Australian Government
Cotton Research and Development Corporation

CAPITAL ITEM FINAL REPORT

Part 1 - Summary Details

CRDC Project Number: DAQ 1304
Project Title**: Pathogen Identification** Capital item: Nikon SMZ800 Stereo Microscope, fibre optics illuminator set

Project Commencement Date: 1 May 2013 **Project Completion Date:** 30 June 2013

Research Program:

Part 2 – Contact Details

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Signature of Research Provider Representative: _____

(Please return to CRDC with a completed financial statement within 40 Business days from purchase of Capital Item)

Part 2 – Final Report Guide

1. Background

In 2012 I attended a Fusarium laboratory workshop and learnt how to identify different *Fusarium* species. This trip was funded by CRDC. As a result of this trip it was obvious that the very old microscope that I am currently using to view fungal pathogens and identify them is inadequate for observation of the spore structures required to differentiate *Fusarium* species. The microscope I am currently using is a Leitz which is approximately 40 years old. I have been advised by the Leitz service person that once the bulb blows it cannot be replaced as bulbs are no longer made for this model.

The benefits of the capital expenditure include the ability to clearly observe microbial structures such as the foot shape of *Fusarium* macroconidia and number of septa plus the ability to measure spore size, also used for diagnostic purposes, using specialised software. There are many *Fusarium* species that colonise fuzzy cotton seed. In our current project we investigated the efficacy of a fumigant on *Fusarium oxysporum* f. sp. *vasinfectum* and other *Fusarium* species that colonise fuzzy cottonseed. It was incredibly difficult to diagnose the various Fusarium species using the microscope I currently have plus I do not have any means to photograph the fungi for proof of pathogen or for publication purposes which is required if a new pathogen of cotton is determined. Further to this, we may conduct further work investigating the effect of fumigation on fuzzy cottonseed. To undertake this work a new microscope with camera and relevant software is required.

In our new project one of the milestones is to identify fungi that cause boll rot. A second milestone is to investigate *Verticillium dahliae* diversity. Both these areas of research require microscopic observation of the pathogens to examine spore structure plus the ability to measure spore size. One of the differentiating features of exotic defoliating *Verticillium dahliae* is the length to width ratio of the microsclerotia, which differs to endemic *Verticillium dahliae*. I am the designated diagnostic expert on defoliating *Verticillium dahliae* and as such require the appropriate equipment to successfully undertake this important task.

There is also a need for new equipment to successfully identify pathogens of cotton from samples received from researchers, consultants and growers for general diagnostic enquiries.

2. Objectives

To identify pathogens of cotton and to photograph specific structures to support diagnosis and collate data for future reference.

3. Methods

Diseased cotton stems received for pathogen diagnosis

If stems are received and suspected of being infected with either *Fusarium oxysporum* f. sp. *vasinfectum* or *Verticillium dahliae* then a 10cm section of stem is cut and surface sterilised with 70% Ethanol. The stem is cut lengthwise using a sterile scalpel blade and vascular tissue is examined for brown discolouration. If vascular tissue is discoloured, small segments of discoloured tissue are cut from the stem and plated onto ¼ strength PDA plus Streptomycin media plates. Plates are incubated at room temperature and examined regularly for fungal growth. Once fungal growth is visible to the naked eye, the culture plate is examined under the dissecting microscope to determine if fugal growth is Fusarium or Verticillium by looking at the spore type. Verticillium has verticilate conidiophores and microsclerotia while *Fusarium oxysporum* has microconidia in false heads on monophialides.

Two examples are provided here to demonstrate the process by which this capital item has been used for swift pathogen diagnosis. However, more than 40 samples have been received since the 21 November 2013 for general diagnosis requiring the use of this capital item.

Example 1. 29/11/2013 Roots that appeared swollen with lesions at the crown were sent from Gail Spargo, DAFF Qld for diagnosis. Plants were collected from Peter Galea's property in Emerald, Qld. The lesion area was surface sterilised and diseased tissue was plated onto ¼ strength PDA plus Streptomycin media.

Example 2. 3/2/2014 Stem sample received from NSW DPI from Mal McNiven in Moree, NSW. Request to determine if vascular discolouration was caused by Verticillium or Fusarium?

4. Results. Conclusion

Example 1. 29/11/2013 Swollen root with lesions at crown, Peter Galea's property, Emerald, Qld.

Penicillium sp. and Rhizopus were common fungi isolated from the wounded stem tissue. No primary plant pathogens were isolated.

Microscopically, *Penicillium* sp. produce chains of single-celled conidia (ameroconidia) are produced in basipetal succession from a specialized conidiogenous cell called a phialide (Fig. 1). Species of *Penicillium* are ubiquitous soil fungi, commonly present wherever organic material is available.

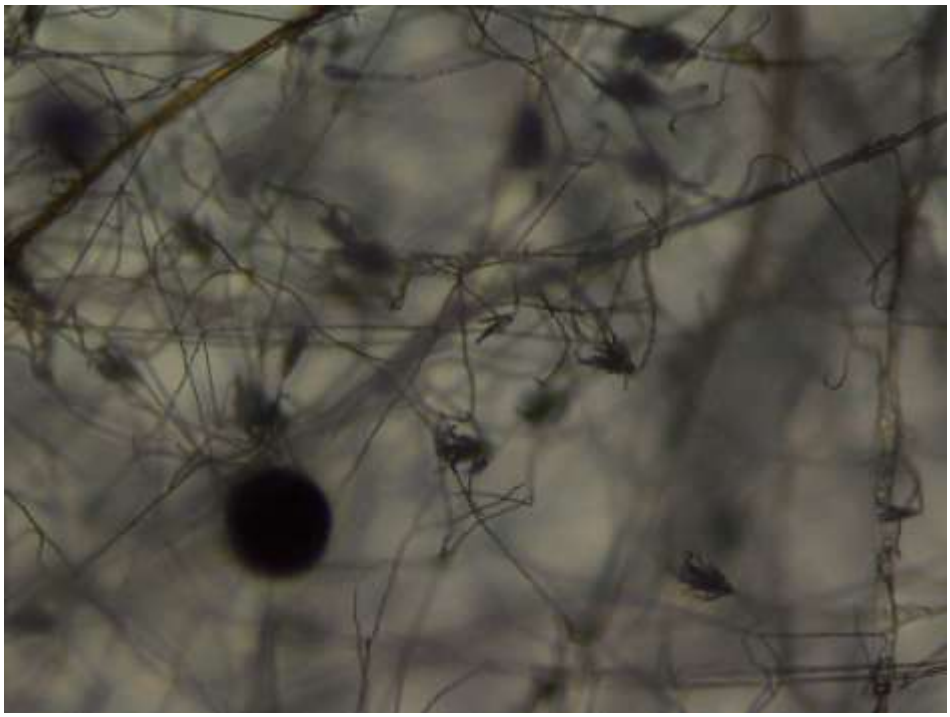

Figure 1. *Penicillium* sp. isolated from a lesion at the crown of a cotton plant

Rhizopus is a genus of common saprophytic fungi on plants. They are found on a wide variety of organic substrates and are commonly isolated from cotton plant tissue as secondary invaders, not cause of disease.

Neither of these fungi would have caused the lesion as they are secondary saprophytic invaders of damaged tissue. Group D herbicides can cause swellings at the base of stems but no herbicides had been used. No primary plant pathogens were isolated. The canker may be a result of the soil cracking open around the cotton stem as the soil was extremely dry. Windy conditions moving the stem against

the abrasive soil may have caused the stem lesions observed which were then invaded by saprophytic soil fungi. These results were communicated to Gail Spargo, Emerald DAFF Qld.

Example 2. 3 Feb 2014 Moree, NSW. Verticillium or Fusarium wilt?

As seen in Figures 2 and 3 the pathogen causing disease was *Verticillium dahliae* as shown by production of verticilate conidiophores and microsclerotia. This result was reported back to Karen Kirkby (NSW DPI) and Stephen Allen (CSD).

Figure 2. Verticilate conidiophores of Verticillium dahliae growing from infected cotton stem

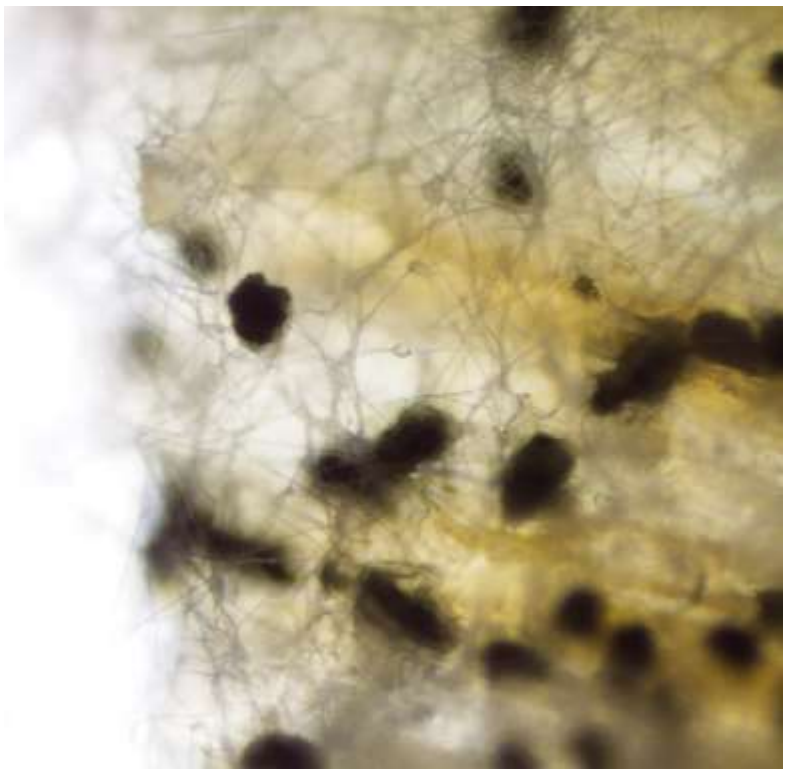

Figure 3. Microsclerotia of verticillium dahliae growing on a piece of infected cotton stem

6. Staff

Linda Smith and Bartley Bauer

7. Extension opportunities

A diagnostic summary of disease survey findings is published annually in the CSD Annual Disease Survey Report.

There could be opportunities to extend new information if new diseases are identified or if endemic diseases are determined to be epidemic or of concern to growers.

8. Publications

No publications at this stage.

Part 3 – Final Report Executive Summary

The objective of purchasing a Nikon stereo microscope was to improve the capability of identifying pathogens of cotton. More than 40 samples for diagnosis have been received at the Ecosciences Precinct laboratory since mid November 2013. The ability to identify pathogens of cotton from diagnostic samples has been greatly improved due to the magnification range of this instrument (5X–378X) and ability to attach a camera to photograph pathogen structures such as the various types of spores produced for use as reference material. Pathogens identified include *Fusarium oxysporum* f. sp. *vasinfectum, Verticillium dahliae, Alternaria macrospora* and *Thielaviopsis basicola*.

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