Australian Government

Cotton Research and Development Corporation

# SUMMER SCHOLARSHIP REPORT: 2014-15 SEASON

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| **1.** | **Project Title**<br>*Thielaviopsis basicola isolates*<br>(Maximum 15 words) | : | Pathogenicity and morphology assays of selected |
| **2.** | **Proposed Start Date**<br>**Proposed Cease Date** | :<br>: | 5 January 2015<br>27 February 2015 |
| **3.** | **Summer Scholar and University** | : | Johanna Nielsen (University of Southern Queensland) |
| **4.** | **Organisation & Location for the project** | : | **NSW DPI, ACRI, 21888 Kamilaroi Highway, Narrabri** |
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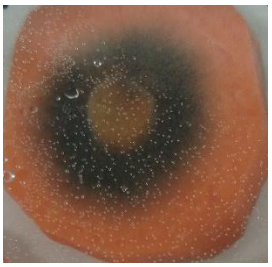
Project Collaborators (Name and Organisation):

# SUMMER SCHOLARSHIP REPORT





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## 1. Executive Summary:

Black root rot, a widespread disease throughout New South Wales, is a seedling disease caused by *Thielaviopsis basicola*. Infection of cotton seedlings by this fungal pathogen symptomized by black, discoloured roots, damages cortical tissue, resulting in slow, stunted growth. Incidence of black root rot is increasing in the Namoi valley. The growth rate and colour of isolates in culture from six major NSW cotton production regions were assessed to determine morphological or behavioural differences between regions. Seedling disease severity, and the biomass weights of infected plants were indicators of isolate pathogenicity. No significant difference was found between or within isolates from varying regions in radial growth rates, colony colour, plant biomass production, or disease severity. It is therefore unlikely that pathogenic or morphological variance is the cause of high incidences of black root rot in the Namoi valley.

## 2. Background:

*Thielaviopsis basicola*, the causitive agent of black root rot, is a soil-borne fungus causing slow, stunted seedling growth. Commercially grown cotton, *Gossypium hirsutum*, is susceptible to infection by *Thielaviopsis basicola*. Distribution of black root rot is widespread and has been detected in all New South Wales cotton production regions, including the Macintyre, Gwydir, Namoi, Barwon, Darling, Macquarie and Lachlan valleys. Black root rot has also been detected in Queensland (Nehl, 2004). *Thielaviopsis basicola* was first detected in Australia during 1942 in a tobacco crop (Simmonds, 1996) and first detected in cotton in 1989. In long-standing cotton growing regions, the Namoi, Gwydir, Macquarie, and Macintyre valleys, black root rot has been detected on almost all farms (Allen, 2001). Incidence of black root rot on surveyed NSW cotton farms during the 2011/12 season was 38.5% (Kirkby, 2013).

Hyphal growth of *Thielaviopsis basicola* primarily affects the root cortex, infecting the plant cell by cell. *Thielaviopsis basicola* infection of cortical cells results in cortical tissue necrosis. Damaged cortical tissue suppresses early season seedling growth. Black discolouration of root systems is the main symptom of black root rot (Allen, 2001). Normal growth resumes at soil temperatures above 24°C and infected cortical cells are sloughed off (King and Presley, 1942). While black root rot is not associated with seedling death in Australia, the slowing of growth results in later maturing crops, or uneven crops. Cotton crops maturing later in the season are exposed to cooler weather, increasing the risk of other yield-reducing diseases.

Early season disease surveys have shown an increase in the severity and incidence of black root rot in the Namoi valley over the last decade. The Namoi valley has the highest incidence of black root rot in NSW cotton regions, however, black root rot is most severe in the Macintyre, followed by the Namoi valley. Studying the morphology and pathogenicity of *Thielaviopsis basicola* isolates from major cotton growing areas, including the Namoi, will determine the role of pathogenicity and morphological differences, if any, in disease severity and incidence.

## 3. Aims and Objectives:

The aims of this project are to:

1. Single spore the 24 Thielaviopsis basicola isolates in the long term storage culture collection
2. Assess morphology of the selected isolates
3. Test pathogenicity of selected isolates

The objective of this project is to determine whether there are morphological or pathological differences contributing to the increased severity and incidence of black root rot of cotton in the Namoi valley. Objectives are related to Farmers: 1.1 Successful crop protection, and will also expand knowledge of *Thielaviopsis basicola* amongst researchers and student.

## 4. Materials and Methods:

*Single Sporing*

Culture vials in long term storage were removed, brought to room temperature and placed into the biohazard safety cabinet. Using a sterilised loop, agar plugs were removed from vials and placed onto carrot juice agar plates. Cultures grown in incubator for 10-14 days before spores were dislodged using distilled water and a glass hockey stick, inoculum was transferred with sterile transfer pipette onto water agar. A hyperdermic needle was used to remove a single chlamydospore and placed onto surface sterilised carrot disks then covered with a 5mm core of carrot stele. Single spored carrots were incubated on moist filter paper in 9cm petri dishes until fungal growth from spore was evident.

*Morphology*

For morphology, isolates subcultured onto TbCEN were incubated at 24*C. For ten days the colour of each fungal growth was assessed and four radial measurements were taken of each colony. These were averaged to obtain an average daily radial growth.

*Testing Pathogenicity*

Innoculum stock of a known level was prepared by dislodging spores from 14 day old culture using a glass hockey stick and distilled water. Spore solutions were sieved through three layers of cheesecloth to remove chlamydospores and debris. Solution was brought to final volume of 300mL and diluted to 1/32 to achieve 175ccf. A soil mixture for each treatment was prepared by adding 5.5kg of twice pasteurised Old 2 soil to 500g of vermiculite and mixed thoroughly in cement mixer innoculum stock. Ten treated cotton seeds were planted in each pot and covered with thin sand layer. Seedlings thinned to five per pot after seven days. Plants grown in temperature controlled growth room for 21 days before assessment. Seedlings washed to remove dirt from roots then root systems assessed on 0-10 scale of discolouration. 0 = 0% root system discolouration, 1 = 1-10, 2 = 11-20, 3 = 21-30, 4 = 31-40, 5 = 41-50, 6 = 51-60, 7 = 61-70, 8 = 71-80, 9 = 81-90 and 10 = 91-100. Wet biomass weights were taken, shoots and roots separately, then dried and weighed to obtain dry biomass weights.

*Data Analysis*

Results were analysed using SPSS data analysis software. Differences were considered significant where $P<0.05$.

## 5. Results:

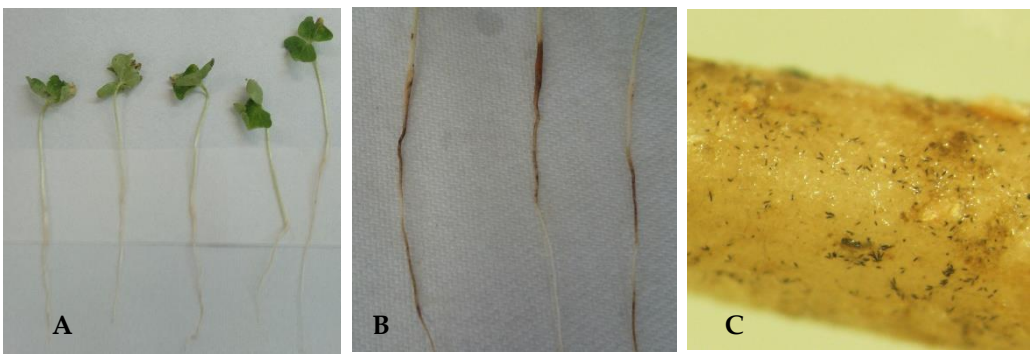
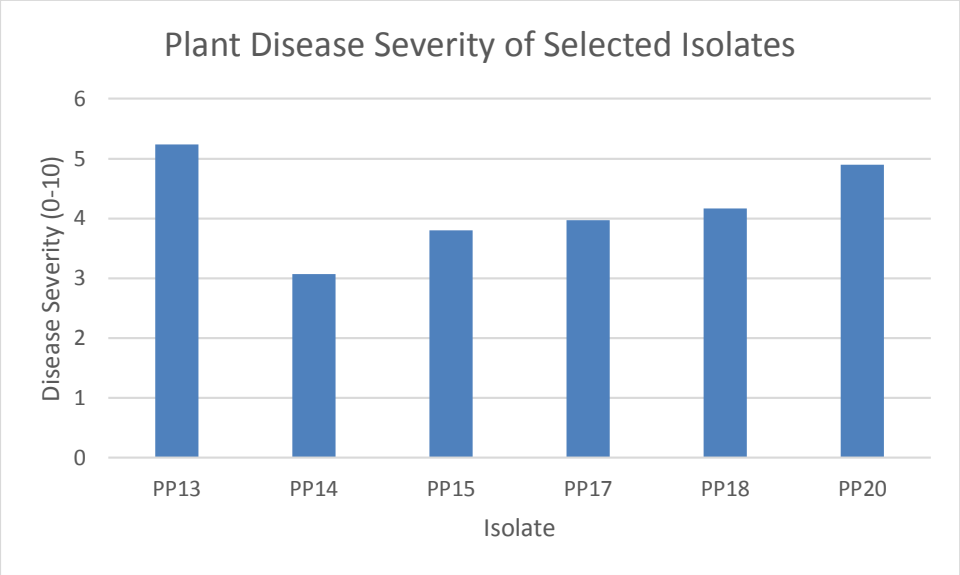
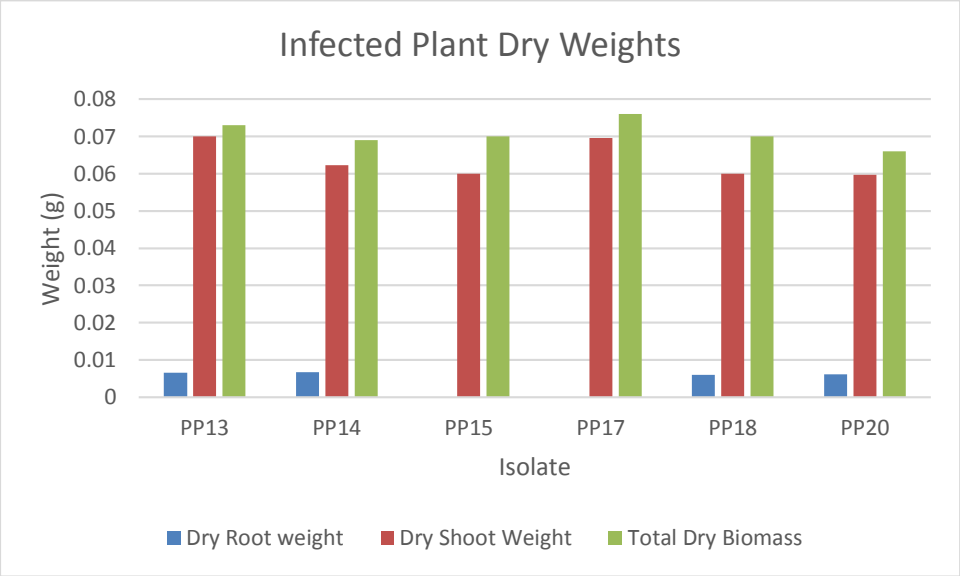



**Fig 1:** Roots of 21 day old cotton seedlings. (A) Control cotton seedlings with no root discolouration. (B) Roots of cotton seedlings grown in PP19 inoculated soil. (C) PP13 spores on seedling root under microscope.



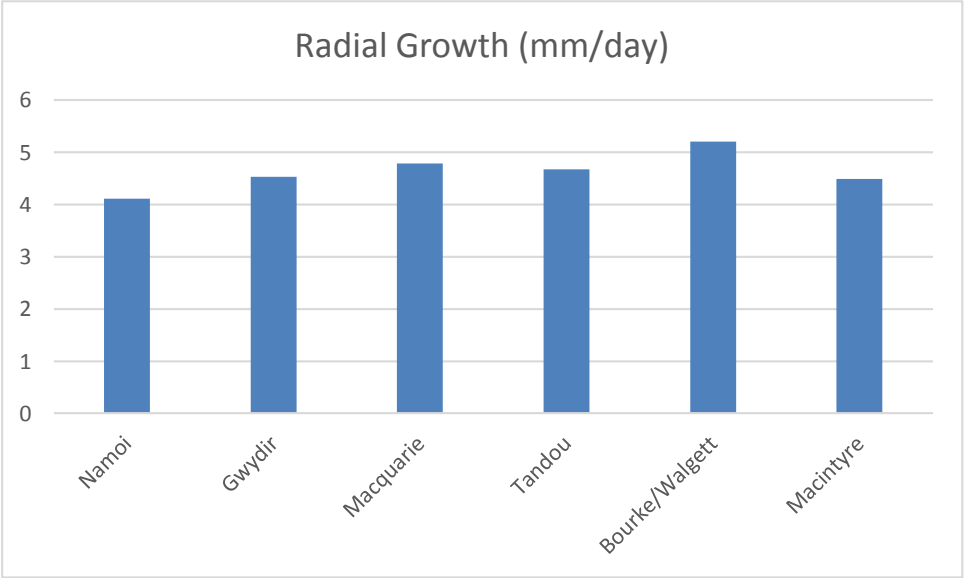

Mean seedling disease severity was higher in Namoi isolates (4.2) than the Macquarie (4.15). Isolates PP13 from the Macquarie region displayed greater variation than Namoi isolates (PP15, 17, 18 and 20). There was no significant difference in disease severity between the six isolates or within regions (P>0.05).

**Fig 2:** Assessed average disease severity of black root rot in cotton seedlings grown in soil inoculated with the selected isolate.




There is no significant difference between the dry biomass weights. The greatest and least total biomass weights were from Namoi isolates (PP17 and PP20 respectively) which had a region mean average of 0.0705g. The Macquarie region dry biomass weight average (0.071) was higher than the Namoi average.

**Fig 3:** Dry shoot, root and total biomass weights of plants grown in soils inoculated with the above specified isolate.




Daily radial growth rates were not significantly different between or within the cultures of the six regions studied. Isolates from the Bourke/Walgett regions had the greatest growth rate (5.21 mm/day) and also the greatest standard deviation (0.83). Namoi region isolates had the lowest average daily radial growth rate (4.11).

**Fig 4:** Daily radial growth averages of cultures grown from the Namoi, Gwydir, Macquarie, Tandou, Bourke and Macintyre valley isolates.

## 6. Discussion and Conclusions:

To ascertain whether there was a correlation between the disease severity and incidence of a region, inoculated plant disease severity and weights were assessed. Culture growth rates and colour were studied in relation to isolate morphology. Surveys carried out during the 2010/11 season showed incidence of black root rot was highest in the Namoi and Macintyre valleys (Kirkby, 2013).

Average disease severity (Fig. 2) was greater in the Namoi (4.2), with less variation between replications. Macquarie isolates showed lesser disease severity (4.15) but the variance between the replications was greater. However there was no significant difference within region results or between regions. Results from 2010/2011 disease surveys where severity was highest in the Macintyre (3.05) and Namoi valleys (2.98) would have predicted black root rot severity to be significantly higher in the Macintyre and Namoi valleys. Black root rot disease severity is positively correlated with inoculum concentration (Holtz and Weinhold, 1994), however due to the long history of cotton cropping in the Namoi valley, disease severity on cotton roots has reached saturation point.

The weights of shoots and roots, or total plant biomass production has been used as an indicator of growth suppression. Had there been a significant difference between plant biomass production this should have correlated with disease severity. Plants inoculated with isolates from the Macquarie region had an average plant biomass weight greater than plants inoculated with isolates from the Namoi region (Fig. 3). However, there was no significant difference.

Daily radial growth averages were assessed for isolates from the Namoi, Macquarie, Macintyre, Gwydir, Bourke and Walgett, and Tandou regions. While there was no significant difference between the daily growth rate between regions, or within region, the Bourke and Walgett region isolates had the greatest daily radial growth rate (Fig. 4). Daily radial growth of the isolates on agar is indicative of the rate in which *Thielaviopsis basicola* would colonise a plant, hyphae infecting cell by cell. As more isolates from the Namoi region were included in this study, assessing additional isolates from other regions would give a more accurate region average. Colony colour results have not been presented in this report as the average colour in both the Namoi and Macquarie isolates was black.

There has been no significant difference in the pathogenicity or morphology of the selected isolates, therefore there is no correlation between black root rot disease incidence in the Namoi valley and pathogenicity of Namoi *Thielaviopsis basicola* isolates. The lack of significant variation between isolates from the same region would suggest that genetic variation in *Thielaviopsis basicola* colonies between regions is unlikely. Further studies will need to be completed but it is not likely that the incidence and severity of black root rot experienced in the Namoi valley is due to pathogenic or morphological variation in *Thielaviopsis basicola*.

## 7. Highlights:

I have immensely enjoyed being a part of this research project, which has been an incredible learning experience. The biggest lesson I learnt was that while experiments may not always give the results you're looking for, there is always so much to learn - as I certainly did. I was particularly glad to see my microbial techniques vastly improve in the eight weeks I was in the pathology laboratory. Thank you to the CRDC and NSW DPI Biosecurity for the funding which enabled me to take part in this project. Dr Karen Kirkby and Peter Lonergan, thank you for your academic support. For technical advice, thank you to Beth Cooper and Sharlene Roser.

## 8. Future Research:

This summer project is part of a larger project, in which all 24 isolates will be single spored, and pathogenicity and morphology assays will be completed. Including more isolates from each region would give a more accurate view of each region, in differences between and within regions. Genotypic differences of these isolates are currently being studied as part of this project. A comparative study of the Schwabe characteristics of these isolates would contribute to the understanding of *Thielaviopsis basicola* differences across Australian cotton growing regions.

## 9. Presentations and Public Relations:

Results from this project will be presented as a poster report at the 2nd Australian Cotton Scientist's Conference (September, 2015). An article will be posted on the University of Southern Queensland's webpage April, 2015.

## 10. Reference List: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