels in field

The inclusion of rotation crops in this field had agronomic and economic benefits. Long term rotations with non-host crops such as wheat and sorghum assisted in the significant reduction of *V. dahliae* inoculum in the soil. The addition of sorghum into the rotation contributed to improved soil stability and another means of income.

Farm F – Effect of management practices on inoculum levels
No significant difference in Verticillium inoculum levels in the soil between the three treatments at each of the two farm sites. The *V. dahliae* pathogen is able to survive in finer residue such as petioles, leaves and bracts. Raking the trash only served to spread the pathogen further around the field.

Farm F - Quantitative assessment of Verticillium wilt symptoms in plants growing in soil of different inoculum levels
There was no difference in disease symptom expression at 355 ppg, 204 ppg or 141 ppg. Further studies using lower ppg of each VCG1A, VCG2A and VCG4B strains will be done in the new project.

Objective 3 Independent and impartial evaluation of existing and novel seed treatments for seedling disease and black root rot

Milestone 3.1 Independent and impartial evaluation of existing and novel seed treatments for seedling disease and black root rot

The industry standard seed treatment remains the most effective treatment.

Objective 4. Assess winter biofumigation crops for the potential to suppress black root rot and Verticillium wilt

Milestone 4.1 Assess winter biofumigation crops for the potential to suppress black root rot and Verticillium wilt

The beneficial effect of biofumigation blends in a cotton rotation is determined by the biomass or yield of the crop, concentration of the glucosinolates in the cover crop, the effectiveness of mulching, incorporating and sealing the topsoil and the fumigation process itself. Adequate moisture is needed to grow the biomass in yields high enough to have an effect, as well as timely application of irrigation after incorporation to activate the biofumigation process. Biofumigation crops are not a feasible rotation in times of water shortage or on farms with very long irrigation runs.

Objective 5: improve Australia's preparedness for incursions of exotic bacterial blight strains

Milestone 5.1 Blight differential lines screened to confirm varietal reaction to blight inoculum

The integrity of the differential cotton lines for screening different races of bacterial blight is currently being tested through the collaborative effort of NSW DPI pathology, EMAI and Dr Ian Wilson in Canberra.

Milestone 5.2 Develop framework and commence draft contingency plan for hyper virulent bacterial blight

The draft bacterial blight contingency plan has been supplied to CRDC.

Objective 6: Build human capacity in cotton pathology

Milestone 6.1 Professional development of pathology staff

The diagnostic capacity of the NSW DPI Pathology team has been increased due to the training and laboratory exchanges during this project.

Extension Opportunities

8. Detail a plan for the activities or other steps that may be taken:
(a) to further develop or to exploit the project technology.
(b) for the future presentation and dissemination of the project outcomes.
(c) for future research.

Areas of future research include developing a molecular tool to quantify Verticillium inoculum and analyse VCG in cotton soils. Also further research into inoculum levels at depth will be carried out by Dr Karen Kirkby in the new project. Additional work will also be done on quantifying the minimum level of Verticillium inoculum (VCG 1A, 2A and 4B) needed to cause disease symptoms in cotton.

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)

Kirkby, K., Lonergan, P., Cooper, B., Roser, S., Smith, L., Scheikowski, L., Bauer, B., Lehane, J., & Allen, S. (2013). Cotton Pathology Survey 2012-13. *Cotton Pest Management Guide 2013-14* (pp. 126-130).

Kirkby, K., & Anderson, C. (2013). Vascular wilt disease findings. *Primefact*. Retrieved from http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0009/472914/Vascular-wilt-disease-findings.pdf

Kirkby, K. A., Allen, S. J., & Lonergan, P. A. (2013). Three decades of cotton disease surveys in NSW, Australia. *Crop and Pasture Science*, *64*(8), 774-779.

Smith, L. J., Scheikowski, L. J., Bauer, B., Lehane, J., Kirkby, K. A., Lonergan, P. A., Cooper, B. R., Roser, S. E., & Allen, S., J. (2014). Cotton Pathology Survey 2014-15. *Cotton Pest Management Guide 2014-15* (pp. 122-126).

Allen, S., Smith, L., Scheikowski, L., Gambley, C., Sharman, M., Kirkby, K., & Lonergan, P. (2014). Integrated Disease Management *Cotton Pest Management Guide 2014-15* (pp. 116-117).

Allen, S., Smith, L., Scheikowski, L., Gambley, C., Sharman, M., Maas, S., Kirkby, K., & Lonergan, P. (2014). Common diseases of cotton *Cotton Pest Management Guide 2014/15* (pp. 118-121).

Moore, J. R., Pratley, J. E., Malone, R., O'Keeffe, K., & Kirkby, K. A. (2015). *Mycorrhizal status in the rotation: the importance to subsequent cotton establishment.* Paper presented at the 17th Australian Society of Agronomy Conference, Hobart.

Chapman, T. A., Chambers, G. A., Kirkby, K., & Jiménez-Díaz, R. M. (2016). First report of the presence of *Verticillium dahliae* VCG1A in Australia. *Australasian Plant Disease Notes*, *11*(1), 1-4. doi:10.1007/s13314-016-0197-2

Hulugalle, N., Nachimuthu, G., Heimoana, V., Finlay, L., Powell, J., Kirkby, K., & Lonergan, P. (2015). Inclusion of corn as a rotation in cotton cropping systems. Paper presented at the ASSI Canberra Meeting

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

Kirkby, K., & Anderson, C. (2013). Progression of Fusarium Wilt in Plants. *Primefact*. Retrieved from http://www.dpi.nsw.gov.au/__data/assets/pdf_file/0007/472903/Progression-of-fusarium-wilt-in-plants.pdf

Kirkby, K. and C. Anderson (2013) Vascular wilt disease findings. Primefact
Retrieved from http://www.dpi.nsw.gov.au/__data/assets/

Part 4 – Final Report Executive Summary

This final report provides an evaluation of laboratory, pot and field trials conducted in the project Diseases of Cotton XI between 2013 and 2016.

The aims of the project were to: quantify the disease enquiries received through Pathway; document the distribution, incidence and severity of disease throughout NSW and carry out surveillance for exotic diseases; collect and maintain a culture collection; investigate the pathogens that cause both black root rot and Verticillium wilt in terms of growth rate, inoculum levels and genetic diversity amongst isolates collected from infected plants from different geographic regions and assess the efficacy of composting gin trash. The project also assessed the efficacy of various management practices including seed treatment, crop rotation with non-hosts and biofumigation crops on inoculum levels in soil.

The author Dr Karen Kirkby would like to acknowledge and thank Dr Steven Harden, NSW DPI biometrician for his assistance in designing and analysing experimental data.

Over the course of the project there were 229 disease enquiries reported through Pathway. Seasonal weather conditions and pathogen inoculum levels played an important role in the distribution, incidence and severity of diseases recorded in the NSW biannual disease surveys. In general, Fusarium wilt remains generally low in NSW; however confirmed diagnosis of Fusarium continued to be reported in new fields and also on new farms. Verticillium wilt is a major concern with a new non-defoliating strain of VCG 2A and the defoliating strain VCG1A being reported in recent years by NSW and QLD pathologists. Reports of Verticillium severity increasing was highlighted during the last few years with yield losses of several bales to the acre being recorded where severe Verticillium patches were present. Black root rot has been highly variable between valleys and seasons. The incidence of Bunchy top and Sclerotinia was also variable. The 2015/2016 season saw high levels of spray drift across the state.

Long term disease data collected between 2001 and 2016 was used to investigate the potential for an interaction between high incidence of black root rot and Verticillium wilt. No interaction was found between incidence levels, however results of pot experiments with dual infection showed a significant increase in the severity of black root rot in young seedlings.

An experiment carried out by Sarah Cooper' during her PhD found no significant difference in the genetic diversity of black root rot isolates collected from different geographic regions. A separate laboratory study found no significant difference in the growth rates of black root rot isolates from different geographic locations. Results of black root rot inoculum levels in both field and pot trials showed there was no significant difference in inoculum levels across the raised bed lines.

Two replicated field trials quantifying where Verticillium inoculum was located found 71% and 77% of the inoculum was concentrated in the top 0-10cm of the soil profile. Also there was no significant difference in inoculum levels across the plant bed. The level of Verticillium inoculum needed to induce disease symptoms was assessed using pot experiments and found that 141 propagules per gram (ppg) of soil was adequate to cause disease symptoms. Lower levels of inoculum still need to be assessed to determine the minimum level.

Management strategies when used alone had varying effects on disease. Seed treatment trials were carried out across NSW valleys and found that the standard industry seed treatment remained the most effective against disease compared to novel products tested.

Time of planting had a significant effect on plant survival. Planting later in mid-October compared to mid-September increased seedling survival and decreased the incidence and severity of diseases like black root rot, *Rhizoctonia* and *Pythium*. The option of planting later when soil temperatures are warmer is not a luxury the southern valley cotton growers have.

Composting gin trash is still a concern if the trash is not composted thoroughly. *Rhizoctonia* and Verticillium pathogens were detected in composted gin trash in a study carried out in 2015; a repeat of an experiment Dr Stephen Allen had conducted in 1998 with similar results.

A field trial with the rotation crops wheat and sorghum showed a significant reduction in Verticillium inoculum levels in the soil after three years. The practice of raking and burning stubble was re-evaluated and was found to have no significant effect on reducing soil inoculum levels.

Biofumigation crops were assessed for efficacy in reducing disease and the results were variable. A limiting factor with biofumigation crops was the availability of water, ability to mulch and irrigate in a timely matter in order to get a biofumigation effect.

During this project the NSW DPI pathology team has built capacity in their skills through phylogenetic training for intermediate users, Biosecurity annual diagnostic workshops as well as laboratory exchanges between QDAFF and EMAI. The cotton industry's capacity to respond to biosecurity incursions has been strengthened by a Hyper virulent bacterial blight contingency plan being drafted and submitted to CRDC. Project outputs were:

- 2 journal articles
- 29 presentations were delivered by the NSW DPI pathology staff at scientific forums, conferences, field days, site visits and grower meetings
- 2 ABC radio interviews discussing the NSW cotton disease surveys
- 5 co-authored Cotton Pest Management Guide articles, 2 DPI Prime Fact sheets as well as contributions to the Cotton Grower Magazine, Spotlight and CottonInfo Fact sheets

Key outcomes included:

- No significant difference in the genetic diversity of the pathogen that causes black root rot
- Identifying the presence of the defoliating strain of *Verticillium dahliae* in Australian cotton
- Better understanding of where Verticillium inoculum is within the soil profile
- Industry standard seed treatment remain the most effective treatment for seedlings
- Time of planting has a significant effect on the establishment of seedlings in fields where disease inoculum is present
- Crop rotation with non-hosts such as wheat and sorghum can significantly reduce Verticillium inoculum in soils
- Integrated disease management remains the best strategy for reducing the risks associated with disease in cotton