Australian Government
Cotton Research and Development Corporation

FINAL REPORT 2013

Part 1 - Summary Details

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

CRDC Project Number: **CRC1002A**

Project Title: Diseases of Cotton X

Project Commencement Date: 2009 **Project Completion Date:** 2013

CRDC Program: Crop Protection

Part 2 – Contact Details

Administrator: Dr Cara Brooks
Organisation: NSW DPI
Postal Address: Locked Bag 21, Orange, NSW, 2800
Ph: 0263 913651 **Fax:** **E-mail:** cara.brooks@dpi.nsw.gov.au

Principal Researcher: Dr Karen Kirkby
Organisation: NSW DPI
Postal Address: ACRI, Locked Bag 1000, Narrabri, NSW, 2390
Ph: 0267 992454 **Fax:** 0267 991503 **E-mail:** karen.kirkby@dpi.nsw.gov.au

Supervisor: Dr Leigh Pilkington
Organisation: NSW DPI
Postal Address: Private Bag 4008, Narellan, NSW, 2567
Ph: 0246 406333 **Fax:** **E-mail:** leigh.pilkington@dpi.nsw.gov.au

Signature of Research Provider Representative:

Part 3 – Final Report

Acknowledgements
The principal researcher extends sincere thanks to Peter Lonergan, Bethany Cooper and Sharlene Roser who have each provided invaluable technical assistance across all aspects of the project. Your efforts made achieving the project milestones possible.

The principle researcher thanks all of the grower co-operators whose assistance and collaboration made this project possible. The principle researcher also extends gratitude to Cotton Catchment Communities CRC, Cotton Research and Development Corporation and Cotton Australia, who provided substantial investment in the professional development of the principle researcher, and in the project.

Background

1. Outline the background to the project.

Three of the most economically damaging and recalcitrant diseases affecting cotton in New South Wales are Fusarium Wilt, Verticillium Wilt and black root rot. Soil-borne diseases are difficult to treat with traditional methods and for practical purposes are impossible to eradicate. However by gaining a better understanding of the pathogens ecology or life cycle, management practices can be modified in an economically and environmentally sustainable way to reduce disease impact.

Previous work has indicated that the soil type cotton is grown in has an impact on the disease expression of these three fungi. Soil borne fungal plant pathogens can overwinter as either saprophytes or is dormant structures such as chlamydospores and sclerotia. Having these mechanisms means the fungi are difficult to control and virtually impossible to eradicate. All three fungi are thought to be able to survive for long periods, for example *Verticillium dahliae* has been reported to survive for at least 10 years as micro-sclerotia. Survival however is mitigated by number of environmental factors such as soil type. There is little information available on the survival of these fungi in different soil types grown under cotton. The proposed research would look at the survival of these pathogens in different soil types. This fundamental information on the ecology of the pathogens is vital for making informed management decisions on crop rotation.

The increase in the price of fertilisers as well as a growing understanding for the need of environmentally sustainable agriculture has meant a growing interest in the use of nitrogen fixing crop species for crop rotation with cotton. It is one of the aims of this proposed research to investigate the potential of alternative crop plant species.

Crop rotation has been shown to affect the chemistry, physics and biology of the soil. Indeed crop rotation is an accepted way of potentially improving soil health and disease suppression. The increase in fertiliser costs, economic and environmental impacts have led to a growing interest in rotation crops such as

legumes that may improve soil fertility. Work is currently under way at Queensland DPI looking at the effect of crop rotation on Fusarium wilt in cotton. However there is little information on the interaction between crops and Verticillium wilt and black root rot.

Objectives

2. List the project objectives and the extent to which these have been achieved.

Tasks	Completion date	% Complete
Objective 1:		
1.1 Conduct 2010/2011 disease surveys in NSW	15/04/2011	100
1.2 Conduct 2011/2012 disease surveys in NSW	15/04/2012	100
1.3 Update disease symptoms image collection	30/05/2013	100
1.4 Conduct 2012/2013 disease surveys in NSW	30/05/2013	100
Objective 2:		
2.1 Conduct 2010/2011 annual seed treatment fungicide trial	15/12/2010	100
2.2 Conduct 2011/2012 annual seed treatment fungicide trial	15/12/2011	100
2.3 Conduct 2012/2013 annual seed treatment fungicide trial	15/12/2012	100
Objective 3:		
3.1 Plant species screened in glasshouse studies for the effect on inoculum levels and disease development in cotton	30/06/2010	100
3.2 Laboratory studies completed of the potential of *Thielaviopsis basicola* chlamydospores to regerminate	30/06/2010	100
3.3 Possible soil amendments screened for the effect on chlamydospore and sclerotia germination completed using glasshouse studies	30/06/2010	100
3.4 If available, soil amendments trialled in the field	30/05/2013	100
Objective 4:		
4.1 Long term enforced fallow experiment continued at ACRI	30/05/2012	100
4.2 Nurse crops investigated to restore mycorrhizal fungi in long fallow situations	30/06/2012	100
Objective 5:		
5.1 Isolates of *V. dahliae*, *T. basicola* and *F. oxysporum vasinfectum* collected. Laboratory assays completed.	30/05/2013	100
5.2 The effects of environmental factors on survival of inoculum assessed in glasshouse studies.	30/05/2012	100
5.3 Investigate black root rot pathogen effects on cotton growing in different soil types	30/04/2013	100
5.4 Re-isolate 1995-2006 cultures stored in long term storage collection.	30/06/2012	100
Objective 6:		
6.1 Identify fields with significant levels of both Verticillium wilt and black root rot	30/05/2013	100

6.2 Development of methods to determine the effects of *V. dahliae* and *T. basicola* alone and when together	30/05/2013	100
Objective 7:		
7.1 Laboratory and glasshouse experiments undertaken to identify the pathogen.	30/05/2012	100
Objective 8:		
8.1 Obtain blight differentials from USA	30/05/2012	100
Objective 9:		
9.1 Respond to industry disease issues as they arise	30/05/2013	100

Methods

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

Objective 1 – Disease Surveys
Early Season Disease Surveys:

Fields from each cotton region (Bourke/Walgett, Macintyre, Gwydir, Namoi, Macquarie, Lachlan and Murrumbidgee) were sampled in November/December each season. A total of 200 plants from each field were sampled using a step-point method across two transects. In summary 100 plants were sampled per transect line from each field. The first sampling is done 50 m into the field at the tail drain end where a GPS coordinate is recorded. The second sampling is done by walking across 10 m and up the row 20 m. This pattern of walking to the right 10m and up the row 20 m is repeated until 10 sampling sites have been covered (Figure 1.7). At the 10 sampling points, 10 plants were carefully removed from soil using an asparagus knife to loosen the soil. Each plant was inspected for black root rot on tap roots and symptoms rated on a scale of 1 to 10, along with the presence of Rhizoctonia, Pythium and exotic diseases.

At each sampling point, the number of plants present along 1m of the row was recorded to calculate seedling mortality rates. Seedling mortality was calculated as the percentage of established plants relative to the number of seeds planted.

Figure 1.7 Disease survey transect used in disease surveys. 100 plants are surveyed along each transect with two transects per field. (Image C. Anderson, 2010).

Late Season Disease Surveys:

Fields from each cotton region (Bourke/Walgett, Macintyre, Gwydir, Namoi, Macquarie, Lachlan and Murrumbidgee) were sampled in March/April each season. Transect sampling was similar to that described for early season surveys, however stems were cut near the base of 10 plants and assessed for symptoms of Verticillium wilt, Fusarium wilt, boll rot, Bunchy top, herbicide/fertilizer damage and exotic disease. Confirmation of pathogens was made using laboratory isolation techniques on selective media.

Objective 2 – Continue to evaluate existing and novel fungicide treatments for seedling disease and black root rot.

Seedling diseases 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*, *Rhizoctonia*, black root rot, and *Fusarium* causing seed rot and/or pre and post- emergent damping off (collapse and death of seedling). Cotton plants in the early stages of growth are more susceptible to seedling disease as cells in the hypocotyl and tap root continue to thicken. As seedlings mature (after two-leaf stage) plants become more resistant, particularly to *Rhizoctonia* and *Pythium*, except under wet and very cool conditions (Nehl, Allen, Mondal, & Lonergan, 2004a).

Each year NSW DPI evaluates the effectiveness of seed treatment fungicides and combinations against seedling disease. The industry standard fungicide seed treatment for cotton for many years was a mixture of Terraclor® (Quintozene- PCNB) (active against *Rhizoctonia*) and Metalaxyl-M (Apron®) (active against *Pythium* spp.). Following the release of Dynasty® a new fungicide seed treatment in 2005/2006 from Syngenta®, registered to protect seeds, roots and emerging seedlings. Dynasty® replaced the historical industry standard. Metalaxyl-M remains a component of

Dynasty®. PCNB was included in the 2010/2011, 2011/2012 and 2012/2013 seed treatment trials as a measure of *Rhizoctonia* pressure.

Materials and Method

The 2010/2011 and 2011/2012 trials each had a total of 20 treatments (19 seed treatments and a control of black seed) using Sicot 71BRF on all sites except Mungindi where conventional Sicot 71 was used. Details of seed treatments and product formulation for 2010/2011 season are detailed in (Table 2.1 and Table 2.2), for 2011/12 (Table 2.3 and Table 2.4) and for 2012/2013 (Table 2.5 and Table 2.6).

In 2010/11 field sites were located at ACRI Narrabri, Hillston, Warren, Mungindi and QLD (Downs). At the ACRI Narrabri site Sicot 71BRF was sown at 100 seeds per 13m plots over two planting dates i.e. early (17/09/10) and normal (13/10/10). At Hillston and Warren seeds were sown (25 to 26/9/10 and 11/10/10 respectively) at 120 seeds per 14m plots. At Mungindi Sicot 71 was sown (30/09/10) at 120 seeds per 14m plots. The Downs was planted on (29/10/10) with 71BRF at 152 seeds per 10 m plots. The number of replicates for each site in 2010/11 was: ACRI Narrabri (8), Hillston (11), Warren (10), Mungindi (14) and Downs (6). At the Downs site a total of 16 treatments were used. Treatments included all in listed in Table 2.1.1 except the Untreated + Cruiser, Bion, Dynasty + Bion and Dynasty Plus.

In 2011/12 field sites were located at ACRI Narrabri, Breeza, Hillston, Warren, Mungindi and Downs. At the ACRI Narrabri site Sicot 71BRF was sown into moisture at 100 seeds per 12m plots over 2 planting dates i.e. early (07/09/11) and normal (12/10/11). Planting of Sicot 71 BRF was done at Breeza (13/10/11) into moisture, Hillston (4/10/11) into dry and Warren (12/10/11) into moisture at 120 seeds per 14m plots. At Mungindi Sicot 71 was sown (24/09/11) into moisture at 120 seeds per 14m plots. Planting date for the Downs site was (25/10/11) using 71BRF at 152 seeds per 10 m plot. In 2011/12 season the number of replicates for each site was: ACRI Narrabri (8), Breeza (4), Hillston (6), Warren (6), Mungindi (8) and Downs (6).

In 2012/2013 field sites were at ACRI Narrabri, Hillston, Warren, Mungindi and the Downs QLD. The trails at ACRI were sown with 74BRF on the 14th September 2012 into moisture and again on the 15th October 2012 which was watered up. The trial at Hillston was planted on 26th September and at Warren on the 25th September. At Mungindi Sicot 730 was planted 5th October. Planting date for the Downs site was 31st October 2012 using 152 seeds per 10 m plot using 74BRF. The number of replicates for each site was: ACRI Narrabri (8), Hillston (12), Warren (4), Mungindi (12) and Downs (6).

Each season, intra-field variation in pathogen populations was accounted for by using completely randomised block designs at each site. Surviving plants were counted at 3 and 6 weeks after sowing at ACRI Narrabri site and after 6 weeks at the remaining sites. Data was analysed using GenStat, 11th Edition, REML spatial modelling.

Table 2.1 Seed Treatments used in seasonal trials

Treatment
1. Untreated
2. Quintozene(PCNB) (Terraclor®)
3. Fludioxonil + Metalaxyl + Azoxystrobin (Dynasty® Cotton Seed Treatment)
4. Dynasty® CST + SYN524 (Dynasty® Plus)
5. Fludioxonil (Maxim® 100FS) – component of Dynasty® CST
6. Metalaxyl-M (Apron® XL 350ES) – component of Dynasty® CST
7. Azoxystrobin (Dynasty® 100FS) – component of Dynasty® CST
8. SYN524 – experimental fungicide Syngenta®
9. Fludioxonil + Metalaxyl-M
10. Fludioxonil + Azoxystrobin
11. Fludioxonil + SYN524
12. Metalaxyl-M + Azoxystrobin
13. Metalaxyl-M + SYN524
14. Azoxystrobin + SYN524
15. Untreated + Thiamethoxam (Cruiser® 600FS)
16. Trifloxystrobin (DC-094) (Rate 1) – experimental fungicide Bayer CropScience
17. Trifloxystrobin (DC-094) (Rate 2)
18. Trifloxystrobin (DC-094) (Rate 3)
19. 1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester (Bion® Plant Activator)
20. Fludioxonil + Metalaxyl + Azoxystrobin + 1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester (Dynasty® CST + Bion® Plant Activator)

Table 2.2 Product formulation, active ingredients and recommended rates

Source	Formulation (proprietary names)	Active Ingredient	Recommended Rate (product)
Syngenta®	Dynasty® Cotton Seed Treatment	Metalaxyl-M 37 g/L Fludioxonil 12 g/L Azoxystrobin 75 g/L	2.00 mL/kg seed
Syngenta®	Apron® XL 350ES	Metalaxyl-M 350 g/L	0.43 mL/kg seed
Syngenta®	Dynasty® 100FS	Azoxystrobin 100 g/L	1.50 mL/kg seed
Syngenta®	Maxim® 100FS	Fludioxonil 100 g/L	0.25 mL/kg seed
Syngenta®	Syn524464 100 FS B-var	Syn-508210 + Syn-508211 100 g/L	0.5g/kg seed
Chemtura Agrosolutions™	Terraclor®	Quintozene(PCNB) 500 g/L	2.15 mL/kg seed
Syngenta®	Cruiser® 600FS	Thiamethoxam 600 g/L	4.60 mL/kg seed
Syngenta®	Bion® Plant Activator	1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester 500 g/L	0.012g/kg seed
Bayer CropScience	DC-094	Trifloxystrobin 13.3%w/w	0.325 mL/kg seed 0.650 mL/kg seed 0.975 mL/kg seed

Table 2.3 Seed Treatments used in 2011-2012 season trial

Treatment
1. Untreated
2. Quintozene(PCNB) (Terraclor®)
3. Fludioxonil + Metalaxyl + Azoxystrobin + 1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester (Dynasty® Complete)
4. 1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester (Bion® Plant Activator) – component of Dynasty® Complete
5. Fludioxonil (Maxim® 100FS) – component of Dynasty® Complete
6. Metalaxyl-M (Apron® XL 350ES) – component of Dynasty® Complete
7. Azoxystrobin (Dynasty® 100FS) – component of Dynasty® Complete
8. Fludioxonil + Metalaxyl-M
9. Fludioxonil + Azoxystrobin
10. Fludioxonil + 1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester
11. Metalaxyl-M + Azoxystrobin
12. Metalaxyl-M + 1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester
13. Azoxystrobin + 1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester
14. Untreated + Thiamethoxam (Cruiser® 600FS)
15. Trifloxystrobin (DC-094) (Rate 1) – experimental fungicide Bayer CropScience
16. Trifloxystrobin (DC-094) (Rate 1) + Metalaxyl-M
17. Trifloxystrobin (DC-094) (Rate 2)
18. Trifloxystrobin (DC-094) (Rate 2) + Metalaxyl-M
19. Trifloxystrobin (DC-094) (Rate 3)
20. Trifloxystrobin (DC-094) (Rate 3) + Metalaxyl-M

Table 2.4 Product formulation, active ingredients and recommended rates

Source	Formulation (proprietary names)	Active Ingredient	Recommended Rate (product)
Syngenta®	Dynasty® Complete	Metalaxyl-M 37 g/L Fludioxonil 12 g/L Azoxystrobin 75 g/L	2.00 mL/kg seed
		1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester 500 g/L	0.012g/kg seed
Syngenta®	Apron® XL 350ES	Metalaxyl-M 350 g/L	0.43 mL/kg seed
Syngenta®	Dynasty® 100FS	Azoxystrobin 100 g/L	1.50 mL/kg seed
Syngenta®	Maxim®100FS	Fludioxonil 100 g/L	0.25 mL/kg seed
Chemtura Agrosolutions™	Terraclor®	Quintozene(PCNB) 500 g/L	2.15 mL/kg seed
Syngenta®	Cruiser® 600FS	Thiamethoxam 600 g/L	4.60 mL/kg seed
Syngenta®	Bion® Plant Activator	1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester 500 g/L	0.012g/kg seed
Bayer CropScience	DC-094	Trifloxystrobin 13.3%w/w	0.325 mL/kg seed 0.650 mL/kg seed 0.975 mL/kg seed

Table 2.5 Seed Treatments used in 2012-2013 season trial

Treatment
1. Untreated
2. Quintozene(PCNB) (Terraclor®)
3. Fludioxonil + Metalaxyl + Azoxystrobin + 1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester (Dynasty® Complete)
4. 1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester (Bion® Plant Activator) – component of Dynasty® Complete
5. Fludioxonil (Maxim® 100FS) – component of Dynasty® Complete
6. Metalaxyl-M (Apron® XL 350ES) – component of Dynasty® Complete
7. Azoxystrobin (Dynasty® 100FS) – component of Dynasty® Complete
8. Fludioxonil + Metalaxyl-M
9. Fludioxonil + Azoxystrobin
10. Fludioxonil + 1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester
11. Metalaxyl-M + Azoxystrobin
12. Metalaxyl-M + 1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester
13. Azoxystrobin + 1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester
14. Untreated + Thiamethoxam (Cruiser® 600FS)

Table 2.6 Product formulation, active ingredients and recommended rates

Source	Formulation (proprietary names)	Active Ingredient	Recommended Rate (product)
Syngenta®	Dynasty® Complete	Metalaxyl-M 37 g/L Fludioxonil 12 g/L Azoxystrobin 75 g/L 1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester 500 g/L	2.00 mL/kg seed 0.012g/kg seed
Syngenta®	Apron® XL 350ES	Metalaxyl-M 350 g/L	0.43 mL/kg seed
Syngenta®	Dynasty® 100FS	Azoxystrobin 100 g/L	1.50 mL/kg seed
Syngenta®	Maxim®100FS	Fludioxonil 100 g/L	0.25 mL/kg seed
Chemtura Agrosolutions™	Terraclor®	Quintozene(PCNB) 500 g/L	2.15 mL/kg seed
Syngenta®	Cruiser® 600FS	Thiamethoxam 600 g/L	4.60 mL/kg seed
Syngenta®	Bion® Plant Activator	1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester 500 g/L	0.012g/kg seed

Objective 3 – Continue to evaluate IDM strategies for the control of black root rot including crop rotation and soil amendments.

Soil amendments trialled in the field.

Biofumigation Trial:

In a completely randomized plot design, 4 treatments were replicated 6 times. The trial covered 96 rows, consisting of 24 plots of 8 rows. Two transects were taken from each plot, one at the head ditch end and the other at the tail ditch end. The trial was divide into tiers with plots 1 to 12 in tier one and 13 to 24 in tier two (Figure 3.4.1). In summer 2008/2009 sunflower was sown across the entire trial to establish an approximately uniform starting point of black root rot inoculum.

In February 2009, the sunflowers were turned in and left to fallow. On the 4th May 2009, a total of 192 soil cores were taken using a 5cm diameter down pipe. For each sampling position 4 x 4cm cores were taken from rows 3, 4, 5 and 6 in the 0-15 cm region of each bed top. The 4 cores were merged into one sample. Soil cores from the 4 rows were blended at both head and tail ditch ends giving 48 samples in which the black root rot inoculum levels were quantified.

On the 5th May 2009, Ag-Muster Canola, Tyrone Chickpeas, Namoi Vetch were planted across the trial including plots of bare fallow in a randomized design. Thereafter, 48 soil samples from the 24 plots were analysed each season. Samples from plots 1 to 12 (tier one) were taken 15 meters from the head ditch and the second transect taken 15 meters toward the head ditch from field mid point maker. Samples from plots 13 to 24 (tier two) were taken 15 meters toward the tail drain from field mid point maker and the second transect taken 15 meters from the tail drain.

Subsamples of the soil from the cores were split and used in the selective medium TbCEN to quantify *T. basicola* chlamydospore numbers in the soil using the method described by Specht and Griffin (1985) and Chittaranjan and Punja (1993). Reagents and concentrations used are listed in Table 3.4.1 In short, 10 g of sub-sampled soil was suspended in 0.1% distilled water agar (DWA), providing a 1 in 10 soil dilution. Using a mechanical shaker, the samples were shaken vigorously for approximately 10 minutes.

One mL of soil suspension was pipetted into each Petri plate (90mm), after which 25 mL of molten medium was dispensed into each Petri dish using sterile tubing attached to a peristaltic pump, using a swirling motion to mix soil and medium whilst dispensing. Five plates were used for each soil collection and 5 containing no soil as controls. Plates were incubated at 23^{o}C under continuous darkness and inspected after 7 to 10 days for the presence of colonies growing on the medium. Counts were adjusted to colony forming units (CFU) per gram of soil by multiplying by the dilution factor of 10.

Inconsistent enumeration of black root rot inoculum levels in soil using the TbCEN assays and discussions with international pathologists, it was decided that relative

comparisons of infectivity of soils would be estimated using the carrot disc method described by Honess (1994) for soil samples taken after 2011. Although the carrot disc method does not accurately quantify inoculum levels in soil it does provide conservative estimates for comparison. In short carrots, approximately 20-30 mm in diameter were prepared first by transversely slicing into discs of equal width (5-6 mm thick), then surface sterilizing and rinsing in sterile water. Discs were then stored in sterile water for up to 30 minutes before use. Five wells were made in the cortical tissue of each carrot disc using a 6mm cork borer. Care was taken not to bore all the way through the disc. There were 5 carrot discs per plate with 4-6 replicates. From the shaken 1 in 10 soil dilutions per treatment, 0.1ml of soil was placed in each well of the carrot discs. Pathogen populations can be expressed on a weight or volume basis known as colony forming units (CFU) per gram of soil or CFU/cm^3 of soil. Estimated population per cm^3 of *T. basicola* is calculated as the average number colonies per plate multiplied by the dilution factor.

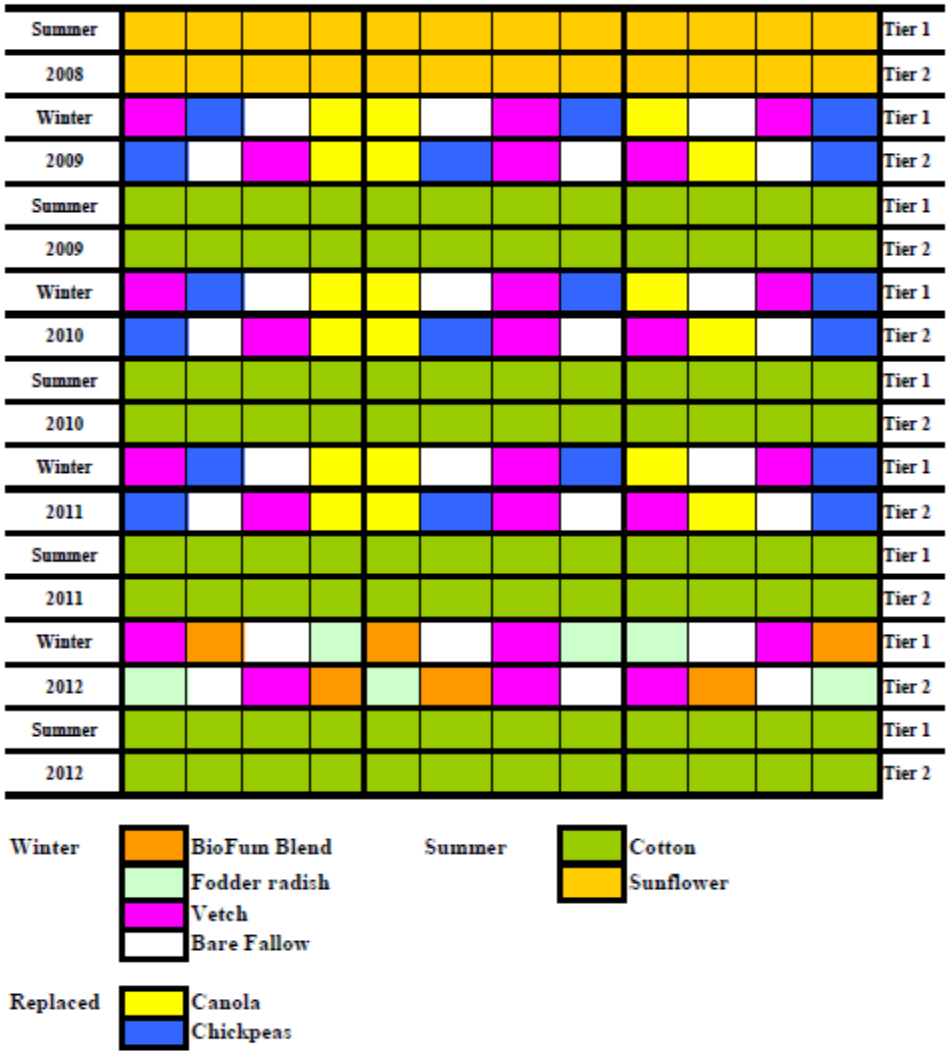


Figure 3.4.1 Experimental design of the long term biofumigation trial at ACRI, Narrabri, NSW.

Table 3.4.1 Reagents used in TB-CEN semi-selective medium for determining soil populations of *T. basicola* in soil collected from ACRI Field 4.

Reagent	Concentration
Agar (commercial grade)	1.50%
Calcium carbonate (CACO3)	1.0g/L
Streptomycin sulphate	500mg/L
Penicillin G potassium salt	100,000U/L
Chlortetracycline hydrochloride	50 mg/L
Nystatin	125,000 U/L
Etridiazol (added as Terrazole 35 WP)	400 mg/L
Carrot extract (100%)	100 mL/L

The remainder of the soil was used in glasshouse and growth room pot experiments to establish the effect of treatments on severity of black root rot on cotton. For each pot experiment, a completely randomized design was conducted in a controlled environment to examine the effects of each treatment on Black root rot. Grapevine tubes were lined with patty cake liners and filled with soil collected from each treatment. Into these, 12 Sicot 43 BRF seeds were placed on level soil surface and covered with vermiculite and sand mix (1:1 ratio). Tubes were watered from below using small individual take away containers under each tube. To ensure the seed being used was viable, germination tests were carried out by placing 300 Sicot 43 BRF seeds on wet paper towel (100 on each paper towel) and rolled into a tube, placed into a plastic container then into an incubator set at 22^{O}C, no light. After 10 days, seed viability (90.3%) was calculated as the average percentage of seeds that had germinated.

Severity of disease was established twenty one days after emergence using the method described by (D. B. Nehl, Allen, Mondal, & Lonergan, 2004b), with the roots of up to 10 seedlings per treatment being rated for disease severity on a 0 to10 scale (as described earlier). Roots were cut from the plant at the soil line and placed into envelopes, along with the aboveground shoot material. Shoots and roots were placed into the dehydrator for 48 hours, before obtaining dry weights. Samples of root were then placed into Petri dishes and covered with distilled water. The presence or absence of chlamydospore chains of *T. basicola* were established by observing the root material with a dissecting microscope.

In summer 2009/2010, cotton was planted across the whole trial. Stand counts and severity of black root rot disease were determined approximately 6-8 weeks after germination. Stand counts were calculated as the average number of plants established in 1 meter transects in each tier in each treatment. After disease severity was rated, roots were then cut from the plant at the soil line and placed into envelopes, along with the aboveground shoot material. Shoots and roots were placed into the dehydrator for 48 hours, before obtaining dry weights. Cotton

growth was maintained, cut out early, harvested and the trial site prepared for biofumigation crops to be planted in winter.

No soil cores were taken (Winter 2010) due to staff changes and wet field conditions. On the 24/05/2010 Namoi Vetch, Tyrone Chickpea was planted across the trial. Then on the 25/05/2010 Ag-Muster Canola was planted across the trial. The trial came up on rainfall. Weeds were a problem across all the plots. Due to mixed cropping, wet conditions and fertilizer placement less biomass was produced by all the crops. Biofumigation crops were turned in and beds prepared for cotton to be planted in summer 2010.

In summer 2010/2011, cotton was planted across the whole trial and stand counts and black root rot severity determined as described earlier. Defoliant was applied to the cotton 25th March 2011. Cotton was picked then the stubble slashed. The trial was cultivated and biofumigation crops were planted the end of April. After irrigation, high weed load and imperfect watering up meant the trial was sprayed out the second week of May and treatments were replanted 8th June. Biomass was cut on the 16th September 2011 before the crops were slashed and turned in on the same day. Data from the biomass cuts was recorded (dry matter converted from grams per plant to tonnes per hectare). Where possible soil samples were collected from each plot and used to grow plants in the glasshouse and growth room to assess disease severity and biomass growth. In summer 2011/2012, cotton was planted across all plots and stand counts and b lack root rot severity determined as described earlier.

After the summer 2011/2012 cotton crop, the treatments were altered in winter 2012 from canola, chickpea, vetch and fallow to biofum blend (40% Doublet fodder radish, 50% Carinata Brassica and 10% Achilies White Mustard), Doublet fodder radish, vetch and fallow. The vetch was planted 21/5/12 prior to rain on the 25/5/12. The biofum blend and radish were planted 1/6/12. Good rain followed in June, July and August with a total of 126.8 mm up to 25/8/12. Aboveground biomass cuts were taken from 2 meter transects randomly selected within each plot. Biomass was weighed and then dehydrated. From this the dry mass was calculated as tonnes per hectare. The biofumigation crops were slashed and turned in on the 30/8/12 with moisture in the soil. Cotton was planted across all treatment plots on 15/10/12.

Growth room pot experiment: (4 soil samples / plot/tier / treatments / replicate = 192 pots). The glasshouse experiment commenced 17/9/12 and was used to determine the base line levels of inoculum in the soil within each treatment as indicated by rating disease severity on seedling roots as described earlier.

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 dry weight and root dry weight

(where applicable). Statistical significance was assessed and reported at the 5% probability level. For pot experiments, when the residual graphs indicated, data was analysed using logit.

Rotation Trial:

The long term rotation trial was set up in Old 2, ACRI in the winter of 2010. This long term rotational trial assessed crop plant species for rotation that may reduce disease development. The experimental design consists of 6 treatments (canola, chickpea, faba bean, oats, wheat and winter fallow/summer cotton), replicated 5 times in a completely randomised design. The trial covered 240 rows (30 plots of 8 rows) (Figure 3.4.5). Soil samples were taken from plots before treatments (rotations) were imposed, with no significant difference (P=0.850) between plots for black root rot inoculum levels in the soil. Chickpea, canola, faba bean and oats were planted at the end of May 2010 and bare plots worked the first week of September 2010 in preparation for cotton being planted in October 2010. Due to weed issues the chickpea and faba bean plots were removed in September 2010. Soil samples after rotations were taken to be used in pot experiments to evaluate incidence and severity of black root rot.

After planting, plants were assessed both in the field and in soil collected from the field and then grown in the glasshouse. Field assessments included the average disease severity of black root rot, incidence of Verticillium wilt, plant numbers and shoot dry weight. Data collected from plants grown in the glasshouse included average disease severity of black root rot, shoot and root dry weight.

Summer 2010 cotton was planted in the plots that had previously been fallow. All other plots remained fallow during winter. Summer 2011/2012 a buffer of sunflower was planted in the western 16 rows and cotton (variety 43RRF) was planted in all of the plots. Winter rotation crops were planted in 2012 and cotton planted in the fallow treatment in summer 2012/2013.

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

Figure 3.4.5 Long term rotation field plan cycle.

Objective 4 – Continue to investigate the effect of long bare fallows on mycorrhizal colonisation of cotton.

4.1 Long term enforced fallow experiment continued at ACRI.

Field site: The long term trial was set up in Field 4, Australian Cotton Research Institute (ACRI), Narrabri, in the summer 2005/2006 cotton season to investigate the impact of cropping versus long bare fallow on inoculum levels in the soil (Figure 4.1.1). The trial site was 4.6ha, consisting of 10 plots with a total of 80 rows, each 148.6m long. The rows were divided in half lengthways to form 2 tiers (within each plot) with tier 1 being towards the head ditch and tier 2 being towards the tail ditch (Table 4.1.1). In the first year (2005/2006) all rows in each plot were planted to sunflower. The experiment consisted of 2 treatments: bare fallow and crop rotations sown each winter and summer cotton growing season. For each season, rows 1-8, 17-24, 41-48, 57-64 and 73-80 were bare fallow while rows 9-16, 25-32, 33-40, 49-56 and 65-72 were planted to the rotating crops. Cotton planted early, late and both with the addition of Bion was planted in summer 2006/2007. After this a single planting date of cotton was carried out. The long term effects of treatments commenced in winter 2009. Soil cores were collected across all plots and sown to cotton in the glasshouse for assessment of VAM colonisation and black root rot severity and associated stand counts and shoot weight.

Summer 2010/2011 cotton season had a wet start and continued rain throughout the early growing season resulted in no black root rot assessment of plants in the field as plants had outgrown the symptoms by the time conditions dried up. Consequently, VAM colonisation and black root rot severity was assessed from plants growing from seed sown into soil collected from each treatment plot and grown in a temperature and light controlled growth room and glasshouse.

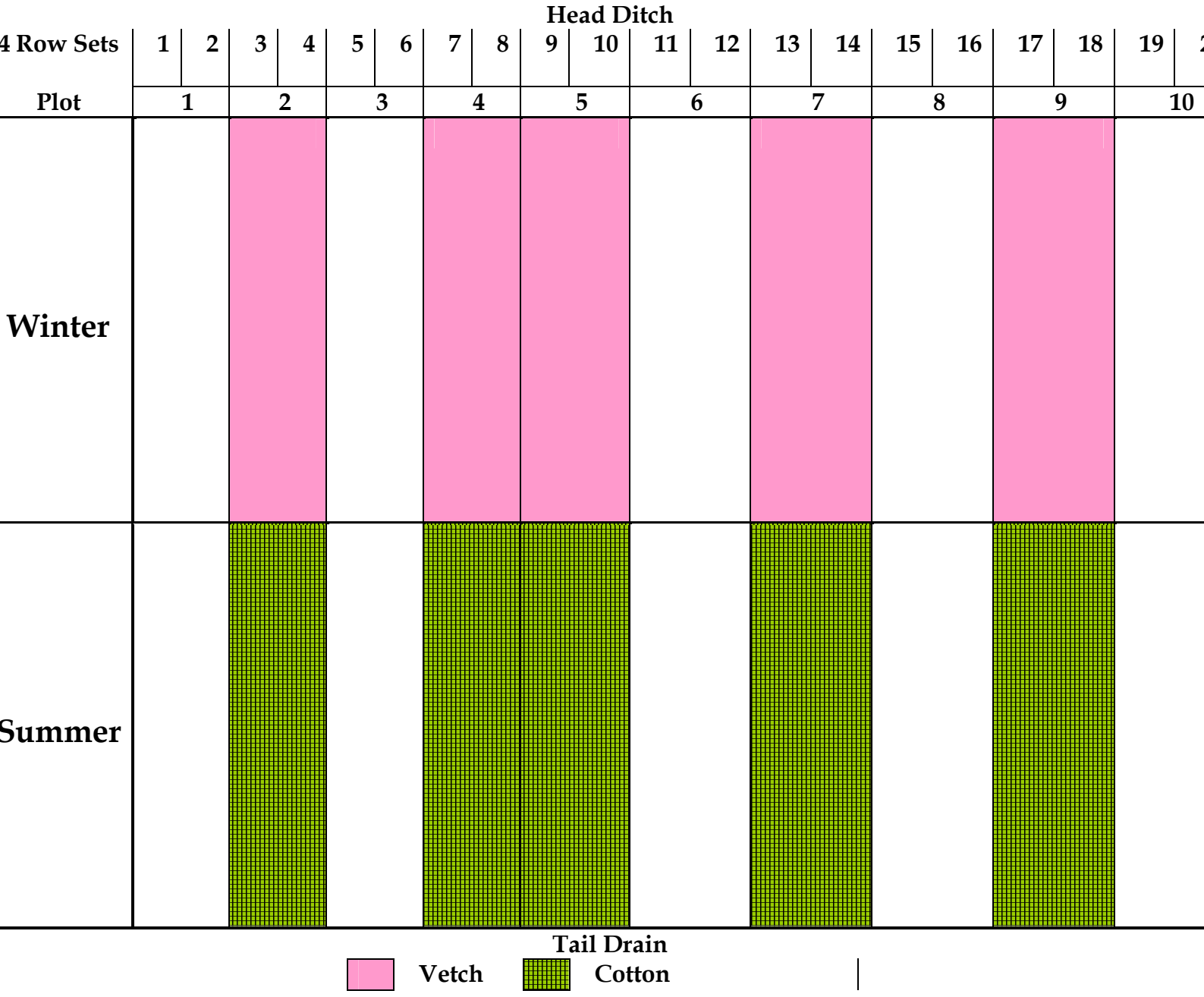


Figure 4.1.1 Layout of the long term VAM trial in Field 4, ACRI.

VAM assessment:
Whole plants were gently removed from pots and washed under tap water to remove soil and debris. Roots were cut from the plant and placed in staining tubes, with tubes being stacked into a stainless steel basket. Empty staining tubes were used to fill the basket when there were less than 30 samples. The basket was placed in a glass dish and 10% KOH (w/v) was added ensuring all the roots were covered by washing the roots to the bottom of the tubes with a stream of tap water before adding KOH. The glass dish was placed into a water bath for 1 hour at 90°C.

The KOH was removed from the root samples by washing with running tap water, before leaving the basket to stand in distilled water for a few minutes at room temperature. The water was removed and replaced with 1L of 2% HCL (necessary for dye to fix to fungal structures) and left for at least 5 minutes at room temperature, ensuring all the roots were well covered. The HCL was removed and replaced with a staining solution: acidic glycerol (500mL glycerol, 475mL H_2O, 25mL 25 HCL) containing 0.05% trypan blue.

Once roots were completely submerged in the stain, they were placed in a water bath for 20 mins at 90°C. The staining solution was drained. Excess staining solution was washed from the samples and tubes using tap water. The roots were covered with acidic glycerol for de-staining and returned to the 90°C water bath for 30 minutes (or alternatively left overnight at room temperature). The basket containing the vials was tapped vigorously against the side of sink to move the roots to one side of the staining tubes. The roots were washed into vials using a wash bottle containing acidic glycerol. Each vial had approximately 10mL of acidic glycerol with the root samples.
The vial containing the roots and acidic glycerol were poured into a 90mm Petri dish with grid lines marked on the bottom of the dish to form 0.5 in. squares. These gridlines were used as a mechanism for the systematic selection of observational points. Colonisation was assessed by a modified method described by Giovannetti & Mosse (1980). Infection was then measured by recording the number of root/gridline intersects at 100 random points for each plant.

Black root rot assessment:
The severity of black rot disease was assessed approximately 3 to 5 weeks after emergence. Soil was loosened around the base of plants in the field using an asparagus knife. Ten plants were lifted from the soil and the tap roots examined for symptoms of black root rot and rated from 0 to 10 for each (D. B. Nehl, et al., 2004b). The number of cotton plants per 10m was counted in rows 3, 4, 5 and 6 of each plot and above-ground plant material separated from root material, placed in paper bags and dehydrated for 2 days to obtain dry shoot weights (g/plant).

For glasshouse and growth room assessments, soil was taken from the 10 treatments and placed into 20 grapevine tubes lined with patty cake liners with 2 pots per treatment. A total of 12 seeds (Sicot 43 BRF) were placed on the soil surface and topped with a mix of sand/vermiculite (1:1 ratio). Tubes were water

individually by placing round take away containers under each tube and placed in a growth room, set at 12 hours light and a maximum temperature of 22°C, 12 hours dark and a minimum temperature of 18°C. After 1 day, the sand mix was dampened down and containers watered to the top again. Tubes were then water every couple of days. Three weeks after emergence, dry shoot biomass and disease severity was assessed as described above.

A modified TbCEN medium method described by Specht and Griffin (1985) and Chittaranjan and Punja (1993) was used to determine the inoculum levels of *T. basicola* per gram dry soil collected from fallow and crop plots. *T. basicola* were isolated using this semi-selective medium TB-CEN, which suppresses other fungi and bacteria. Two soil samples were taken from mid distance in each plot. In short, 10 g of sub-sampled soil was suspended in 0.1% distilled water agar (DWA), providing a 1 in 10 soil dilution. Using a mechanical shaker, the samples were shaken vigorously for approximately 10 minutes. One mL of soil suspension was pipetted into each Petri plate (90mm), after which 25 mL of molten medium was dispensed using sterile tubing attached to a peristaltic pump, using a swirling motion to mix soil and medium whilst dispensing. Five plates were used for each soil collection and 5 containing no soil as controls. Plates were incubated at 23°C under continuous darkness and inspected after 7 to 10 days for the presence of colonies growing on the medium. Counts were adjusted to colony forming units (CFU) per gram of soil by multiplying the number of colonies by the dilution factor of 10.

Design and statistical analysis:
Completely randomised designs were used for all glasshouse and growth room experiments. Field experiments used completely randomised block designs. Results from field experiments were analysed using analysis of variance with spatial analysis (ASREML) applied with planned comparisons of treatments. Liner and nonlinear regression models were fitted to experiments with comparisons of symptoms and other parameters.

Objective 5 – Provide information on the survival of pathogen inoculum in soil.

5.1 Establishing the culture collection:

Samples were collected from infected plants on commercial cotton farms during 2008 to 2013 cotton seasons during disease surveys. Pathogens causing diseases such as black root rot, Alternaria, Verticillium wilt, Fusarium wilt, Rhizoctonia, Pythium, sudden wilt and boll rots were isolated and subcultured.

Pathogens were isolated on selective media (Table 5.1.1) and identity confirmed by microscopic examination. Plates were subcultured until clean cultures were obtained. Clean single strain isolates were preserved in triplicate in long term storage using silica gel, sterile water method or both. Each of the triplicate samples is stored in separate locations to preserve samples for long term research. One of the samples is stored laboratory; one in the -80^{o}C freezer in potting shed and the other is stored off site (e.g. DAFF QLD or UNE). Information recorded for each isolate included: pathogen, GPS coordinates of where sample was taken from, farm/field name, time of sampling and crop history.

Table 5.1.1 Selective media used for isolating various pathogens of cotton

Media	Pathogen	Disease
V8 agar	Promote *Alternaria* spp. Sporulation	Alternaria leaf spot
PDA - potato dextrose agar	General isolation media - carbohydrate/sugar rich	
10% PDA	Improved isolation of pathogens from plant material - reduced carbohydrate/sugar	
50% PDA	Improved isolation of pathogens from plant material - reduced carbohydrate/sugar	
WA - water agar	Suitable to initiate single spore cultures	
CJA - carrot juice agar	Growth of *Theilaviopsis basicola*	Black root rot
TbCEN agar - *Theilaviopsis basicola* carrot etridiazole nystatin agar	Semi-selective media for recovery of *T. basicola*	Black root rot
ESA - ethanol streptomycin agar	Selective media for isolating *Verticillium dahliae*	Verticillium wilt
Komada's media	Promote *Fusarium oxysporum vasinfectum* sporulation	Fusarium wilt
PCA - potato carrot agar	Isolation of *Pythium*	Seedling disease
NA - nutrient agar	General isolation media - bacteria	
SPA - sucrose peptone agar	Isolation and growth media for *Xanthomonas axonopodis* pv. *Malvacearum*	Bacterial blight
PCDA - potato carrot dextrose agar	Isolation and growth media for *Xanthomonas axonopodis* pv. *Malvacearum*	Bacterial blight

5.2 The effects of environmental factors on survival of inoculum assessed in glasshouse studies.

A pilot experiment was set up to evaluate the average disease severity of black root rot caused by the pathogen *T. basicola* in different soil types grown in the glasshouse. The aim of the two experiments was to assess the average disease severity of black root rot of plants grown in soil collected from four different cotton fields from two cotton regions with the highest levels of black root rot; the Namoi and Macquarie Valley was evaluated. Soil was collected from different geographical locations known to have naturally high levels of black root rot: Namoi

(ACRI Old 2) and (Warilea Field 3), Macquarie (Dulla Dulla Field 1) and (Dulla Dulla Field 3).

Soil from each location was potted up into 5 (117x170 mm black plastic pots with seven 12x15 mm holes in the side at the base) pots. Each pot had 25 (Sicot43BRF) seeds planted before being covered with a sand/vermiculite/potting (1:1:1 mix) and pressed down. Round pot saucers were paced under each pot to facilitate watering from the base. Two experiments were done in the glasshouse and growth room at low and high temperatures. The air temperature was set in the glasshouse for 15-25^{O}C and this was not adjusted before doing a second experiment. Air temperature in the growth room were 17-21^{O}C and 20-25^{O}C range. The average disease severity was assessed in each pot on the 0-10 scale.

5.3 Evaluated the survival of the three pathogens in different soil types under a range of environmental conditions throughout New South Wales.

Initially three pathogens were to be investigated, however due to staff changes and a prolonged period where there was no professional officer it was decided that efforts would concentrate on *Thielaviopsis basicola*, the pathogen causing black root rot of cotton.

A completely randomised pot experiment (Figure 5.3.1) was conducted at Australian Cotton Research Institute, Narrabri, Australia. Growth room conditions were set at 12 hours light, 12 hours dark with temperature set to be conducive for disease development. The minimum temperature was 16^{o}C and maximum temperature of 26^{o}C. Pots were individually watered from the base using saucers and maintained in a growth room.

Fungal isolates and inoculum preparation

Inoculum was made using a modified method from (Mauk & Hine, 1988). Generally, *T. basicola* isolate PP237 collected previously and isolated from infected seedling roots was used for inoculating the three different soil types used in this experiment. Inoculum was prepared by mechanically dislodging the culture with sterile distilled water and a 40mm glass hockey stick. The isolate was grown on 10% carrot juice agar for 14 days. Using a Sedgwick Rafter chamber, the concentration of chlamydospore/ chain and fragments (ccf) were counted and the total concentration of inoculum adjusted to 100 ccf per ml. The inoculum stock solution was mixed thoroughly and divided into three lots of 200mls.

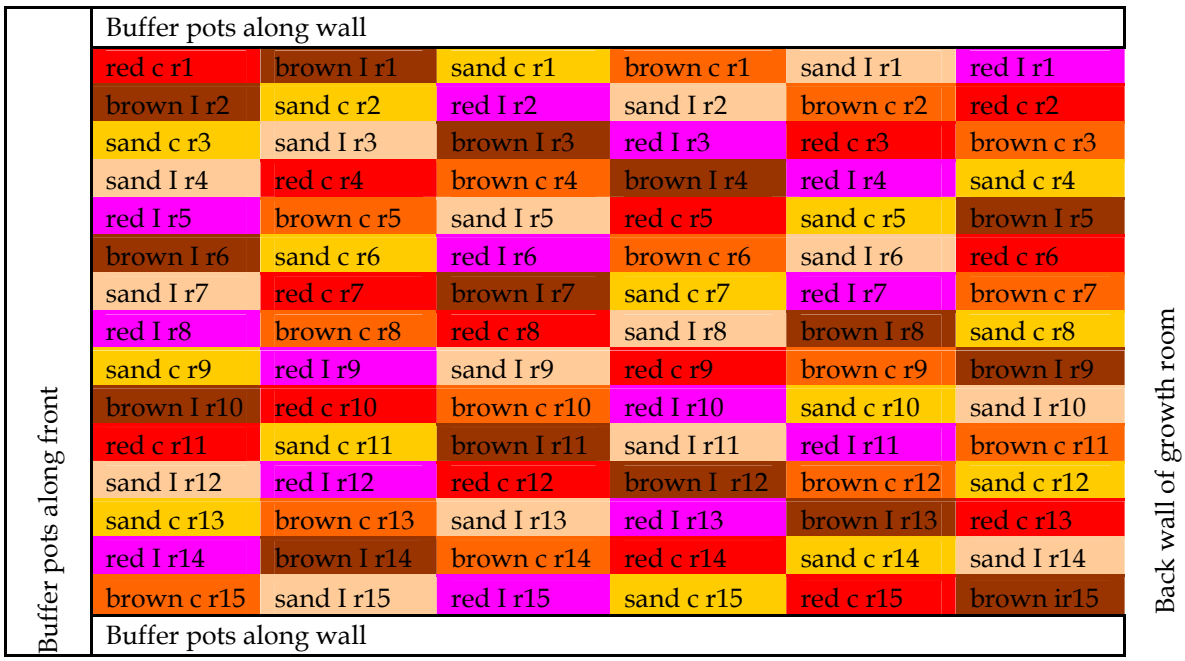


Figure 5.3.1 Randomised pot design for growth room experiment, with buffer pots surrounding experiment.

Soil preparation

Soil was collected from three cotton fields near Narrabri. Each field had soil of different colour and structure. Soils were characteristically described as red, brown and sand. Each soil was further characterised using the Munsell® Color Chart with red (4/6), brown (4/4) and sand (5/8).

The pH of each soil type was tested using a TPS Conductivity/pH meter. Soil from each field was then pasteurised twice. Half of the pasteurised soil was kept for controls while the balance was inoculated with *T. basicola* at a level of 100ccf/gram soil.

Forty five pots were filled with the pasteurised soil that was inoculated while another forty five were filled with pasteurised soil. The 6 treatments were: 15 pots filled with the inoculated sand soil, 15 pots with sand soil that was not inoculated, 15 pots filled with inoculated red soil, 15 pots filled with red soil that was not inoculated, 15 pots filled with inoculated brown soil and 15 pots filled with brown soil that was not inoculated.

Inoculating soil: 200mls of inoculum (100ccf/ml) was added to 3L vermiculite and mixed thoroughly in a plastic tray before being added to a commercial cement mixer. Fifteen litres of damp sand soil was then added to the cement mixer and mixed for at least 5 minutes. The pasteurised, inoculated sand soil mix was potted into 15 labelled pots. The cement mixer was washed out and the process repeated for the red and brown soil types. For the controls of each soil type, the method was the same except that 200mls of distilled water was added to the vermiculite and mixed thoroughly

before being mixed in with each soil type. Fifteen seeds (Sicot 74BRF) were planted into each pot and covered with a thin layer of river sand.

Plants were monitored daily and days to emergence recorded for each pot. Twenty one days after planting, seedlings were gently removed from the pots and the roots washed with tap water over a sieve to remove any soil and debris. The tap roots from each seedling were assessed for disease severity using the method described by Nehl et al. (2004a). In short, the scale of 0 to 10 represents the percentage length of the tap root blackened (see Table 5.3.1). Seedlings were cut into shoots and roots and wet weight (ww) and dry weights (dw) recorded.

Table 5.3.1 Black root rot disease severity scale

Plants are rated on a 0 to10 scale			
0	Clean tap root system	**6**	>50% and ≤60% tap root discolouration
1	>0 and ≤10% tap root discolouration	**7**	>60% and ≤70% tap root discolouration
2	>10% and ≤20% tap root discolouration	**8**	>70% and ≤80% tap root discolouration
3	>20% and ≤30% tap root discolouration	**9**	>80% and ≤90% tap root discolouration
4	>30% and ≤40% tap root discolouration	**10**	>90% and ≤100% tap root discolouration
5	>40% and ≤50% tap root discolouration		

Objective 6 – Investigate Verticillium dahliae interaction with black root rot.

6.1 Identify fields with significant levels of both Verticillium wilt and black root rot.

The long term data base records and collates information collected from the disease surveys. Fields with significantly high incidence of more than 30% Verticillium wilt and black root rot were identified.

6.2 Development of methods to determine the effects of *Thielaviopsis basicola* and *Verticillium dahliae* alone and together.

Basic information on inoculum levels in the soil profile in cotton beds and within furrows is needed. To address this issue the pathology unit has purchased a soil corer and will commence gathering this information in the Diseases of Cotton XI project. Several experiments have been proposed to help determine the effects of each pathogen alone and combined.

Objective 7 – Investigate bacterial seed rots identifying the pathogen(s) and potential means of infection.

7.1 Laboratory and glasshouse experiments undertaken to identify the pathogen.

As a result of staff changes there was a substantial period where there was no project leader (pending my recruitment). In order to best meet this milestone a collaborative approach was negotiated with Dr Moazzem Khan (Research Scientist) with Department of Employment, Economic Development and Innovation (DEEDI) to collaborate on this milestone. The pathology unit collected boll samples during the 2011/2012 disease surveys and these were sent to Dr Khan pathogen identification and means of infection identified.

Objective 8 – Re-establish Australia's capacity to screen for exotic races of bacterial blight.

8.1 Obtain blight differentials from USA.

Dr Kirkby provided USDA with all the information on the 10 differential lines needed to be imported into Australia. Unfortunately USDA only had 5 of the 10 lines, those being Acala 44, Stoneville 20, Mebane B-1, Gregg and Empire B4. USDA-ARS National Plant Germplasm System has also been contacted about the missing lines. Dr Peggy Thaxton was able to supply the missing 5 lines (Stoneville 2B-S9, 1- 10B, 20-3, 101-102B and DPxP4) from Mississippi State University.

Objective 9 – Respond to industry disease issues as they arise.

As a participant of the 2012 Future Cotton Leaders Program, Dr Kirkby had the great opportunity to work on a project that benefited the Australian cotton industry. There are many people that receive disease related enquiries and in the past this occasionally led to confusion about who has responded. In order to address disease issues as they emerge Dr Kirkby streamlined responses using a coordinated approach. The aim of the project was to see that concerns were addressed as they arose by developing, implementing and maintaining a communication model called PathWAY that "captures and quantifies disease enquiries from the cotton industry".

During the development phase, a letter of invitation was sent to representatives from researchers, pathologists, entomologists, virologists, consultants and funding bodies from across borders and agencies were invited to participate in this communication model. These people would be referred to as key points of contact (POC).

Once the POC were established, a model for capturing disease enquiries was established and set to the network. A formalised method of capturing the information enabled quantitative data to be collected.

"Improve Communication Through Industry Partnerships"

Industry Points Of Contact (POC) Communication Model

Disease Enquiry	Response	Collaborate	Communicate	Action	Respond	Quantify
Grower Consultant Industry Organisation Extension Research	Immediate	Inform all POC of enquiry	Respond to the source of enquiry	Deliver information to source of enquiry. **Additionally** email all POC with the nature of enquiry, valley/region & response.	Rapid response to disease enquiries.	Disease enquiry data captured in POC email list and available to all
	Considered	Inform all POC of enquiry	Request information from all POC	Collate & deliver information to source of enquiry. **Additionally** email response to all POC.	Responses from all POC collated and delivered to source of enquiry.	

NOTE: POC must maintain confidentiality & should only share nature of disease enquiry, region & response.

PathWAY was supported by CRDC in assistance in developing the brand. Articles featuring PathWAY findings have been featured in Spotlight in each article since it was launched. The best practice symbol is also associated with the tool.

Results

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

1.1 2010/2011 Cotton Disease Surveys

Results of the disease surveys from NSW and QLD are collated each year and published in the Cotton Pest Management Guide.

Cotton Pathology 2010-2011

S.J. Allen[1], K.A. Kirkby[2], J. Lehane[3], P.A. Lonergan[2], B.R. Cooper[2] and L.J. Smith[4]
Cotton Catchment Communities CRC
[1]Cotton Seed Distributors Ltd., PO Box 17, Wee Waa NSW
[2]NSW Department of Primary Industries, Locked Bag 1000, Narrabri NSW
[3]Agri-Science Queensland, DEEDI, 203 Tor Street, Toowoomba, Qld
[4]Agri-Science Queensland, DEEDI Ecoscience Precinct, GPO Box 46, Brisbane, Qld

Commercial cotton crops across NSW and Queensland were inspected in November-December 2010 and February-April 2011. The incidence and severity of those diseases present were assessed and field history, ground preparation, cotton variety, planting date and seed rate were recorded for each of the 89 and 56 fields that were surveyed in NSW and Queensland respectively. This represents the 28th consecutive season of quantitative disease surveys of cotton in NSW and the 9th consecutive season of cotton disease surveys in Queensland.

The 2010/2011 season generally featured a wet spring, significant flooding in summer and a dry autumn. Consequently, planting was delayed and crop development was slow culminating in a late harvest. The combination of a wet spring and record prices also contributed to a late planting frenzy. These conditions impacted significantly on the incidence and severity of cotton diseases and on the timing of disease surveys (Table 1). The devastating flooding in the Dawson River completely destroyed cotton growing near Theodore and Moura and late season surveys in this area were not possible.

Table 1. *The number of fields surveyed and the timing of surveys for 2010/2011.*

Area/Region	No. of fields	Early season survey	Late season survey
Burdekin	7	16th Feb.	19th May
Emerald	11	24th Nov.	22nd Feb.
Theodore/Moura	9	25th Nov.	Floods destroyed all crops
St George/D'bandi	16	14th – 15th Dec.	2nd – 3rd Mar.
Darling Downs	13	16th – 17th Dec.	10th Mar.
Bourke/Walgett	14	18th Nov.	14th – 15th Mar.
Macintyre	12	24th – 25th Nov.	31st Mar. – 1st Apr.
Gwydir	12	4th & 24th Nov., 14th	8th , 28th & 31st Mar.
Namoi	22	22nd , 26th & 29th Nov.	7th, 10th, 11th, 29th Mar, 6th, 7th
Macquarie	12	22nd Dec.	7th Apr., 12th – 13th Apr.
Lachlan	8	10th Nov., 20th Dec.	16th Mar., 18th Apr.
Murrumbidgee	9	21st Dec.	17th Mar.

During these surveys particular attention was given to the detection of Cotton Leaf Curl Virus, Blue disease, Phymatotrichopsis root rot, the hypervirulent strains of the bacterial blight pathogen, the defoliating strains of the Verticillium wilt pathogen and exotic strains of the Fusarium wilt pathogen. None of these diseases and/or pathogens were observed.

VOLUNTEER COTTON– (Carry-over from the previous season)

Information on the occurrence of volunteer cotton was collected during the annual disease surveys and is based on visits to 42 farms in NSW and 18 farms in Queensland during November and December of 2010 (Table 2). The number of farms with (1) mature cotton plants surviving along roadsides, fence lines, along channels and in tail water return systems or drains, (2) volunteer cotton in fallow or rotation fields and (3) mature cotton plants surviving from the previous season or regrowth from stubs (Ratoon cotton?) in current cotton crops, were recorded.

Table 2. *The occurrence of volunteer cotton plants surviving from the previous season on farms in NSW and Queensland in the spring of 2010.*

	1. Along channels, roads, fences	**2. In fallows and rotation crops**	**3. In the current crop (regrowth from stubs)**	**TOTAL**
In NSW	25/42 (60%)	9/42 (21%)	23/42 (55%)	34/42 (81%)
In Qld	7/18 (39%)	1/18 (6%)	5/18 (28%)	10/18 (56%)
Total	32/60 (53%)	10/60 (17%)	28/60 (47%)	44/60 (73%)

The presence of volunteer plants surviving over from the previous season enables pests and pathogens such as aphids and mealy bug and cotton bunchy top to overwinter and initiate new outbreaks in the spring. Wet weather during September 2010 allowed vigorous growth of volunteer cotton in non-cropped areas. Volunteer cotton plants were observed on 44 of the 60 farms visited during the disease surveys (73%).

SEEDLING MORTALITY

As part of the disease survey an estimate of the number of seeds planted per metre is compared to the number of plants established per metre. This comparison produces an estimate of seedling mortality which includes the impact of seedling disease (*Rhizoctonia* and *Pythium* etc.) as well as seed viability, the activity of soil insects such as wireworms, physical problems such as fertiliser or herbicide burn and the effects of adverse environmental conditions.

Mean seedling mortality (Figure 1) for the crops inspected in NSW and Queensland was 31.9% and 25.8%, respectively, (32.5% and 25.8% in 2009-10; 28.8% and 24.9% in

2008-09; 31% and 19.5% in 2007-08). Many growers were able to establish the crop on rainfall with no need to pre-irrigate.

Problems with crop establishment were noted in some areas. These problems included a cold spell in mid-October and the necessity of a quick turnaround between the rain-delayed harvest of a winter crop and the planting of cotton in the same field. The highest incidence of seedling mortality was 36.7% in crops in the Murrumbidgee Valley and the lowest incidence was 18.6% in crops in the Burdekin area of Queensland where planting takes place in late December and January.

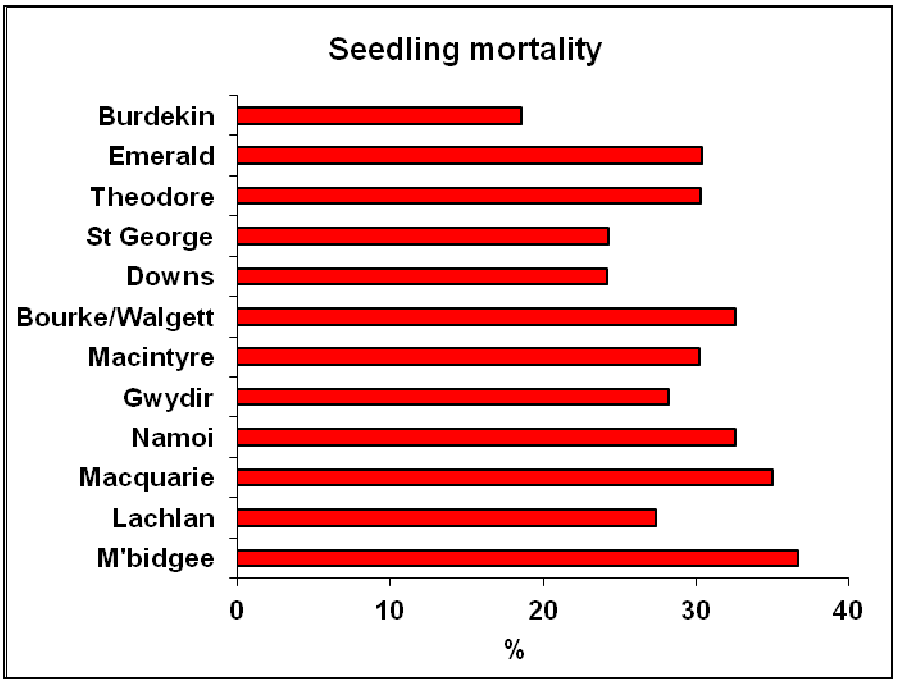


Figure 1. *Mean seedling mortality in the 2010/2011 season. Seedling mortality is derived from the difference between the number of seed planted and the number of plants established.*

FUSARIUM WILT

The wet spring, followed by a cool and wet summer, contributed to an increased incidence of Fusarium wilt and negated the usual benefits associated with a delayed sowing. Conversely, the widespread adoption of the new, more resistant, varieties reduced the potential impact of the disease. Fusarium wilt was very obvious during early season surveys where up to 11% of seedlings had been killed in some fields. Later in the season common symptoms included gaps in the stand, stunted growth and a dark brown discoloration of the vascular tissue in the stem. Wilting, dead and dying plants were not always present as was observed in previous years with more susceptible varieties.

There was one new report of Fusarium wilt east of Goondiwindi in Queensland, two new reports of Fusarium wilt in the Gwydir valley of NSW, the first report of Fusarium

wilt on a farm in the Lachlan Valley and the first report of Fusarium wilt on a farm in the Emerald area. These new reports were confirmed by Dr Linda Smith (Agriscience Queensland, DEEDI) who provides a free, confidential diagnostic service for Fusarium wilt funded by the Australian cotton industry. All five new reports were found to be caused by the Downs strain of the pathogen.

Fusarium wilt was observed in 22 of the 89 crops surveyed in NSW (Figure 2). including eleven of the 12 crops inspected in the Macintyre valley and seven of the 12 crops surveyed in the Gwydir valley. The incidence of Fusarium wilt averaged 8.8% and 9.9% respectively, for these two production areas where four fields had in excess of 25% of plants affected. Though Fusarium wilt is known to be present and widespread in the Macquarie valley and the upper Namoi valley it was not detected in these areas in either the 2009/10 or the 2010/11 disease surveys.

The disease was observed in only 11 of the 47 crops surveyed in Queensland including nine of the 13 crops inspected on the Darling Downs. The incidence of Fusarium wilt averaged 2.8% and 0.6% respectively, for the Darling Downs and St George areas and only exceeded 5% in two fields (Figure 2).

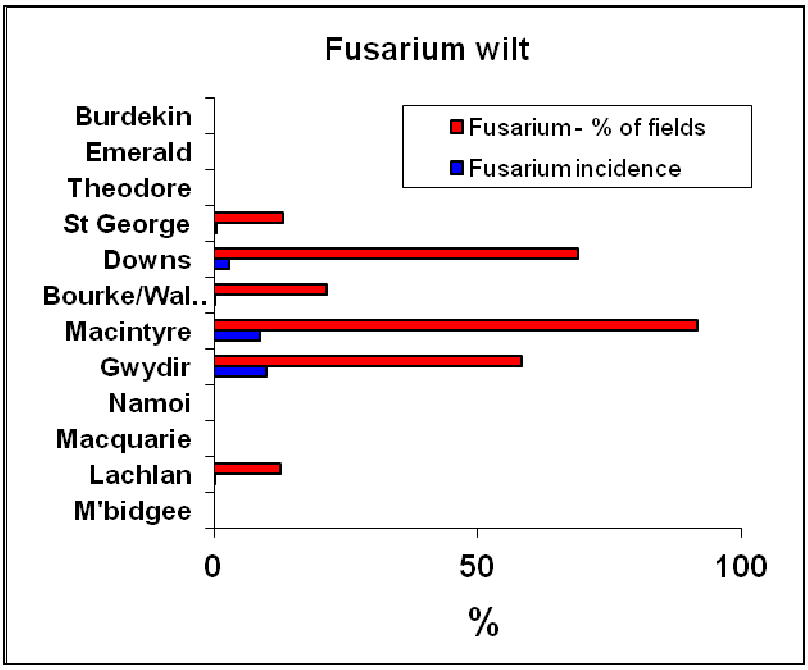


***Figure 2**. The average distribution and incidence of Fusarium wilt of cotton in the 2010/2011 season.*

Transects have been established in fields near Theodore, St George, Boggabilla and Gunnedah. The incidence of Fusarium wilt is assessed along these transects in seasons when cotton is grown in these fields. Assessments during the 2010/2011 show an increase of between 60% and 320% in disease incidence in five of the six transects despite a four-year rotation with cereals, fallows and sorghum in two of the fields. The

only decline in the incidence of Fusarium wilt was observed in a rain-grown crop growing in a field that had not grown cotton for five years.

BLACK ROOT ROT

Black root rot of cotton is favoured by cool weather conditions early in the season. The pathogen colonises the root surface, suppresses the development of secondary roots and stunts seedling growth. When temperatures rise the tap root expands and the blackened root surface is sloughed off and disappears. The seasonal conditions in the spring and early summer of 2010 were very favourable for black root rot.

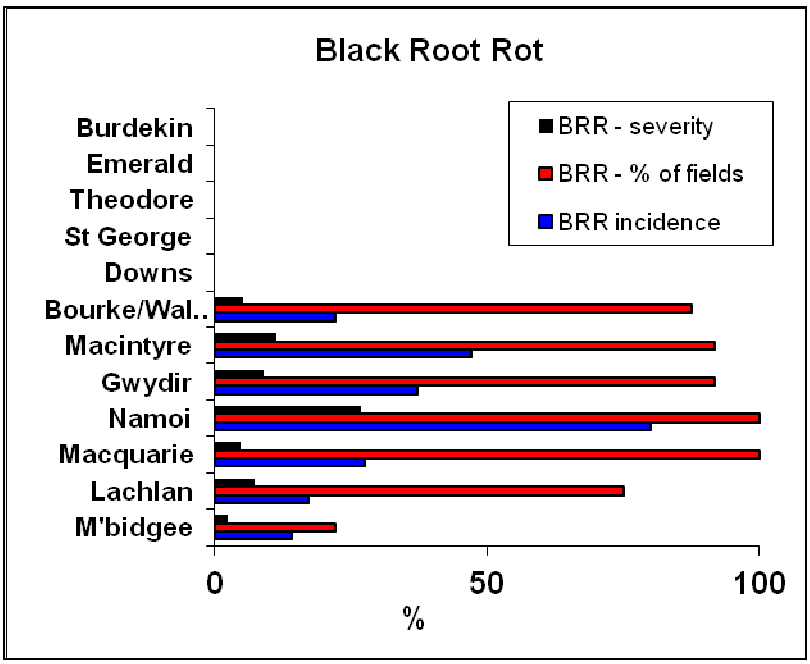


Figure 3. *The distribution, incidence and severity of black root rot in cotton in the 2010/2011 season.*

Black root rot was observed on 93% of farms visited and in 83% of the fields surveyed in NSW (Figure 3). The average incidence within fields was 41% and mean disease severity was 1.18 (11.8% of each tap root blackened). The disease was most common in crops in the Namoi valley where it was observed in all of the fields surveyed. The average incidence within fields was 80% and the incidence exceeded 80% (of plants infected) in ten of the fields. The mean disease severity was 2.69 (26.9% of each tap root blackened). There were also fields in both the Gwydir and Macintyre valleys where the incidence of black root rot exceeded 90%. The incidence of black root rot in crops in the Namoi, Gwydir and Macintyre valleys was substantially higher than ever recorded previously.

Black root rot has previously been observed in all Queensland cotton production areas except the Burdekin. There were several reports of black root rot in cotton crops on the Darling Downs and at St George. However, wet weather delayed the surveys until mid

December by which time the weather had warmed and plants were growing away from the symptoms.

VERTICILLIUM WILT

Verticillium wilt is also favoured by cooler weather and is rarely observed in Queensland production areas. The disease was observed in 36% of fields surveyed in NSW. However, the average incidence was only 4.1% of plants infected (Figure 4). The average incidence of Verticillium wilt of cotton in NSW was estimated in previous surveys to be 3.8% and 3.7% for the 2008/09 and 2009/10 seasons.

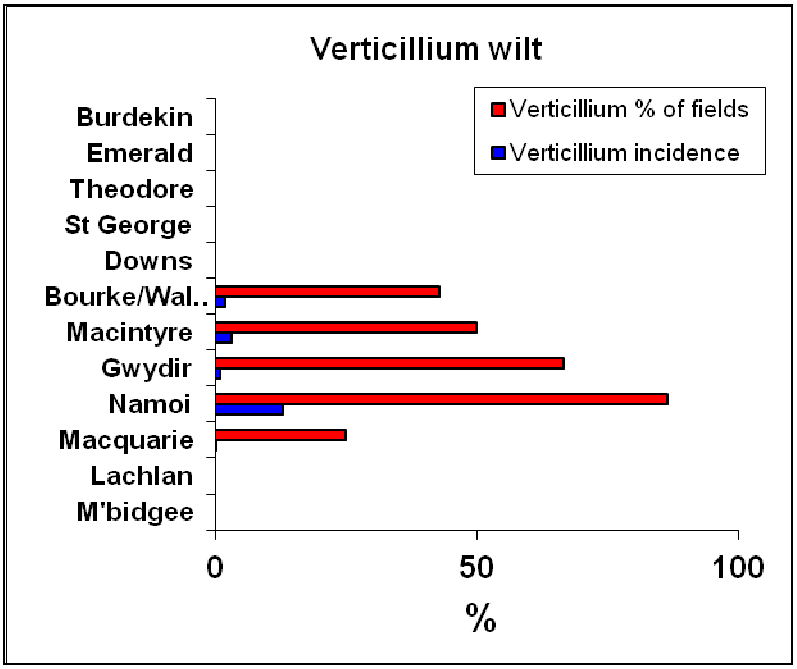


Figure 4. *The distribution and incidence of Verticillium wilt of cotton in the 2010/2011 season. The disease was present in many areas but the incidence was generally low.*

Verticillium wilt was observed in 86% of fields surveyed in the Namoi valley where the average incidence of the disease was 13.1% of plants infected (compared to 14.0% and 12.7% in the previous two seasons). The worst affected fields had 41%, and 71% of plants with symptoms. It is interesting to note that these fields had had 68% and 96.5% of plants with black root rot at the beginning of the season. The interactions between the pathogens that cause black root rot and Verticillium wilt and the effect of that interaction on cotton needs to be investigated.

BOLL ROTS

The reported incidence of boll rots (Figure 5) can be affected by planting date, survey date, distribution of bolls on the plant and the size and density of the crop canopy as well as by the timing and intensity of wet weather. Overcast weather and waterlogging contributed to low retention early in the season and few low bolls in many crops. This resulted in a lower incidence of the boll rots that develop when soil is splashed up onto low bolls.

Delayed maturity in crops on the Darling Downs and at Emerald resulted in few open bolls and consequently a lower incidence of boll rots at the time of survey. In contrast, the average incidence of boll rot in the more advanced crops in the St George and Dirranbandi area was 5.25% and exceeded 10% in four of the 16 crops surveyed.

The average incidence of boll rots was recorded as 0.7% for NSW and 2.7% for Queensland (2.7% and 1.9% in 2008/09; 9.7% and 7.3% in 2009/10).

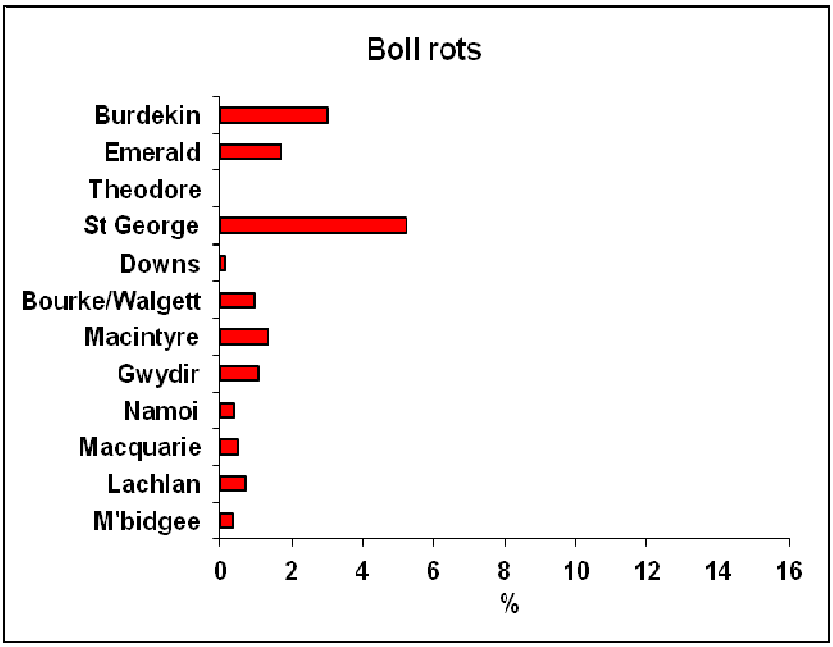


Figure 5. *The average incidence of boll rots in each of the cotton production areas for the 2010/2011 season. These figures may under-estimate the final incidence as assessments are usually completed after the final irrigation and several weeks before harvest.*

BUNCHY TOP

According to the 2007/2008 disease survey cotton bunchy top was observed in 10% of crops inspected in Queensland and in 14% of fields inspected during the NSW surveys.

During the 2008/09 season symptoms were observed in 7% of crops inspected in Queensland and the average incidence of bunchy top in these crops was <0.1%. Bunchy top was observed in 11% of fields inspected during the NSW surveys where the average incidence was 0.2% of plants with symptoms. The incidence of bunchy top in three crops in the Lachlan Valley was found to be 5%, 4% and 1%.

The 2009/2010 disease survey report indicated that "Bunchy top was commonly observed on volunteer cotton plants surviving over from the previous season" and "A large area of severely affected plants was observed in a field near Theodore". The disease was found in 6% of crops inspected in Queensland and 7% of crops in NSW.

Bunchy top was observed in 73% of crops inspected in Queensland during late February and early March, 2011 (Figure 6) and the average incidence of bunchy top in these crops was 0.53%.Symptoms were apparent on single plants and rarely in patches. The incidence of bunchy top in some fields on the Downs was up to 3%.

In Emerald symptoms were apparent in the upper canopy while at St George and on the Darling Downs affected plants were stunted and largely hidden within the canopy. Bunchy top was seen in 10 of the 13 crops inspected on the Downs. It is interesting to note that the three crops where the disease wasn't observed were all Siokra 24BRF while the 10 infected crops were either Sicot 71BRF or Sicot 74BRF.

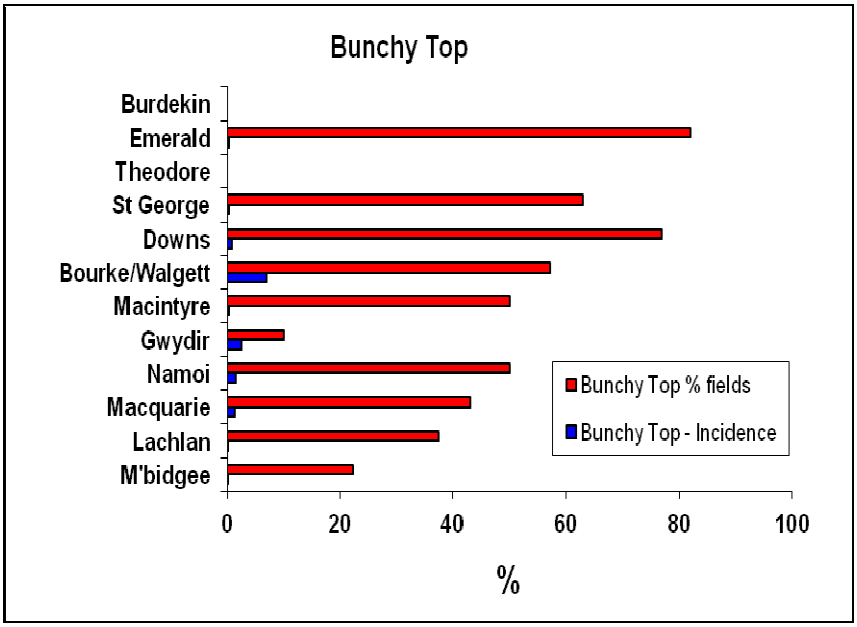


Figure 6. *The distribution and average incidence of bunchy top in each of the cotton production areas for the 2010/2011 season.*

In NSW, bunchy top was observed in 43.0% of crops surveyed in March and April, 2011 (See Table 1) with the average incidence being 2.1%. Bunchy top was particularly apparent in some fields in the Bourke/Walgett region where the average incidence of cotton plants with symptoms was 6.9%. The incidence of the disease in two crops on one farm was estimated to be 48.5% and 43%. The worst affected fields in the Namoi and Gwydir valleys had 28.5% and 25.5% of plants with symptoms. There was an apparent association between a high incidence of bunchy top in a crop and large numbers of volunteers with bunchy top symptoms nearby.

OTHER DISEASES AND DISORDERS

Alternaria leaf spot and premature senescence. Alternaria leaf spot was present at low levels in almost all crops and was generally of minor significance. Premature senescence caused some concern in some crops to the North and West of Emerald.

Wet weather through until early April favoured some premature senescence (2.3% of plants), and leaf spots causing defoliation of lower leaves in some crops in the Burdekin valley of Queensland.

Tobacco Streak Virus (TSV). TSV was observed in nine of the 14 crops inspected in central Queensland in November 2010. The average incidence of the virus was 0.61% with 3% of plants with symptoms in one field.

Seed rot. Symptoms of seed rot include a soft brown rot of developing seed within the bolls that may not become apparent until the bolls either drop or open prematurely. Only one or two locks, or sometimes the whole boll, can be affected. Seed rot appears to be caused by either bacteria or fungi that are introduced into the young developing boll by sucking insects such as the green vegetable bug. The average incidence of seed rot in cotton crops in the Burdekin valley was estimated to be 2.3% (5.6% in the 2010 season).

ACKNOWLEDGMENTS

These surveys were made possible with the financial support of the Cotton Research & Development Corporation, the Cotton Catchment Communities CRC, Cotton Seed Distributors Ltd., NSW Department of Primary Industries and the Queensland Department of Education, Economic Development and Innovation.

1.2 2011/2012 Cotton Disease Surveys

Results of the disease surveys from NSW and QLD are collated each year and published in the Cotton Pest Management Guide.

Cotton Pathology 2011-2012

L.J. Smith[1], J. Lehane[2], K.A. Kirkby[3], P.A. Lonergan[3], B.R. Cooper[3] and S.J. Allen[4]
Cotton Catchment Communities CRC
[1]Queensland DAFF Ecoscience Precinct, GPO Box 46, Brisbane, Qld
[2]Queensland DAFF, 203 Tor Street, Toowoomba, Qld
[3]NSW DPI, Locked Bag 1000, Narrabri NSW
[4]Cotton Seed Distributors Ltd., PO Box 17, Wee Waa NSW

Commercial cotton crops across NSW and Queensland were inspected in November-December 2011 and February-April 2012. Crops in North Queensland were inspected in January and May, 2012. The incidence and severity of those diseases present were assessed and field history, ground preparation, cotton variety, planting date and seed rate were recorded for each of the 100 and 66 fields that were surveyed in NSW and Queensland respectively. This represents the 29th consecutive season of quantitative disease surveys of cotton in NSW and the 10th consecutive season of cotton disease surveys in Queensland.

The 2011/2012 season featured mean daily maximum temperatures (Table 1) and mean daily minimum temperatures (Table 2) that were below normal in most cotton production areas in December, January and February - particularly in December! Monthly rainfall throughout the season was above average for most areas (Table 3) and many fields in NSW cotton production areas were subjected to flooding. The incidence and severity of those diseases of cotton observed in the 2011/2012 season can generally be explained by the weather patterns experienced.

Cotton growers that have been impacted by the extremely severe symptoms of Verticillium wilt, the increasing incidence of Fusarium wilt, the surprise occurrence of Sclerotinia and or the prevalence of boll rots and leaf spots can be confident that a more 'normal', hotter and drier season next year would reduce the incidence and severity of these diseases.

Table 1. Difference (^{0}C) from the long term mean daily maximum temperature for December 2011, January 2012 and February 2012.

	Dec. 2011	Jan. 2012	Feb. 2012
Emerald	- 2.4	- 0.7	- 0.4
Dalby	- 3.0	- 1.3	- 0.2
St George	- 5.6	- 2.7	- 3.4
Goondiwindi	- 3.6	-.2.9	- 0.6
Moree	- 4.8	- 3.2	- 3.4
Narrabri	- 4.9	- 3.0	- 2.5
Trangie	- 3.6	- 1.8	- 2.5
Hillston	- 0.7	- 0.9	- 1.7

Source: Australian Government Bureau of Meteorology 'Climate Data Online'

Table 2. Difference (^{0}C) from the long term mean daily minimum temperature for December 2011, January 2012 and February 2012.

	Dec. 2011	Jan. 2012	Feb. 2012
Emerald	- 1.2	- 1.1	- 0.8
Dalby	- 1.5	- 1.0	- 0.8
St George	- 2.0	- 1.7	- 1.9
Goondiwindi	- 1.3	- 1.1	- 0.5
Moree	- 0.6	+ 0.2	- 0.4
Narrabri	- 1.5	- 1.8	- 1.0
Trangie	- 1.8	- 1.7	- 1.5
Hillston	+ 0.2	- 0.1	- 1.6

Source: Australian Government Bureau of Meteorology' Climate Data Online'

Table 3. Difference (mm) from the long term average monthly rainfall.

	Oct. 2011	Nov. 2011	Dec. 2011	Jan. 2012	Feb. 2012	Mar. 2012
Emerald	- 16.7	+ 1.6	+ 30.7	+ 42.1	+ 1.7	+ 109.9
Dalby	+ 57.2	- 38.6	- 39.5	- 27.7	- 6.0	+ 6.0
St George	+ 11.2	+ 122.2	+ 26.0	+ 33.6	+ 153.8	-47.4
Goondiwindi	+ 41.2	+ 19.6	+ 91.8	+ 54.6	- 39.2	-26.8
Moree	+ 24.2	+ 169.2	+ 77.8	+ 64.2	+ 139.4	- 49.0
Narrabri	- 6.6	+ 88.8	+ 49.8	+ 85.6	+ 143.7	- 47.0
Trangie	+ 41.2	+ 38.0	+ 7.0	+ 27.4	+ 10.5	+ 72.1
Hillston	- 18.5	+ 11.4	+ 59.0	+ 21.8	+ 82.5	+ 208.8

Source: Australian Government Bureau of Meteorology 'Climate Data Online'

VOLUNTEER COTTON– (Carry-over from the previous season)

Information on the occurrence of volunteer cotton was collected during the annual disease surveys and is based on visits to 45 farms in NSW and 25 farms in Queensland during November and December of 2011 (Table 4). The number of farms with (1) mature cotton plants surviving along roadsides, fence lines, along channels and in tail water return systems or drains, (2) volunteer cotton in fallow or rotation fields and (3) mature cotton plants surviving from the previous season or regrowth from stubs (Ratoon cotton?) in current cotton crops, were recorded.

Table 4. *The occurrence of volunteer cotton plants surviving from the previous season on farms in NSW and Queensland in the spring of 2011.*

	1. Along channels, roads, fences	**2. In fallows and rotation crops**	**3. In the current crop (regrowth from stubs)**	**TOTAL**
In NSW	20/45 (44%)	16/45 (36%)	25/45 (56%)	32/45 (71%)
In Qld	11/25 (44%)	2/25 (8%)	5/25 (20%)	13/25 (52%)
Total	31/70 (44%)	18/70 (26%)	30/70 (43%)	45/70 (64%)

The presence of volunteer plants surviving over from the previous season enables pests and pathogens such as aphids, mealy bug and cotton bunchy top to overwinter and initiate new outbreaks in the spring. Volunteer cotton plants were observed on 45 of the 70 farms visited during the disease surveys (64%).

Cotton Industry Biosecurity Plan – Crop Surveillance for Priority Pests

During these surveys particular attention was given to surveying fields for the presence/absence of exotic diseases including Cotton Leaf Curl Virus, Blue disease, Phymatotrichopsis (Texas) root rot, the hypervirulent strains of the bacterial blight pathogen, the defoliating strains of the Verticillium wilt pathogen and exotic strains of the Fusarium wilt pathogen. None of these diseases and/or pathogens were observed.

SEEDLING MORTALITY

As part of the disease survey an estimate of the number of seeds planted per metre is compared to the number of plants established per metre. This comparison produces an estimate of seedling mortality which includes the impact of seedling disease (*Rhizoctonia* and *Pythium* etc.) as well as seed viability, the activity of soil insects such as wireworms, physical problems such as fertiliser or herbicide burn and the effects of adverse environmental conditions.

Mean seedling mortality (Figure 1) for the crops inspected in NSW and Queensland was 32.3% and 29.6% respectively in the 2011-12 season, (31.9% and 25.8% in 2010-11, 32.5% and 25.8% in 2009-10; 28.8% and 24.9% in 2008-09). Seedling mortality exceeded 50% in 16 of the crops surveyed. CSD Ltd estimated that 10% of the total area planted had to be replanted.

Seedling mortality is usually lowest (< 20%) in the Burdekin Valley where planting takes place in late December. The mean seedling mortality for the Burdekin in the 2011/12 season was 27.4%.

Problems with crop establishment included wireworm, uneven moisture in the seed bed resulting from rain prior to planting and poor emergence as a result of crusting due to rain soon after planting. Allelopathy was apparent where cotton was planted into freshly incorporated, un-weathered plant residues, (eg rice straw). The highest mean incidence of seedling mortality was 38.4% in crops in the Macquarie Valley and the lowest incidence was 24.2% in crops in the St George area.

Some concern has been expressed about the impact of seed density on stand establishment. Sicala 74BRF has significantly lighter seed than Sicot 71BRF. In Queensland cotton production areas the mean seedling mortality was found to be 28.4% for Sicot 71BRF (25 fields) and 27.6% for Sicala 74BRF (21 fields). In NSW the mean seedling mortality was found to be 32.3% for Sicot 71BRF (39 fields) and 32.5% for Sicala 74BRF (45 fields).

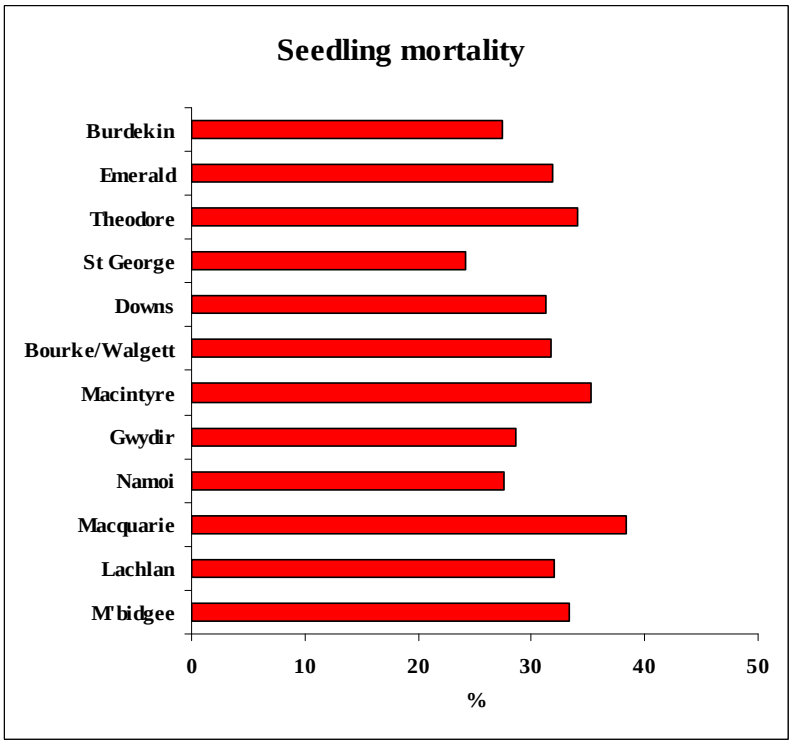


Figure 1. *Mean seedling mortality in the 2011/2012 season. Seedling mortality is derived from the difference between the number of seed planted and the number of plants established.*

FUSARIUM WILT

Fusarium wilt is most severe when October/November rainfall is above normal and when temperatures are below normal – as was experienced during the 2011/2012 season. The disease is least severe when it is hot and dry in spring. The widespread adoption of the new, more resistant, varieties reduced the potential impact of the disease. Fusarium wilt was again obvious during early season surveys. Later in the season common symptoms included gaps in the stand, stunted growth and a dark brown discoloration of the vascular tissue in the stem. Wilting, dead and dying plants were not always present as was observed in previous years with more susceptible varieties.

There was one new report of Fusarium wilt ('Downs' strain) on a second farm in the Lachlan Valley. This new report was confirmed by Dr Linda Smith (Agriscience Queensland, DAFF) who provides a free, confidential diagnostic service for Fusarium wilt of cotton funded by the Australian cotton industry.

Fusarium wilt was observed in 16 of the 100 crops surveyed in NSW (Figure 2). including nine of the 12 crops inspected in the Macintyre valley and six of the 11 crops surveyed in the Gwydir valley. The incidence of Fusarium wilt averaged 16.6% and 16.4% respectively, for these two production areas (8.8% and 9.9% in 2010/2011) and exceeded 60% of plants affected in four of the 16 fields. Though Fusarium wilt is known

to be present and widespread in the Macquarie valley and the upper Namoi valley it was not detected in these areas in any of the last three seasons.

It is interesting to note that black root rot was also present in all 16 of the fields in NSW where Fusarium wilt was recorded. The incidence of black root rot exceeded 50% in 14 of the 16 fields.

The disease was observed in only 15 of the 64 crops surveyed in Queensland including nine of the 12 irrigated crops inspected on the Darling Downs. Fusarium wilt was not observed in any of the rain grown crops that were inspected. The incidence of Fusarium wilt averaged 4.4% and 2.5% respectively, for the Darling Downs and St George areas and only exceeded 5% in five fields (Figure 2).

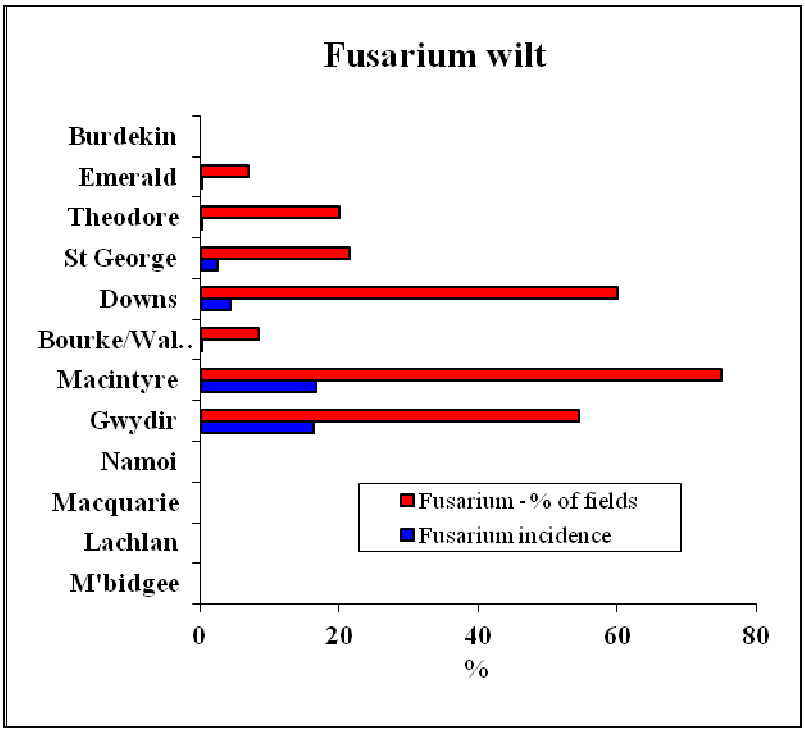


***Figure 2**. The distribution and mean incidence of Fusarium wilt of cotton in the 2011/2012 season.*

Transects have been established in fields near Emerald, Theodore, Moura, St George, Boggabilla, Moree and Gunnedah. The incidence of Fusarium wilt is assessed along these transects in seasons when cotton is grown in these fields. Assessments during the 2011/2012 season show a significant increase in disease incidence in fields near Boomi, St George and some of the fields near Boggabilla but little or no increase in fields near Moura and in other fields near Boggabilla.

BLACK ROOT ROT

Black root rot of cotton is favoured by cool weather conditions early in the season. The pathogen colonises the root surface, suppresses the development of secondary roots and

stunts seedling growth. When temperatures rise the tap root expands and the blackened root surface is sloughed off and disappears. The seasonal conditions in November were generally warmer and wetter than normal and temperatures in December of 2011 were well below average.

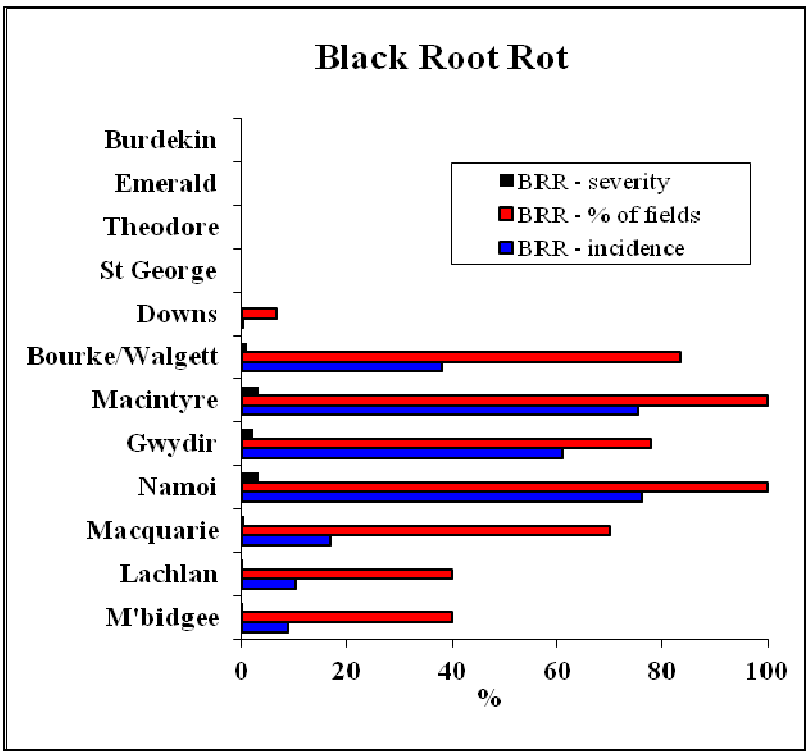


Figure 3. *The distribution, incidence and severity of black root rot in cotton in the 2011/2012 season.*

Black root rot was observed in 73% of the fields surveyed in NSW (Figure 3) including all of the fields visited in the Namoi and Macintyre Valleys. The average incidence within fields was 38% which included 33 fields where more than 50% of plants were affected. Two fields in the Namoi Valley and one field in the Gwydir Valley had over 99% of plants affected. Verticillium wilt was present in 24 (72%) of the 33 fields that had a high incidence of black root rot.

Assessment of disease 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 mean severity of black root rot for fields in the Namoi, Gwydir and Macintyre Valleys was 3.0, 2.4 and 3.1 respectively.

Black root rot has previously been observed in all Queensland cotton production areas except the Burdekin. There were several reports of black root rot in cotton crops on the Darling Downs but it was only recorded in one of the fields surveyed.

VERTICILLIUM WILT

Verticillium wilt is also favoured by cooler weather and is rarely observed in Queensland production areas. The disease was observed in 51% of fields surveyed in NSW. However, the average incidence was only 6.8% of plants infected (Figure 4). The average incidence of Verticillium wilt of cotton in NSW was estimated in previous surveys to be 4.1%, 3.8% and 3.7% for the 2008/2009, 2009/2010 and 2010/2011 seasons.

It has long been recognized that the resistance of cotton to the pathogen that causes Verticillium wilt is temperature sensitive. Resistant varieties become completely susceptible when temperatures drop by 5^0C**.** It is not unusual to see cotton plants with severe foliar symptoms and stunting if the weather in November is cool but symptoms generally disappear during December, as the season warms up. However, summer temperatures in 2011/2012 were well below normal (Tables 1 & 2) and symptoms of Verticillium wilt became even more severe. In the upper Namoi some fields of cotton were exposed to 22 days in December when overnight minimum temperatures were below 15^0C and 100% of plants were infected in some patches**.**

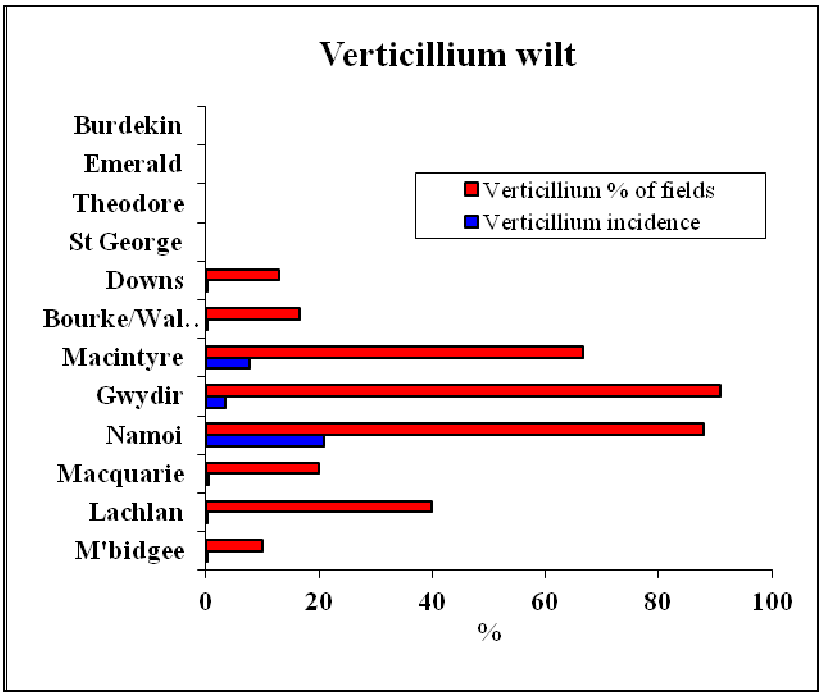


Figure 4. *The distribution and incidence of Verticillium wilt of cotton in the 2011/2012 season. The disease was present in many areas but the incidence was generally low.*

Verticillium wilt was observed in 88% of fields surveyed in the Namoi valley where the average incidence of the disease was 20.8% of plants infected (compared to 14.0%, 12.7 and 13.1% in the previous three seasons). Two fields in the Namoi Valley had over 80% of plants with symptoms and both of these fields had been severely affected by black root rot earlier in the season.

The distribution and incidence of Verticillium wilt increased significantly in crops in the Gwydir and Macintyre Valleys in the 2011/2012 season. The mean incidence of the disease in crops in the Macintyre Valley was estimated to be 7.8% which is higher than that recorded previously and the disease was observed in 67% of the fields inspected. Verticillium wilt was recorded in 90.1% of fields surveyed in the Gwydir Valley with the mean incidence estimated to be 3.4%. The disease was observed for the first time in the Murrumbidgee Valley during the 2011/2012 season.

BOLL ROTS

The average incidence of boll rots was recorded as 1.6% for NSW and 6.8% for Queensland; (9.7% and 7.3% in 2009/2010, 0.7% and 2.7% in 2010/2011).

Boll rots were particularly prevalent in crops in. the central highlands of Queensland where the average incidence was 16.7% with over 20% of bolls affected in four of the 11 crops surveyed in late February. These boll rots developed when bolls that were maturing and opening were exposed to wet weather in late January and early February. Wet weather in March further delayed harvest and resulted in some instances where seeds germinated in the bolls and fibre quality was significantly downgraded.

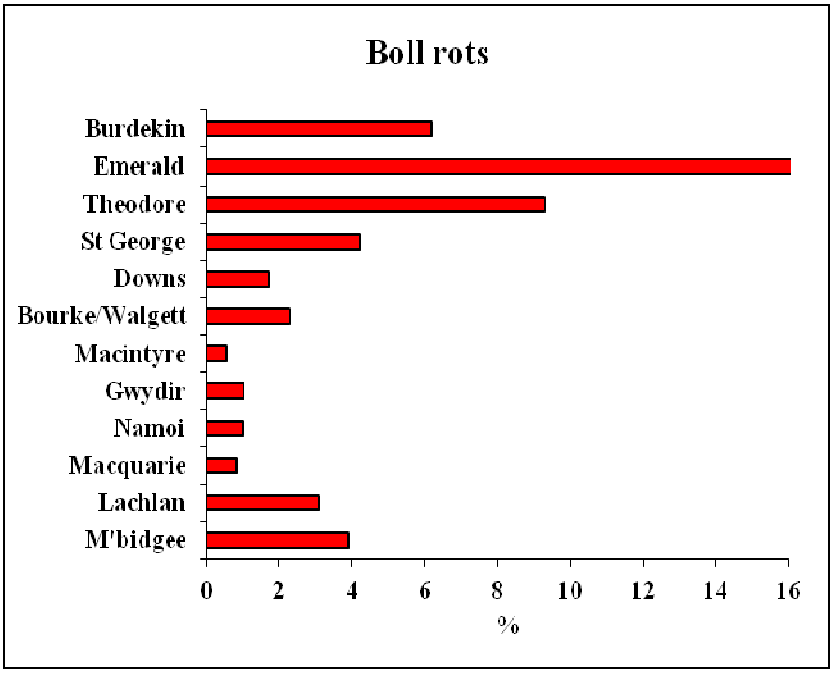


Figure 5. *The average incidence of boll rots in each of the cotton production areas for the 2011/2012 season.*

Phytophthora boll rot occurs when heavy rain splashes soil up onto low maturing bolls or when low bolls are inundated by water during flooding. The significant rainfall and flooding in Southern NSW late in the season (Table 3) contributed to a higher incidence of this disease in the Lachlan and Murrumbidgee Valleys.

Another boll rot, identified as Lasiodiplodia (Diplodia) boll rot, has been noticed in crops growing in the Central Highlands of Queensland in recent years. Affected bolls are covered in 'sooty' black spores. It is found throughout the world in the more humid climates.

SEED ROT

Symptoms of seed rot include a soft brown rot of developing seed within the bolls that may not become apparent until the bolls either drop or open prematurely. Only one or two locks, or sometimes the whole boll, can be affected. Seed rot appears to be caused by either bacteria or fungi that are introduced into the young developing boll by sucking insects such as the green vegetable bug and the cotton lint stainer. Seed rot was observed in 90% of fields inspected in Queensland with a mean incidence of 1.1%. The incidence of bolls affected by seed rot in one field in the Burdekin Valley was estimated to be 6% and the incidence in a field on the Darling Downs was assessed to be 4.3%.

SCLEROTINIA BOLL AND STEM ROT

The extended period of cool, wet weather coinciding with flowering in late January and early February resulted in numerous reports of Sclerotinia boll and stem rot in crops growing under lateral move and centre pivot irrigation in the Macintyre, Namoi and Macquarie Valleys. The disease was also reported in a few furrow irrigated crops. The NSW disease survey found the disease in three fields in the Namoi Valley, five fields in the Lachlan Valley and one field in the Murrumbidgee Valley. The incidence of the disease was generally low with up to 2% of bolls affected in some crops.

Sclerotinia has been seen previously on rare occasions in Australia and is not referred to as a disease of cotton in the international literature. When it does occur it is usually in areas of the field were growth is rank and humidity in the canopy is higher.

The pathogen that causes this disease is widespread, has a large host range which includes sunflower, canola and legumes and can survive for years in the soil as resistant black sclerotia that may vary in size from a few millimeters to several centimeters across.

The sclerotia germinate to produce apothecia (small cream coloured 'golf tees' – not to be confused with bird's nest fungi!) which release clouds of microscopic spores that can only infect the plant through dead or dying flower petals or dead leaves 'hung up' in the canopy. The fungus then grows into healthy plant tissue such as the developing boll and down the fruiting branch towards the main stem producing the hard black sclerotia in and on bolls and on infected branches. The presence of sclerotia is a distinctive feature of Sclerotinia boll and stem rot.

Another fungus that thrives when it is cool and wet or humid is *Botrytis* sp. which may produce a grey powdery mould on dead flowers and leaves. There was some confusion

between *Botrytis* sp. and *Sclerotinia* sp.. *Sclerotinia* sp. only produces spores in the apothecia on the soil surface while Botrytis produces spores on dead plant material within the canopy.

BUNCHY TOP

In contrast with the previous season, the incidence of 'bunchy top' was quite low. Bunchy top was observed in 18.6% of the fields surveyed in Queensland production areas and in 25% of fields surveyed in NSW with the average incidence only 0.05% in Queensland and 0.7% in NSW. It was estimated that 8.5% of plants were affected in one field in the Lachlan Valley.

OTHER DISEASES AND DISORDERS

Tobacco Streak Virus was observed in seven of the 14 crops inspected in central Queensland in November 2011. The average incidence of the virus was 0.1% with 0.5% of plants with symptoms in two fields.

Alternaria leaf spot was present at low levels in almost all crops and was generally of minor significance except in crops in the Burdekin Valley where leaf spots caused significant defoliation of lower leaves.

Premature senescence was noted in 58% of the crops surveyed in Queensland. However, the average incidence was only 1.6%.

ACKNOWLEDGMENTS

These surveys were made possible with the financial support of the Cotton Research & Development Corporation, the Cotton Catchment Communities CRC, Cotton Seed Distributors Ltd., NSW Department of Primary Industries and the Queensland Department of Agriculture, Fisheries and Forestry.

1.3 2012/2013 Cotton Disease Surveys

Cotton Pathology 2012-2013

K.A. Kirkby[1], P.A. Lonergan[1], B.R. Cooper[1], S.E.Roser[1], L.J. Smith[2], L.J.Scheikowski[3], B.Bauer[2], J. Lehane[3], and S.J. Allen[4]

[1]NSW DPI, Locked Bag 1000, Narrabri NSW

[2]DAFF Queensland, Ecoscience Precinct, GPO Box 46, Brisbane, Qld

[3]DAFF Queensland, 203 Tor Street, Toowoomba, Qld

[4]Cotton Seed Distributors Ltd., PO Box 17, Wee Waa NSW

Commercial cotton crops across NSW and Queensland were inspected in October-December 2012 and February-April 2013. The incidence and severity of those diseases present were assessed and field history, ground preparation, cotton variety, planting date and seed rate were recorded for each of the 112 and 48 fields that were surveyed in NSW and Queensland respectively. This represents the 30th consecutive season of quantitative disease surveys of cotton in NSW and the 11th consecutive season of cotton disease surveys in Queensland.

Daily maximum temperatures were well above average from planting through to the end of January, 2013 and then average or below average from February to harvest. The daily maximum temperature exceeded 45C in many areas in mid-January, 2013. At Bourke, in western NSW, temperatures exceeded 35C on 111 days and over 45C on 24 days between 1st October, 2012 and 20th April, 2013.

In contrast the cold spell and rainfall that occurred in NSW cotton production areas on 11th and 12th October, 2012 interrupted planting and produced problems with stand establishment. Widespread rainfall in late January, associated with Cyclone 'Oswald,' was accompanied with a drop in daily maximum temperatures. The cooler weather was further accompanied by above average rainfall in February and March, 2013.

The incidence and severity of plant diseases is determined by environmental conditions. The cooler and wetter 2011/2012 season favoured the appearance of Sclerotinia stem and boll rot, severe Verticillium and Fusarium wilt and problems with boll rot in central Queensland. The hot and dry conditions experienced during much of 2012/2013 suppressed the development of most diseases of cotton. However, the cooler and wetter autumn weather did allow late development of severe Verticillium wilt.

VOLUNTEER COTTON – (Carry-over from the previous season)

Information on the occurrence of volunteer cotton was collected during the annual disease surveys and is based on visits to 54 farms in NSW and 21 farms in Queensland

during October and December of 2012 (Table 1). The number of farms with (1) mature cotton plants surviving along roadsides, fence lines, along channels and in tail water return systems or drains, (2) volunteer cotton in fallow or rotation fields and (3) mature cotton plants surviving from the previous season or regrowth from stubs (Ratoon cotton?) in current cotton crops, were recorded.

Table 1 *The occurrence of volunteer cotton plants surviving from the previous season on farms in NSW and Queensland in the spring of 2012.*

	1. Along channels, roads, fences	**2. In fallows and rotation crops**	**3. In the current crop (regrowth from stubs)**	**TOTAL**
In NSW	22/54 (41%)	13/49 (26%)	40/54 (74%)	44/54 (81%)
In Qld	11/21 (54%)	2/21 (9%)	11/21 (54%)	15/21 (71%)
Total	33/75 (44%)	15/70 (21%)	51/75 (43%)	59/75 (79%)

The presence of volunteer plants surviving over from the previous season enables pests and pathogens such as aphids, mealy bug and cotton bunchy top to overwinter and initiate new outbreaks in the spring. Volunteer cotton plants were observed on 59 of the 75 farms visited during the disease surveys (79%).

Cotton Industry Biosecurity Plan – Crop Surveillance for Priority Pests

During these surveys particular attention was given to surveying fields for the presence/absence of exotic diseases including Cotton Leaf Curl Virus, Blue disease, Phymatotrichopsis (Texas) root rot, the hypervirulent strains of the bacterial blight pathogen, the defoliating strains of the Verticillium wilt pathogen and exotic strains of the Fusarium wilt pathogen. None of these diseases and/or pathogens were observed.

SEEDLING MORTALITY

As part of the disease survey an estimate of the number of seeds planted per metre is compared to the number of plants established per metre. This comparison produces an estimate of seedling mortality which includes the impact of seedling disease (*Rhizoctonia* and *Pythium* etc.) as well as seed viability, the activity of soil insects such as wireworms, physical problems such as fertiliser or herbicide burn and the effects of adverse environmental conditions.

Mean seedling mortality (Figure 1) for the crops inspected in NSW and Queensland was 32.1% and 26.6% respectively in the 2012-13 season, (32.3% and 29.6% in 2011-12 and 31.9% and 25.8% in 2010-11).

Mean seedling mortality was lowest (21.1%) in the Tandou and St George/Dirranbandi areas and highest in crops in the Macquarie Valley (39.1%) and Bourke/Walgett (37.6%). Problems with crop establishment included wireworm, symphlids, the necessity to flush irrigate while seedlings were still emerging, uneven moisture in the

seed bed resulting from rain prior to planting as well as the activity of the seedling disease pathogens.

In southern NSW the most affected fields were those that were either sown into moisture or watered up on 5th and 6th October, 2012 and then received rainfall on 11th and 12th October along with daily minimum soil temperatures < 12°C between 11th and 14th October. The mean seedling mortality in these fields was approximately double (50.5%) that observed in other fields planted either before or after 5th and 6th October, 2012.

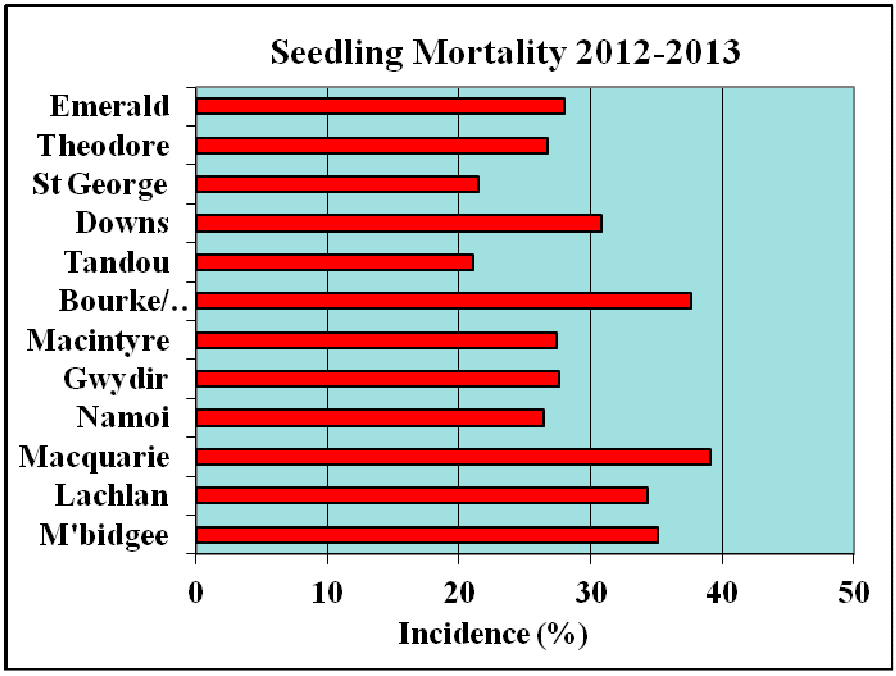


Figure 1. *Mean seedling mortality in the 2012/13 season. Seedling mortality is derived from the difference between the number of seed planted and the number of plants established.*

FUSARIUM WILT

Fusarium wilt (*Fusarium oxysporum* Schlecht f.sp. *vasinfectum* Atk. Sny. & Hans.) is most severe when October/November rainfall is above normal and when temperatures are below normal – as was experienced during the 2011-12 season. The disease is least severe when it is hot and dry in spring. The widespread adoption of the new, more resistant, varieties reduced the potential impact of the disease. Fusarium wilt was again obvious during early season surveys. Later in the season common symptoms included gaps in the stand, stunted growth and a dark brown discoloration of the vascular tissue in the stem. Wilting, dead and dying plants were not always present as was observed in previous years with more susceptible varieties.

There was one new report of Fusarium wilt ('Downs' strain) on a farm in the Murrumbidgee Valley. This new report was confirmed by Dr Linda Smith (DAFF Queensland) who provides a free, confidential diagnostic service for Fusarium wilt of cotton funded by the Australian cotton industry. This represents the first report of Fusarium wilt of cotton in the Murrumbidgee Valley.

Fusarium wilt was observed in 18 of the 112 crops surveyed in NSW including nine of the 12 crops inspected in the Macintyre Valley and seven of the 14 crops surveyed in the Gwydir Valley. The incidence of Fusarium wilt (Figure 2) averaged 11.6% and 5.2% (respectively), for these two production areas (16.6% and 16.4% in 2011-12; 8.8% and 9.9% in 2010/11) and exceeded 30% of plants affected in two of the 18 fields. Though Fusarium wilt is known to be present and widespread in the Macquarie Valley and the upper Namoi Valley it has not been prevalent in fields surveyed in any of the last four seasons.

It is interesting to note that black root rot was also present in 17 of the 18 fields in NSW where Fusarium wilt was recorded. The incidence of black root rot exceeded 40% in 11 of the 18 fields.

The disease was observed in 20 of the 48 crops surveyed in Queensland including 11 of the 12 irrigated crops inspected on the Darling Downs. Fusarium wilt was not observed in the rain grown crops that were inspected. The incidence of Fusarium wilt averaged 3.4% and 1.2% respectively, for the Darling Downs and St George areas compared to 4.4% and 2.4% in the previous season (Figure 2).

Disease survey results over the last five seasons (Figure 2) reveal the impact of favourable weather conditions in the 2011-12 season and the trend in increasing disease incidence that is particularly evident in crops surveyed in the Macintyre Valley and on the Darling Downs.

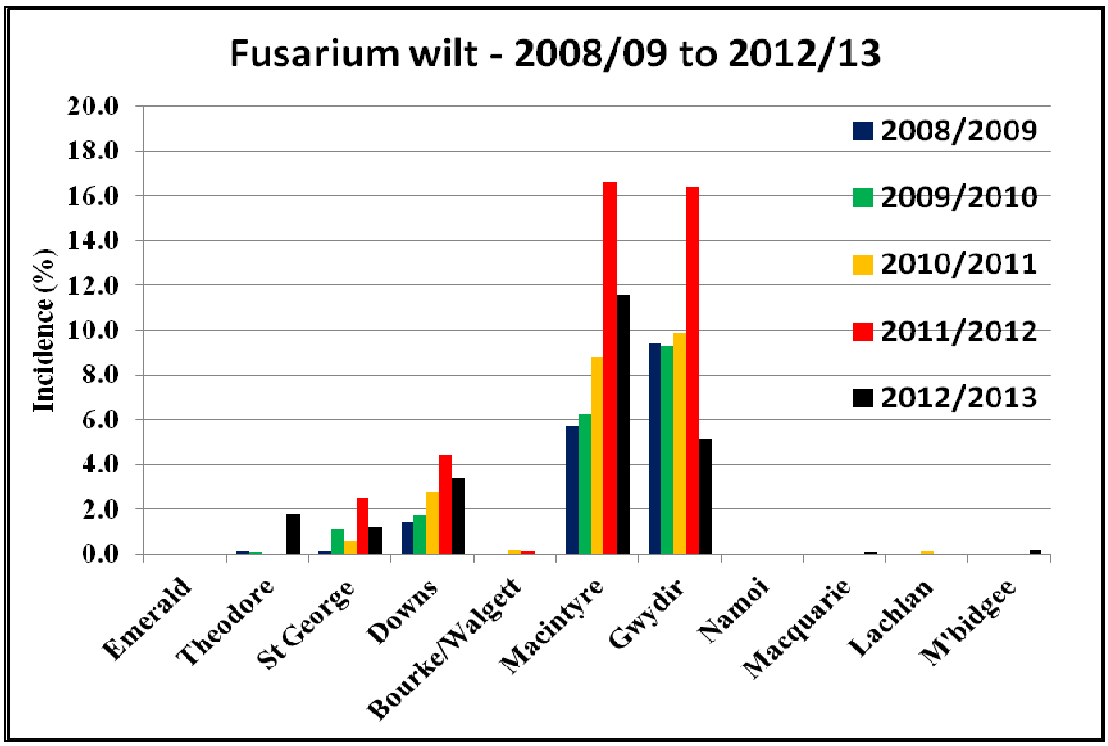


***Figure 2**. The mean incidence of Fusarium wilt of cotton in the five seasons from 2008-09 to 2012-13. Fusarium wilt is present in all cotton production areas listed.*

Transects have been established in fields near Emerald, Theodore, Moura, St George, Boggabilla, Moree, Boomi, Narrabri and Gunnedah. The incidence of Fusarium wilt is assessed along these transects in seasons when cotton is grown in these fields. Assessments during the 2012/13 season showed a decrease in disease incidence in eight of the ten transects. Factors contributing to this observed decrease include the use of varieties with the highest level of resistance and the less favourable hot seasonal weather conditions during the 2012-13 season compared to the cooler and wetter 2011-12 season.

BLACK ROOT ROT

Black root rot of cotton (*Thielaviopsis basicola* [Berk.] & Br.) is favoured by cool weather conditions early in the season. The pathogen colonises the root surface, suppresses the development of secondary roots and stunts seedling growth. When temperatures rise the tap root expands and the blackened root surface is sloughed off and disappears. Black root rot of cotton was apparent early in the 2012-13 season but the severity of symptoms declined with the above average spring temperatures – except where the warm temperatures caused seed beds to dry back too quickly and an extra irrigation was required to establish the crop.

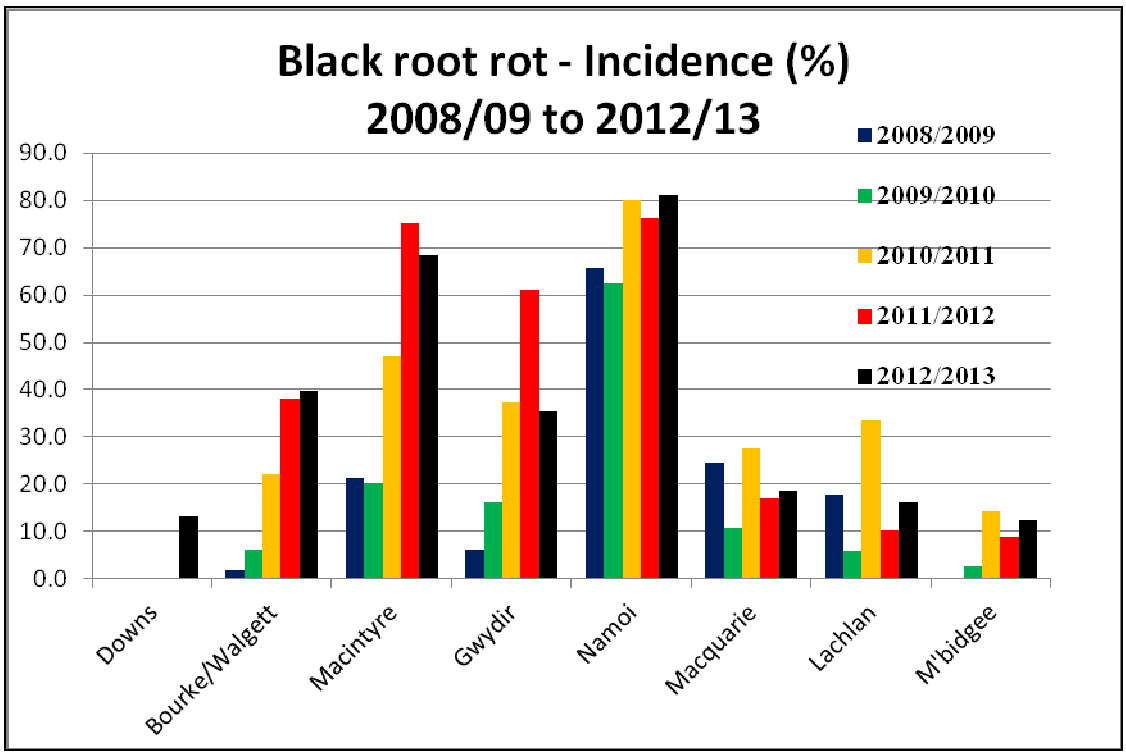


Figure 3. *The incidence of black root rot of cotton in the five seasons from 2008-09 to 2012-13.*

Disease survey results over the last five seasons (Figure 3) reveal the impact of favourable weather conditions in the 2011-12 season and the trend in increasing disease incidence that is particularly evident in crops surveyed in the Bourke/Walgett area, Macintyre Valley, Gwydir Valley and Namoi Valley.

The average incidence of black root rot within fields was 40% for NSW. This included 48 fields where more than 50% of plants were affected and four fields in the Namoi Valley, three fields in the Macintyre Valley, one field in the Murrumbidgee Valley and one field at Tandou where all plants were infected. The disease was found in all of the fields visited in the Namoi and Macintyre Valleys, 86% of fields surveyed in the Gwydir Valley; 64% of fields in the Bourke/Walgett area, 50% of fields in the Tandou area, Macquarie Valley and Lachlan Valley and 21% of fields in the Murrumbidgee Valley. Verticillium wilt was present in seven (78%) of the nine fields that had a high incidence of black root rot.

Assessment of disease 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 mean severity of black root rot for fields in the Namoi, Gwydir and Macintyre Valleys was 3.6, 1.1 and 3.5 respectively (3.0, 2.4 and 3.1 in 2011-12).

Black root rot has previously been recorded in all Queensland cotton production areas except the Burdekin. The disease was observed in eight of the 14 fields surveyed on the Darling Downs and two of the 15 fields surveyed in the St. George/Dirranbandi area. The mean incidence of black root rot in crops on the Darling Downs was estimated to be 13.1%.

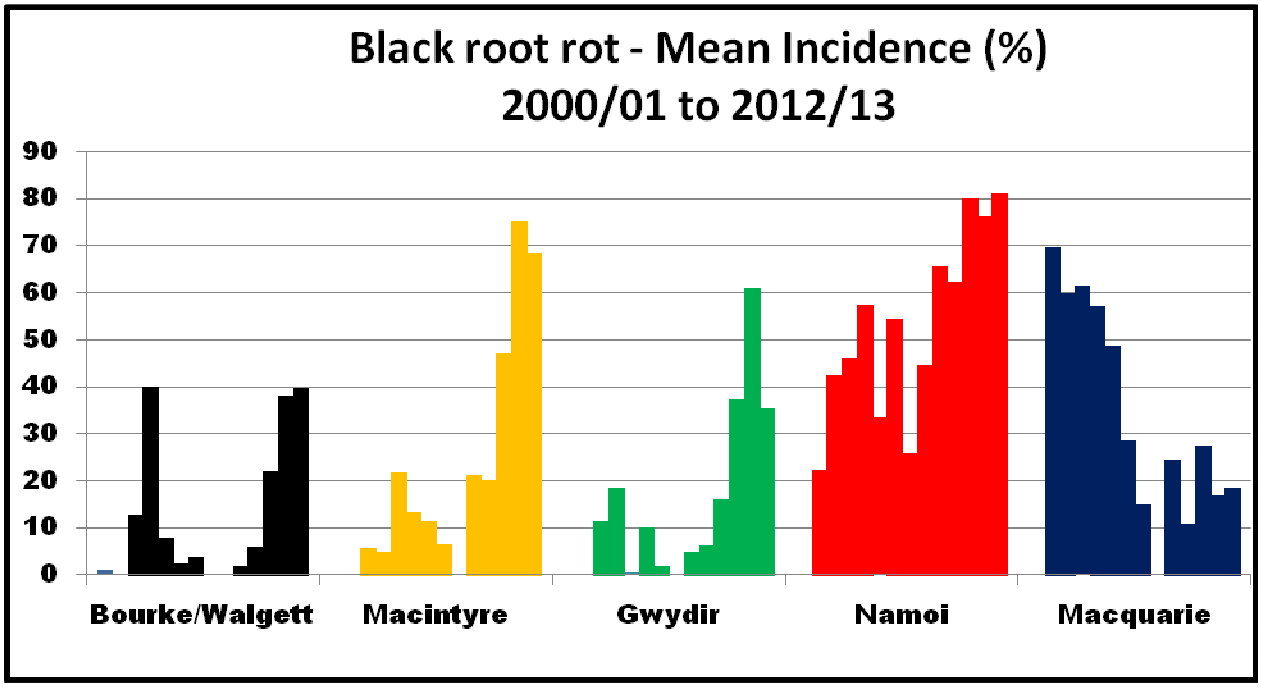


Figure 4. *The incidence of black root rot of cotton in NSW production areas in the 13 seasons from 2000-01 to 2012-13. The impact of the drought between 2003 and 2010 is very obvious.*

A review of the mean incidence of black root rot in the established cotton production areas of NSW since the 2000-01 season (Figure 4) indicates that black root rot was more prevalent in the Macquarie Valley than in the Namoi Valley prior to the drought years between 2003 and 2010. The reduced cropping and enforced long fallows during the drought resulted in a dramatic reduction in the mean incidence of black root rot. The impact of the drought was least severe in the Namoi Valley and most severe in the Bourke/Walgett area. As mentioned previously – during the last five seasons since the drought the incidence of black root rot has increased significantly.

VERTICILLIUM WILT

Verticillium wilt (*Verticillium dahliae* Klebahn) is also favoured by cooler weather and is rarely observed in Queensland production areas. The disease was observed in 38% of fields surveyed in NSW during the 2012-13 season. However, the average incidence was only 5.3% of plants infected (Figure 5). This can be compared with average incidences of 6.8%, 3.7%, 3.8% and 4.1% in the 2011-12, 2010-11, 2009-10, and 2008-09 seasons (respectively). Symptoms of Verticillium wilt observed during the 2012-13 season were not as severe as those seen in the cooler and wetter 2011-12 season.

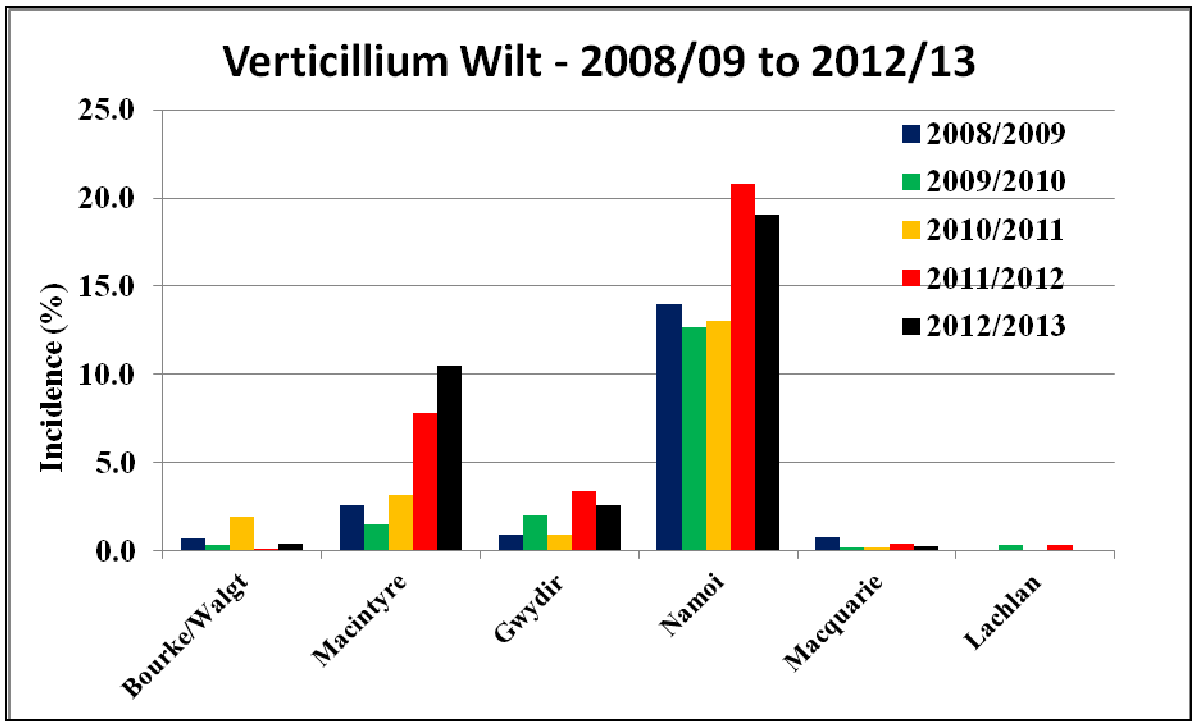


Figure 5. *The distribution and incidence of Verticillium wilt of cotton 2008/09 to 2012/13. The disease was present in many areas but the incidence was generally low.*

Verticillium wilt was observed in all of the fields surveyed in the Macintyre Valley during the 2012-13 season and the average incidence was 10.5% of plants affected (Figure 5). The disease was observed in 86% of fields surveyed in the Namoi valley, 57% of fields in the Gwydir Valley and 21% of fields in the Bourke/Walgett area where the average incidence of the disease was 19.5%, 2.6% and 0.6% (respectively). Two fields in the Namoi Valley had over 75% of plants with symptoms and both of these fields had been severely affected by black root rot earlier in the season.

The distribution and incidence of Verticillium wilt has increased significantly in crops in the Namoi, Gwydir and Macintyre Valleys in the 2011/12 and 2012-13 seasons (Figure 5). The disease was observed for the first time in the Murrumbidgee Valley during the 2011/12 season.

BOLL ROTS

The average incidence of boll rots in the 2012-13 season was recorded as 0.9% for NSW and 1.5% for Queensland; (1.6% and 6.8% in 2011-12; 0.7% and 2.7% in 2010/11; 9.7% and 7.3% in 2009/10). Only 2.6% of bolls were affected in crops in the Macintyre Valley and 2.4% of bolls in crops in the Emerald area. It should be remembered that the disease surveys are completed in February and the final incidence of boll rots at harvest may be significantly higher.

The most common boll rot in NSW production areas is Phytophthora boll rot, which develops when soil is splashed up onto low opening bolls. Boll rots are most severe in Emerald and Theodore when opening bolls are subjected to extended periods of wet and cloudy weather and harvest is delayed.

Rainfall in Emerald exceeded 2.0mL on only three days in January, 2013 and five days in February, 2013 compared to eleven days in January, 2012 and seven days in February, 2012. Despite the fact that many of the crops in the Emerald area were planted in September and early October, 2012 and bolls were maturing and opening in January, 2013 the mean incidence of boll rots (2.4%) was well below the mean incidence of boll rots observed in the previous season (16.7%).

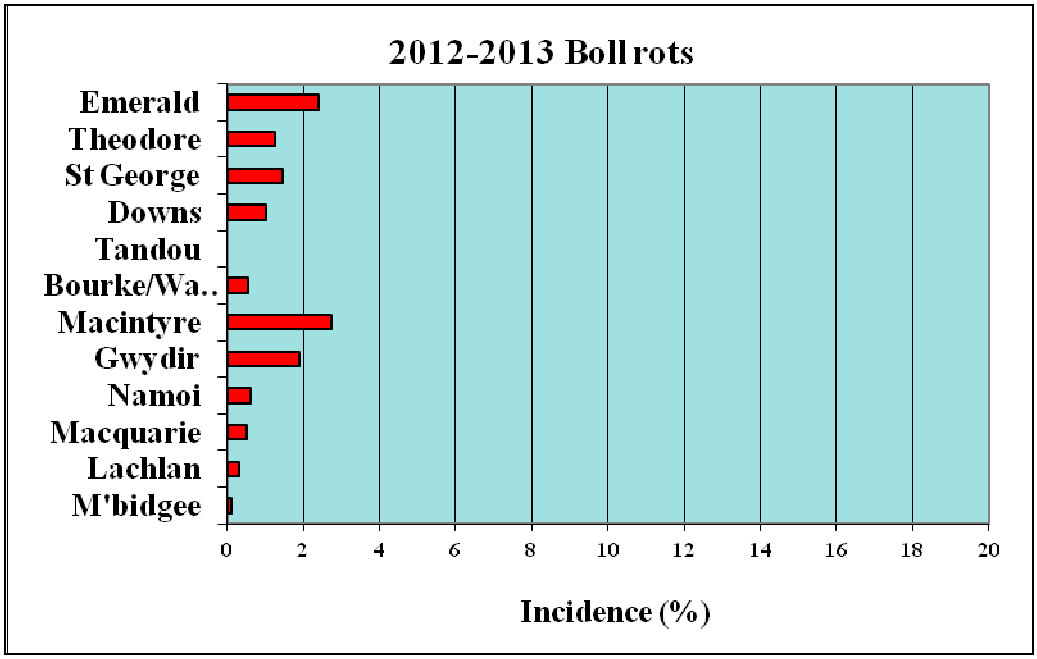


Figure 6. *The average incidence of boll rots in each of the cotton production areas for the 2012/13 season.*

RENIFORM NEMATODE

Stunted seedlings and roots with 'nodules' (Figure 7) were collected from fields near Theodore in Queensland during the annual disease survey in November, 2012. Nematologist Jenny Cobon of DAFF Queensland confirmed the presence of the reniform nematode (*Rotylenchulus reniformis*).

Figure 7. Egg sacks of the reniform nematode on seedling roots *(photo Damien Erbacher)*

Subsequent intensive sampling has found the nematode to be widespread in the Theodore area. The reniform nematode had previously been observed in a field near Emerald during the November, 2003 disease survey and was still present in this and other adjacent fields in 2012-13. Reniform nematode is considered to be a major pest of cotton in parts of the USA.

OTHER DISEASES AND DISORDERS

Bunchy top was observed in only two (4.2%) of the fields surveyed in Queensland production areas and in 31% of fields surveyed in NSW with the average incidence only 0.01% in Queensland and 1.7% in NSW. It was estimated that 24.5% of plants were affected in one field in the Walgett area of NSW.

Seed rot was observed in 23 of the 48 (48%) crops inspected in Queensland. The average incidence was only 0.4% although 4.5% of bolls were affected in one field near Emerald.

Tobacco Streak Virus was observed in two of the 11 crops inspected in central Queensland in November 2012. The incidence of the virus was very low.

Alternaria leaf spot was present at low levels in almost all crops and was generally of minor significance.

Premature senescence was noted in 29% of the crops surveyed in Queensland. However, the average incidence was only 0.7%.

Sclerotinia boll and stem rot was not observed during disease surveys in the 2012-13 season.

ACKNOWLEDGMENTS

These surveys were made possible with the financial support of the Cotton Research & Development Corporation, Cotton Seed Distributors Ltd., NSW Department of Primary Industries and the Department of Agriculture, Fisheries and Forestry Queensland. The cooperation of cotton growers is greatly appreciated.

Exotic diseases:

No exotic diseases have been observed during the course of this project. Dr Kirkby undertook a 3 week trip to Texas, USA during July/August 2011 funded by the Department of Agriculture, Fisheries and Forestry (DAFF). The purpose of the trip was to undergo training in diagnosing these two diseases and gaining knowledge to prepare National Diagnostic Protocols for these two diseases. The overseas travel commenced on 16th July 2011 and finished on 8th August 2011. Between these dates Dr Kirkby visited:

- Dr Steve Hague (Plant Breeder) - shown around the university, discussed the upcoming activities and tour of the farm. Steve organised meetings with several key scientists, both at Texas A&M University and with the USDA.
- Professor James Starr (Plant Pathologist) - discussed protocols for bacterial blight, then I met with Dr Richard Percy (Research Leader), Dr John Yu (Geneticist), Dr Lori Hinze (Research Geneticist), Dr All Bell (Pathologist).
- USDA germplasm curator Dr James Frelichowski, Dr David Stelly (Geneticist) and Associate Professor Keerti Rathore (Institute for Plant Genomics & Biotechnology). Toured the facility, growth rooms, glasshouses and field trials as well as met with graduate students.
- Dr Gino Medrano (USDA Research Plant Pathologist), Dr Jinggao Liu (Research Chemist – Cotton Pathology), Dr Robert Stipanovic (Research Leader), Associate Professor Gaylon Morgan (State Extension Cotton Specialist). Had discussions and initiated conversation regarding collaborative work. USDA are very keen to do this.
- 23rd July – 29th July 2011 – College Station and a 1030 mile road trip with Dr. Thomas Isakeit visiting farms with disease.
- 30th July – 8th August 2011 – Lubbock to work with Dr Terry Wheeler on Bacterial Blight then back to Narrabri

Travelling to the natural range where these diseases occur in the USA allowed Dr Kirkby to gain first hand experience in diagnosing the diseases, experience that could not be gained in Australia. The benefit to the cotton industry is having a local diagnostician with specialist experience with these organisms, hence improving the biosecurity of the industry. Development of national standard diagnostic protocols for Texas root rot and hypervirulent bacterial blight has increased Australia's capacity to respond quickly should an exotic disease incursion occur. Acquisition of the full set of bacterial blight differential cultivars now enables designation of race to bacterial isolates in the event of an incursion.

The development of and leadership in assembling and delivering the National Diagnostic Protocols for these two pathogens ensures that the methods are validated and maintained by NSW DPI. These protocols will be implemented should an incursion occur in Australia. The biosecurity profile of Australian Cotton Research Institute (ACRI) and NSW DPI will be enhanced with the cotton industry and with the Commonwealth Department Agriculture, Fisheries and Forestry (DAFF). This will be of benefit in attracting external funding in the future.

1.4 Update Disease Symptoms image collection

Images have been taken of many disease symptoms and also the pathogens that cause disease. A lot of images were used in the newly published Cotton Symptoms Guide that was launched at the last CRC Forum in Narrabri. The image collection will continue to be added to during 2012/2013 season.

Figure 1.4.1. *Front cover of the updated disease symptoms guide.*

Objective 2 – Continue to evaluate existing and novel fungicide treatments for seedling disease and black root rot.

2.1 2010/2011 annual seed treatment fungicide trials:

Plant stands were not significantly different regardless of seed treatment at the three week count, however after six weeks in the early planted trial mean number of plants were significantly higher (P=0.048) in plots treated with Dynasty® Plus, Dynasty® CST and Apron® + Azoxystrobin (76, 76 and 75 plants respectively) in comparison to the untreated control with 68 plants (Figure 2.1.1). Seedling mortality after six weeks averaged 29% across the trial.

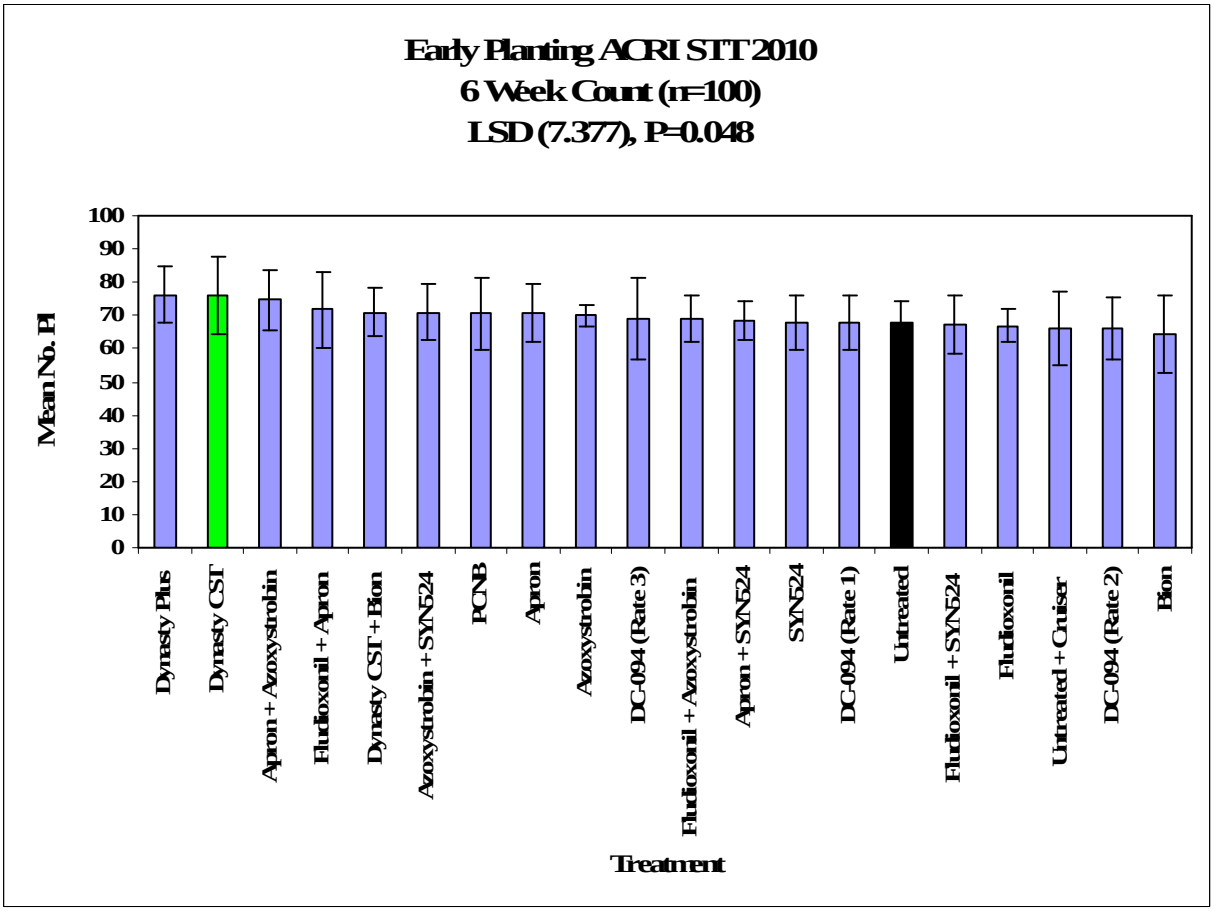


Figure 2.1.1 Mean stand counts six weeks after early sowing (17/09/10) following seed treatment with various fungicides at ACRI Narrabri site. Significant differences between seed treatments (P=0.048). The industry standard seed treatment is highlighted in green.

In the trial planted later (Normal) on the 13/10/10, plots treated with Dynasty® CST, Azoxystrobin, Apron® + Azoxystrobin, Dynasty® Plus, Fludioxonil + Apron® and Azoxystrobin + Syn524 had significantly (P<0.001) higher mean number of plants (83,81,81,81,80,80 plants respectively) after six weeks in comparison with the untreated control with 73 plants (Figure 2.1.2). Seedling mortality after six weeks averaged 25% across the trial.

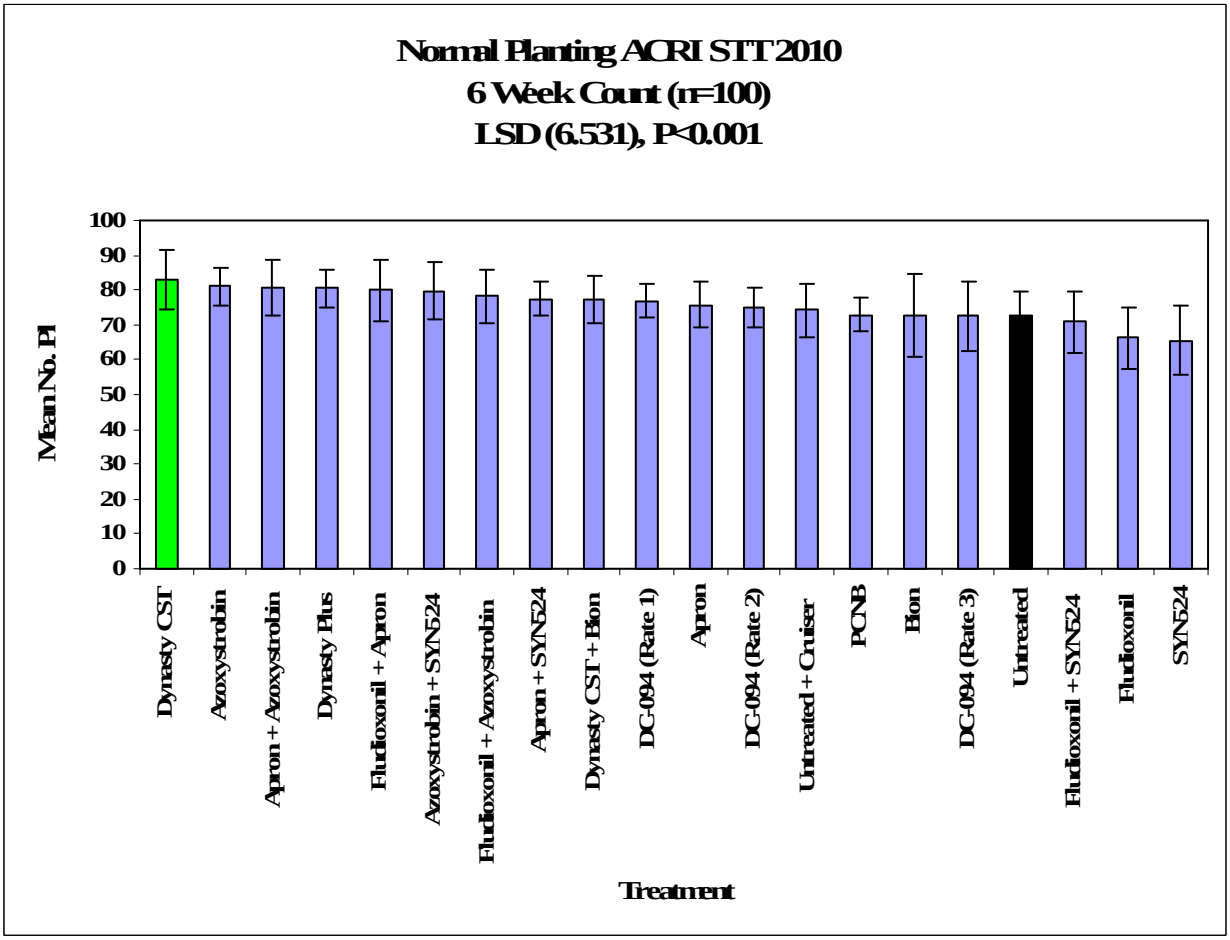


Figure 2.1.2 Mean stand counts six weeks after normal sowing date (13/10/10) following seed treatment with various fungicides at ACRI Narrabri site.

At the Mungindi site, seed treatments had a significant (P<0.001) effect on reducing seedling mortality in comparison to untreated plots (Figure 2.1.3). PCNB and Syn524 had no effect; however plots treated with the remainder of seed treatments had significantly higher stand counts compared to untreated control which had the lowest mean number of plants of 53. The lack of effect of PCNB (known to be effective against *Rhizoctonia*) indicated that *Pythium* spp. may have been the dominant pathogen early this season. This was consistent with observations of Pythium damage on plants. Seedling mortality averaged 37% across the trial which was 11% higher than last season.

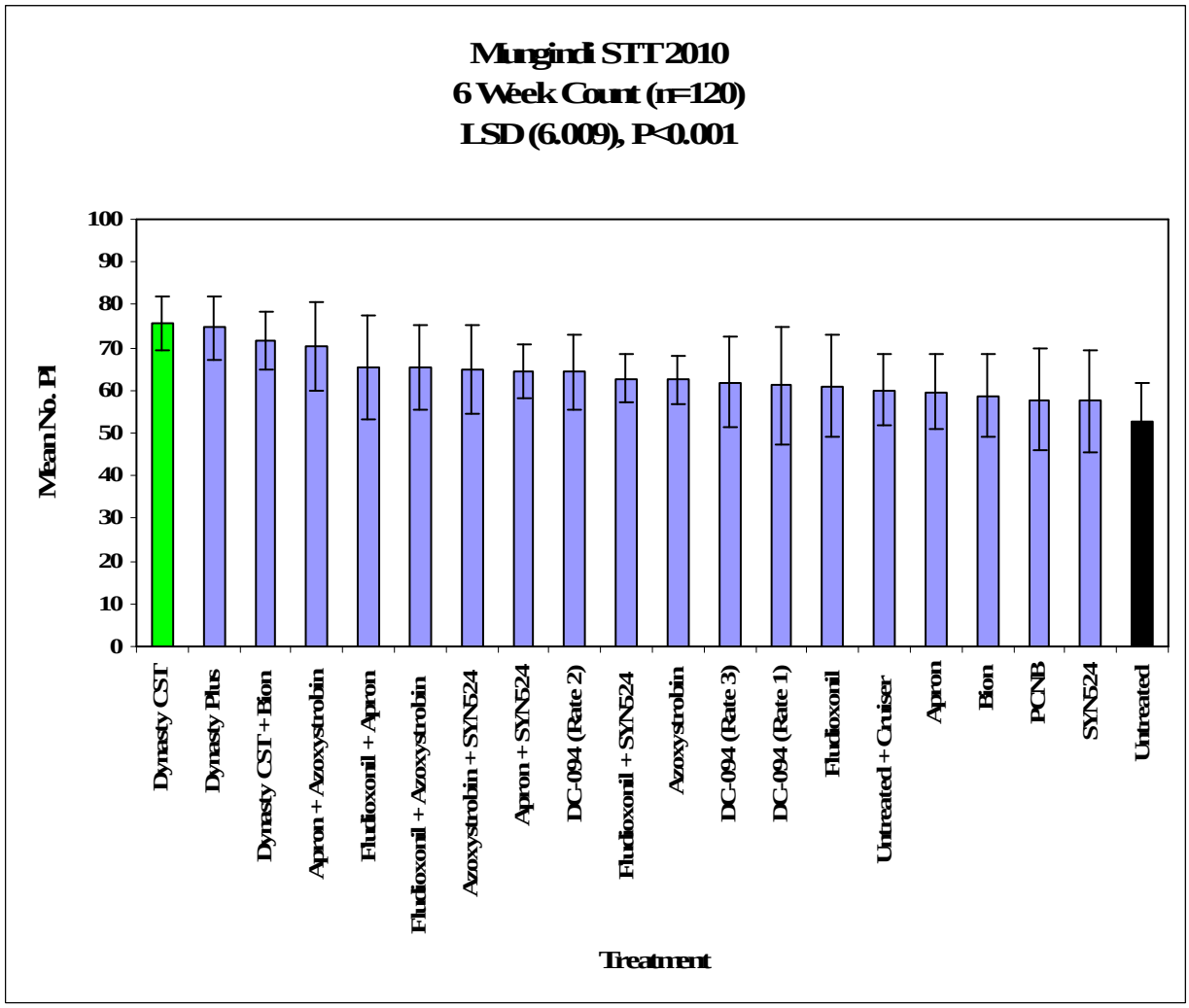


Figure 2.1.3 Mean stand counts six weeks after sowing (30/09/10) following seed treatment with various fungicides at Mungindi site.

At the Hillston site, stand counts were much lower with untreated plots recording a mean number of plants of 46. Of the 20 treatment, 12 had higher mean number of plants than that of the untreated control (Figure 2.1.4). At Hillston, seedling mortality averaged 47%.

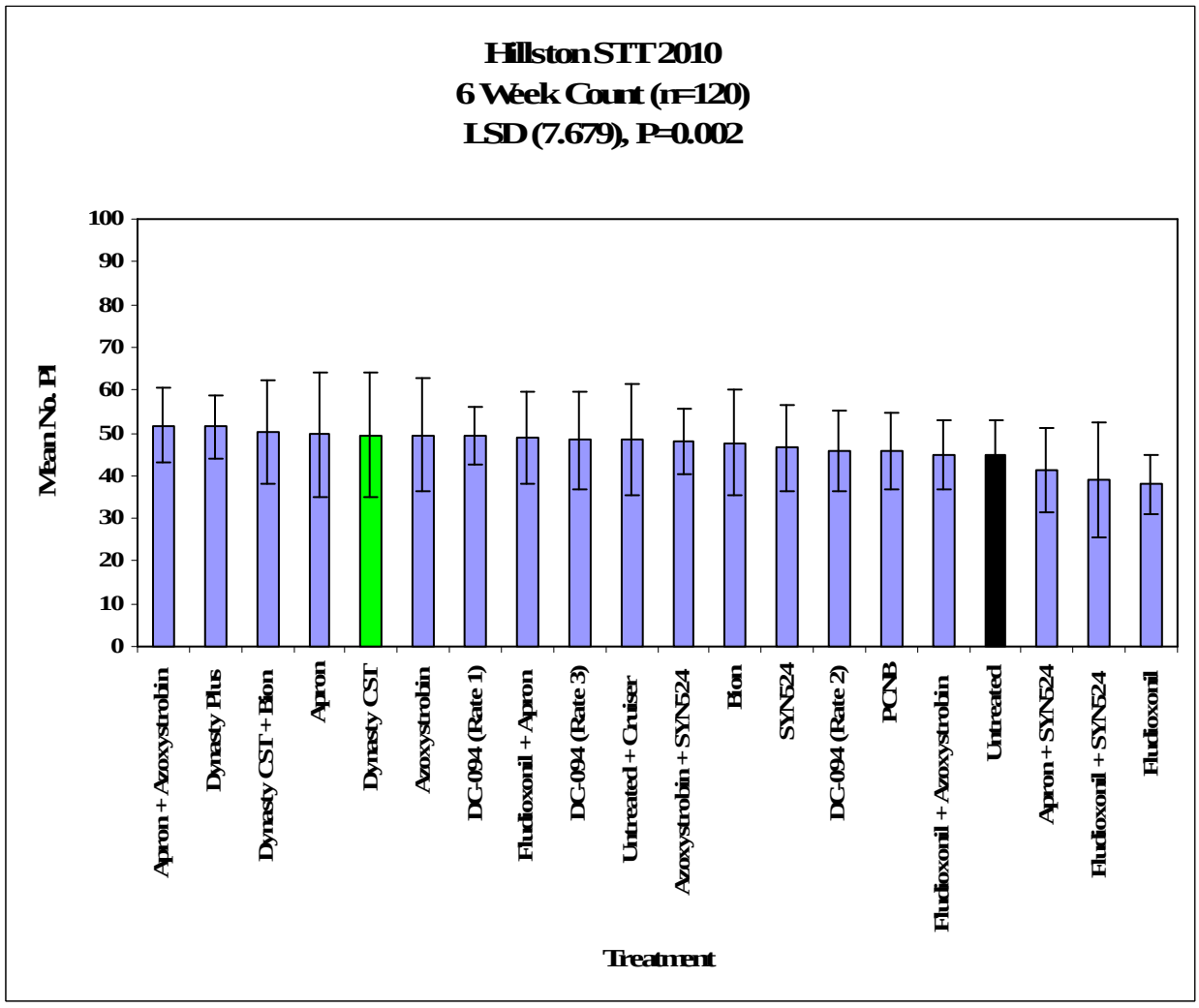


Figure 2.1.4 Mean stand counts six weeks after sowing (25/09/10 & 26/09/10) following seed treatment with various fungicides at Hillston site. Significant differences between seed treatments (P=0.002).

At the QLD Downs site, seedling mortality was moderate (Figure 2.1.5). Higher survival in plots treated with Apron and Apron combinations indicates the dominant pathogen was *Pythium* spp. Mean % of plants across the trial was 65%.

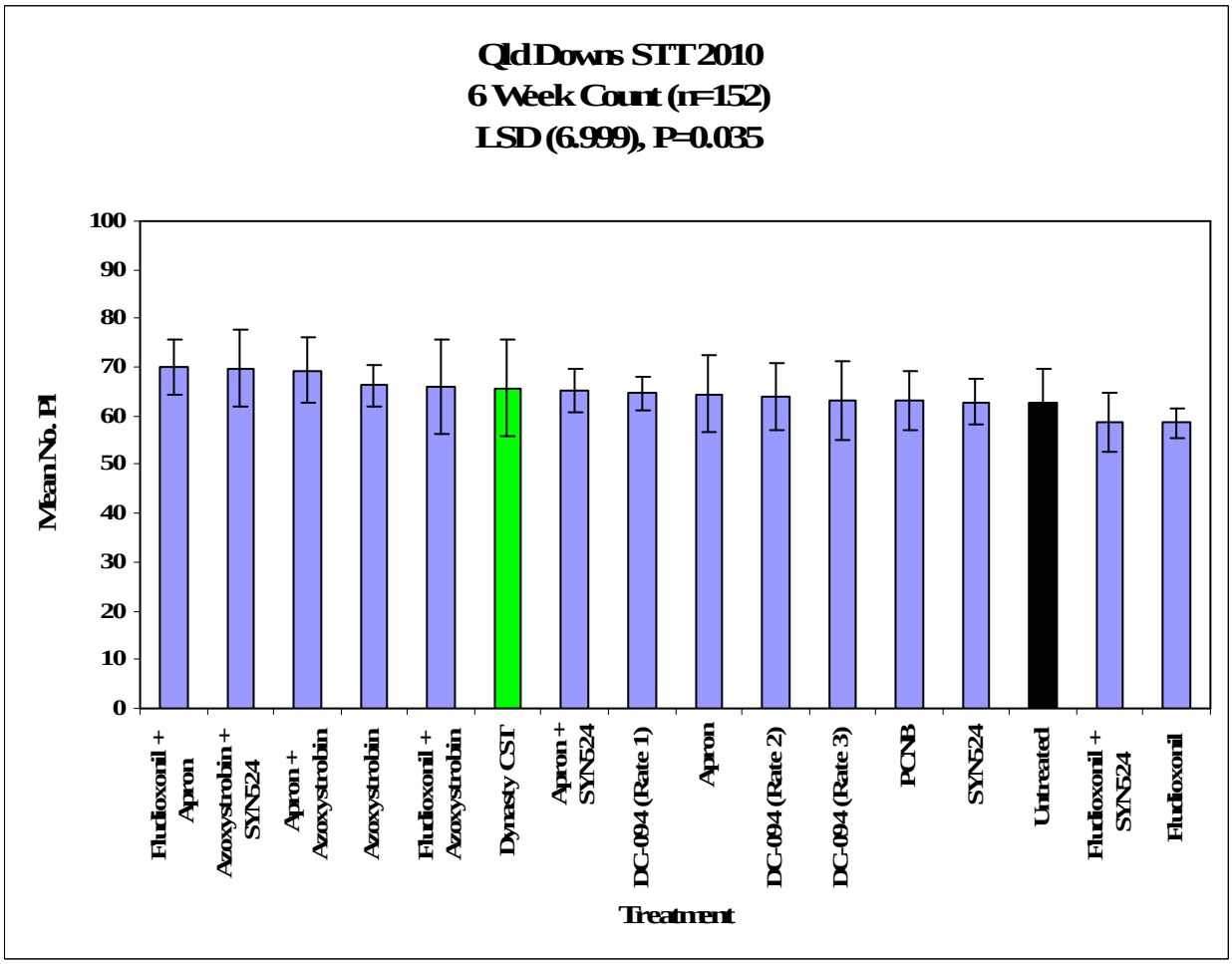


Figure 2.1.5 Mean stand counts six weeks after sowing (29/10/10) following seed treatment with various fungicides at QLD Downs site.

2.2 2011/2012 annual seed treatment fungicide trials:

At ACRI Narrabri, the trails were sown on the 7th September 2011 and again on the 12th October 2011. Plant stands treated with Bion alone were significantly lower than all other treatments at the three week count. There was no difference between the standard seed treatment Dynasty® Complete and the remaining treatments (Figure 2.2.1). The mean number of plants in the plots treated with Dynasty® Complete, untreated and Bion® was 38, 35 and 26 plants respectively.

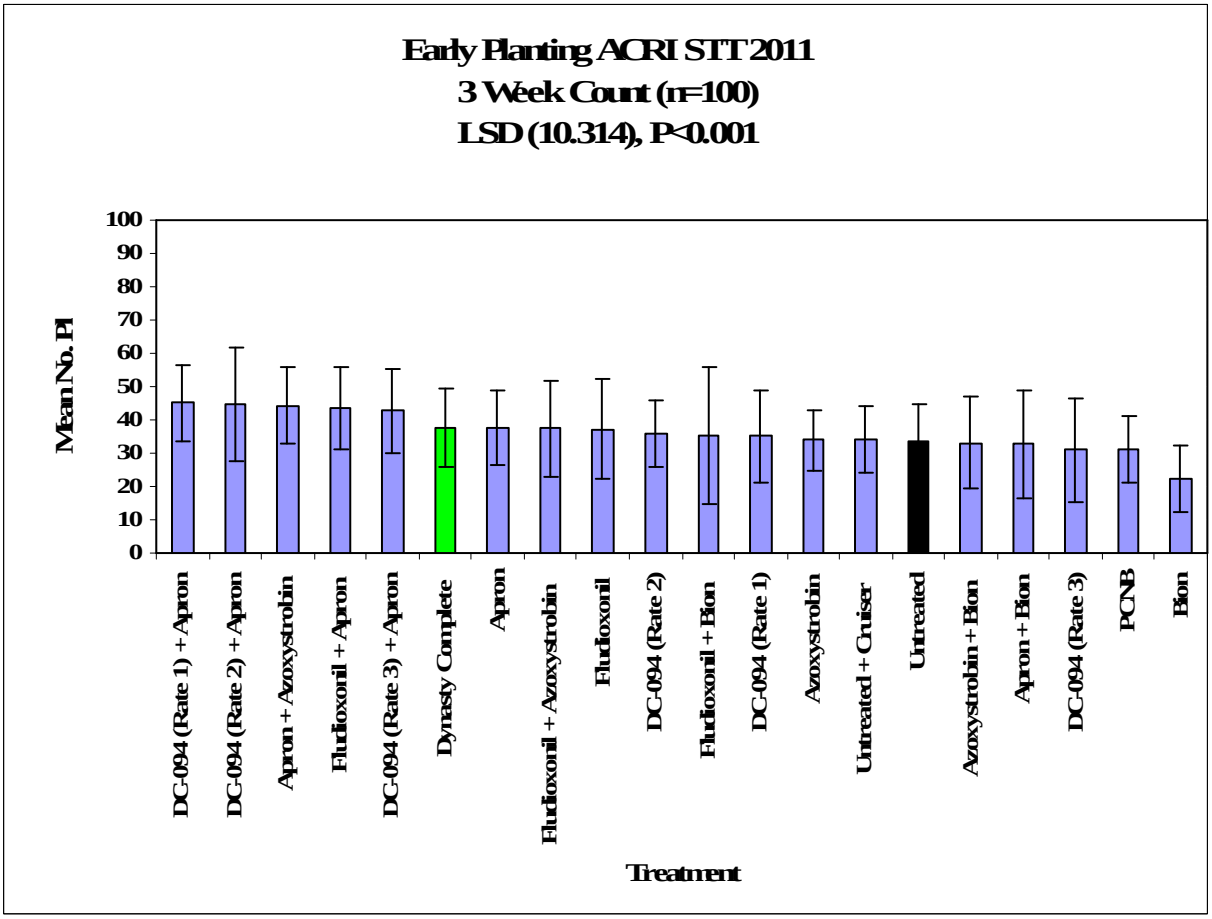


Figure 2.2.1 Mean stand counts three weeks after early sowing (07/09/11) following seed treatment with various fungicides at the ACRI Narrabri site.

The mean number of plants after six weeks growth in the early planted trial remained low with the mean for the trial being 33 plants. Soil and air temperatures were low providing favourable conditions for pathogens such as *Rhizoctonia*. The number of plants was significantly lower (P=0.004) in plots treated with Bion®, PCNB, DC-094 (Rate 3), Apron® + Bion®, Azoxystrobin + Bion® and Untreated (Figure 2.2.2). Seedling mortality after six weeks averaged 68% across the trial.

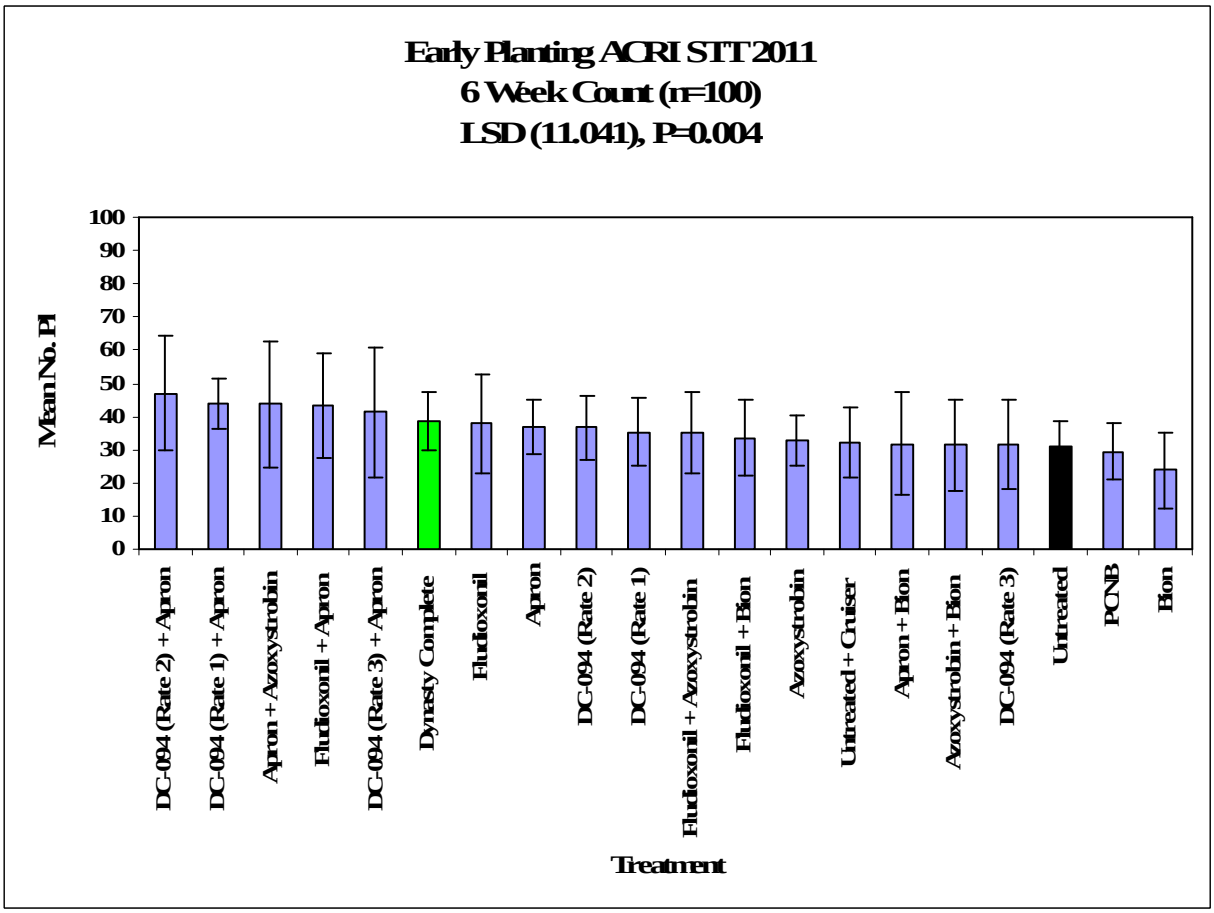


Figure 2.2.2 Mean stand counts six weeks after early sowing following seed treatment with various fungicides at the ACRI Narrabri site.

In the trial planted later, considered normal time for planting (12/10/11), after three weeks there was no significant difference (P=0.083) between treatments, with average seedling mortality after six weeks being 27% across the trial. The six week count also recorded no significant difference (P=0.125) between treatments with a mean mortality of 28%.

At the Mungindi site, seedling mortality was extremely high approximately 70%) regardless of treatment (Figure 2.2.3). The lack of effect of PCNB (known to be effective against *Rhizoctonia* indicated that *Pythium* spp. may have been the dominant pathogen early this season. This was consistent with observations of *Pythium* damage on plants. Mean % of plants across the trial was low at 24%.

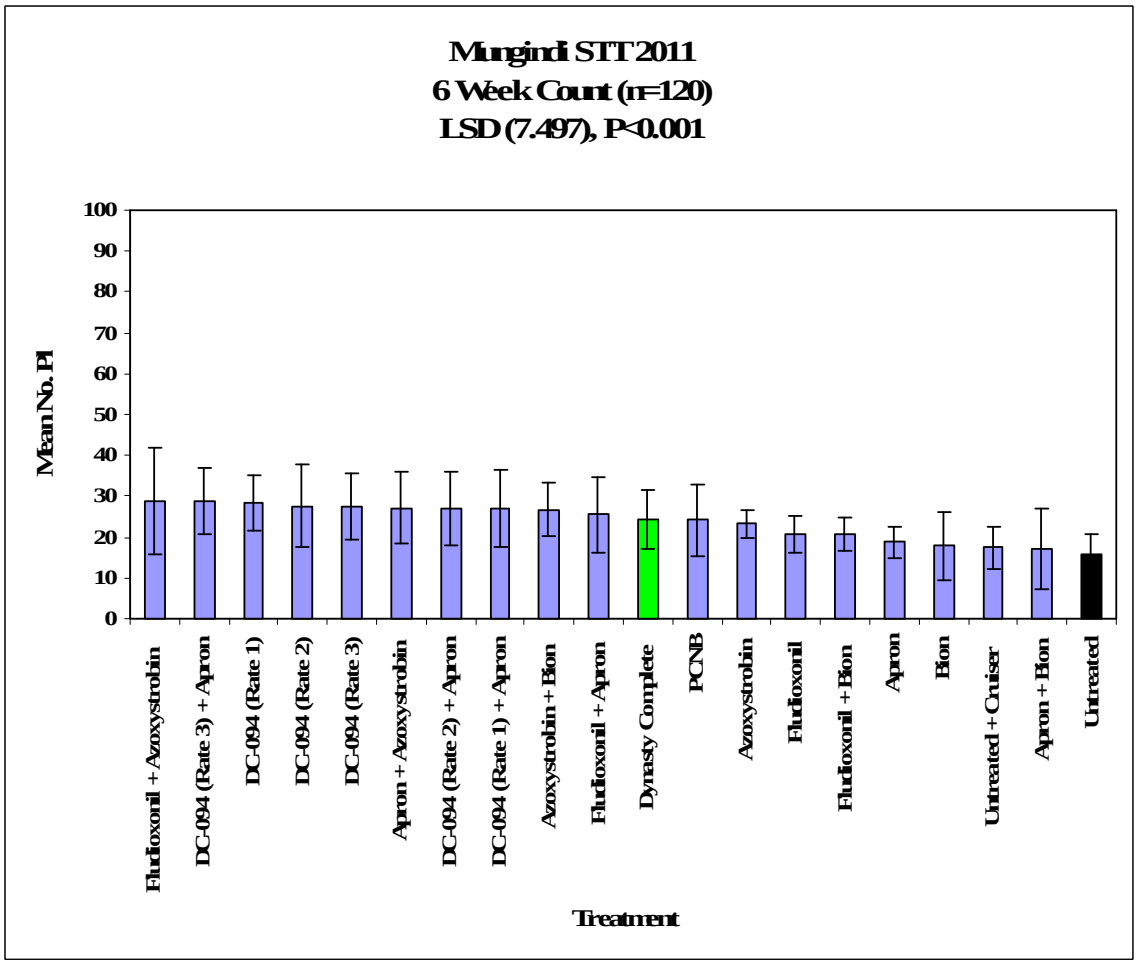


Figure 2.2.3 Mean stand counts six weeks after sowing (24/09/11) following seed treatment with various fungicides at Mungindi site.

At the QLD Downs site, average seedling mortality was mild at 20% (Figure 2.2.4).

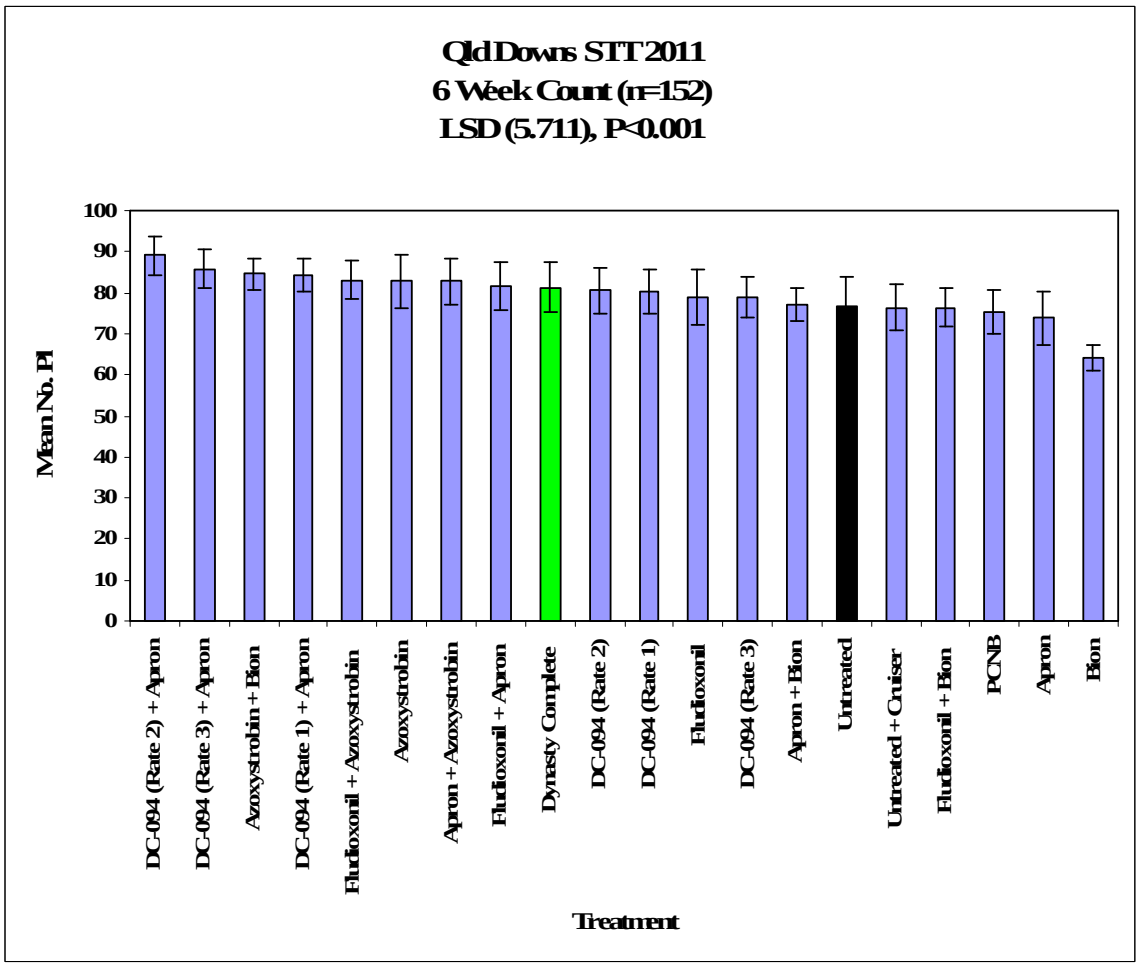


Figure 2.2.4 Mean stand counts six weeks after sowing (25/10/11) following seed treatment with various fungicides at QLD Downs site.

Due to the wet weather conditions the trial sites at Breeza, Hillston and Warren were not evaluated.

2.3 2012/2013 annual seed treatment fungicide trials:

Seedling mortality was significantly different (P<0.001) at both the early three week counts (Figure 2.3.1) and six week counts (Figure 2.3.2). For both the mean number of plants was higher where seeds had been treated with Apron, Apron + Azoxystrobin, Apron + Bion, Dynasty® Complete or Fludioxonil + Apron.

Mortality was high when planted early, with the average mortality after 6 weeks being 72%. The higher plant numbers under Apron and Apron combination treated plots indicates the dominant pathogen in this field may have been *Pythium* spp.

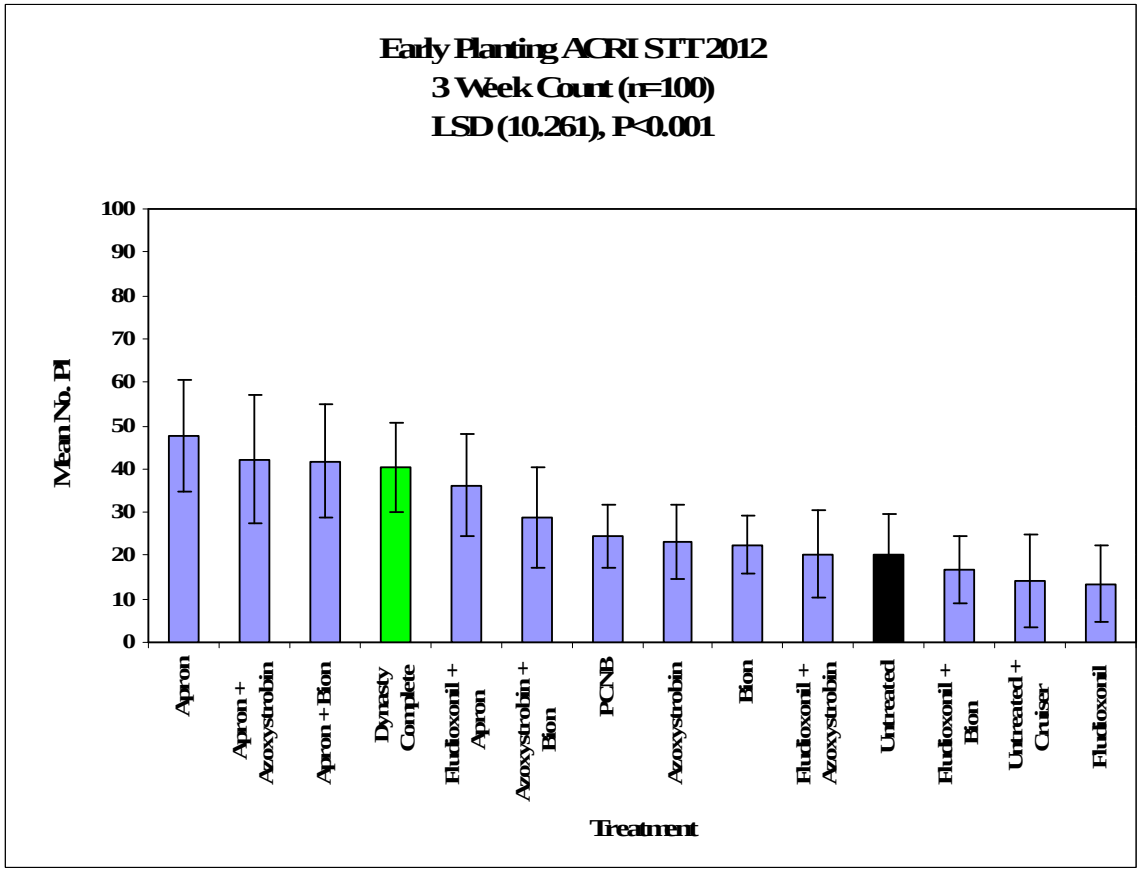


Figure 2.3.1 Mean stand counts three weeks after sowing following seed treatment with various fungicides at the ACRI Narrabri site.

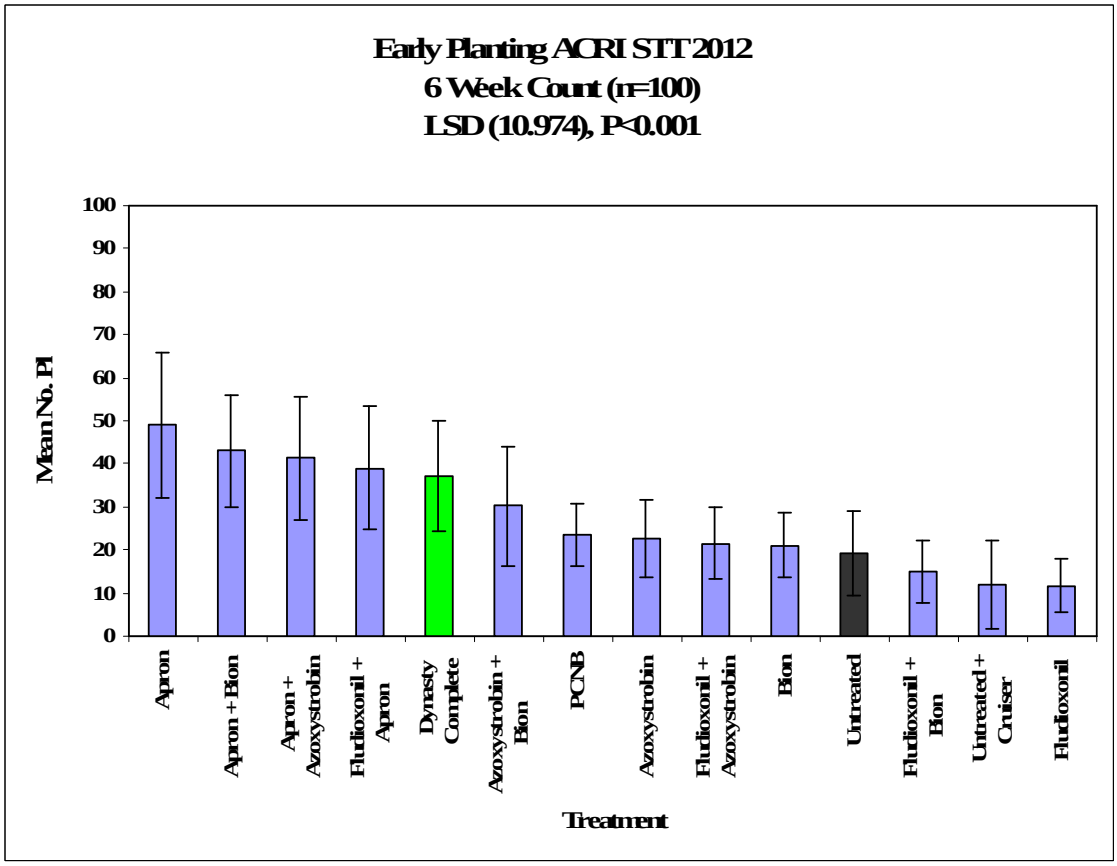


Figure 2.3.2 Mean stand counts six weeks after sowing following seed treatment at the ACRI Narrabri site.

There was no significant difference between treatments in the normal planting date trial after three weeks. After six weeks the mean number of plants was significantly higher (P=0.038) than the untreated control in all treatments except Azoxystrobin + Bion, Bion, Untreated + Cruiser and Fludioxonil + Bion (Figure 2.3.3).

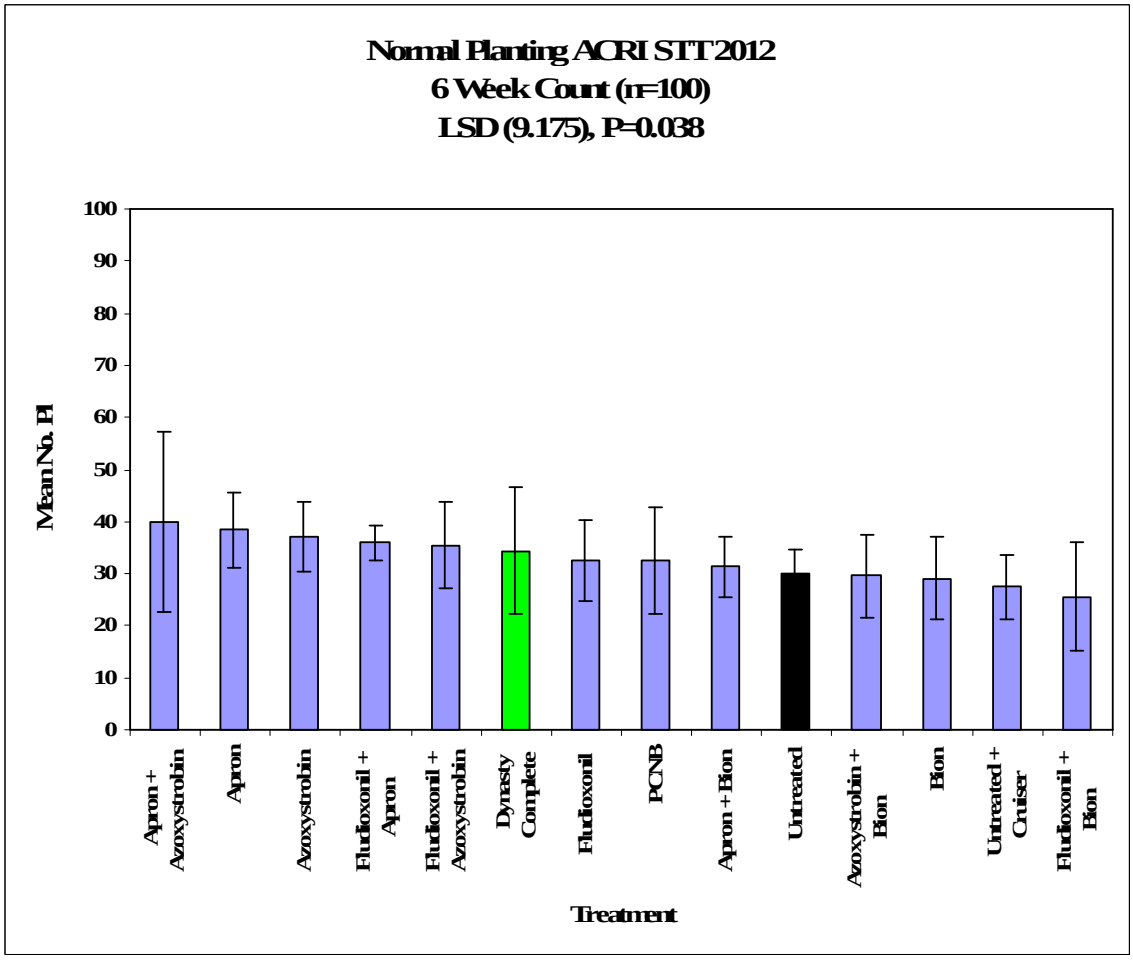


Figure 2.3.3 Mean stand counts six weeks after normal planting date following seed treatment with various fungicides at ACRI Narrabri site.

Initial stand establishment at ACRI Narrabri was very poor in both the early (average after six weeks 72%) and normal planting dates (average after six weeks 67%). This can largely be attributed to planting issues which only became apparent after planting and emergence. The cone seeder was refurbished in the months prior to planting. All calibrations were checked; however a different chain configuration was detected after stand issues were seen. Differences between treatments and planting dates are difficult to assess given the planting issues. Any significant differences seen in these two trials should be regarded with this in mind.

At Warren, the average seedling mortality was 47%. Plant stands were significantly higher (P<0.001) when seed was treated with Apron and Apron combinations except Apron + Bion (Figure 2.3.4). Higher stand counts under Apron products suggests the dominant pathogen at the Warren site was *Pythium* spp.

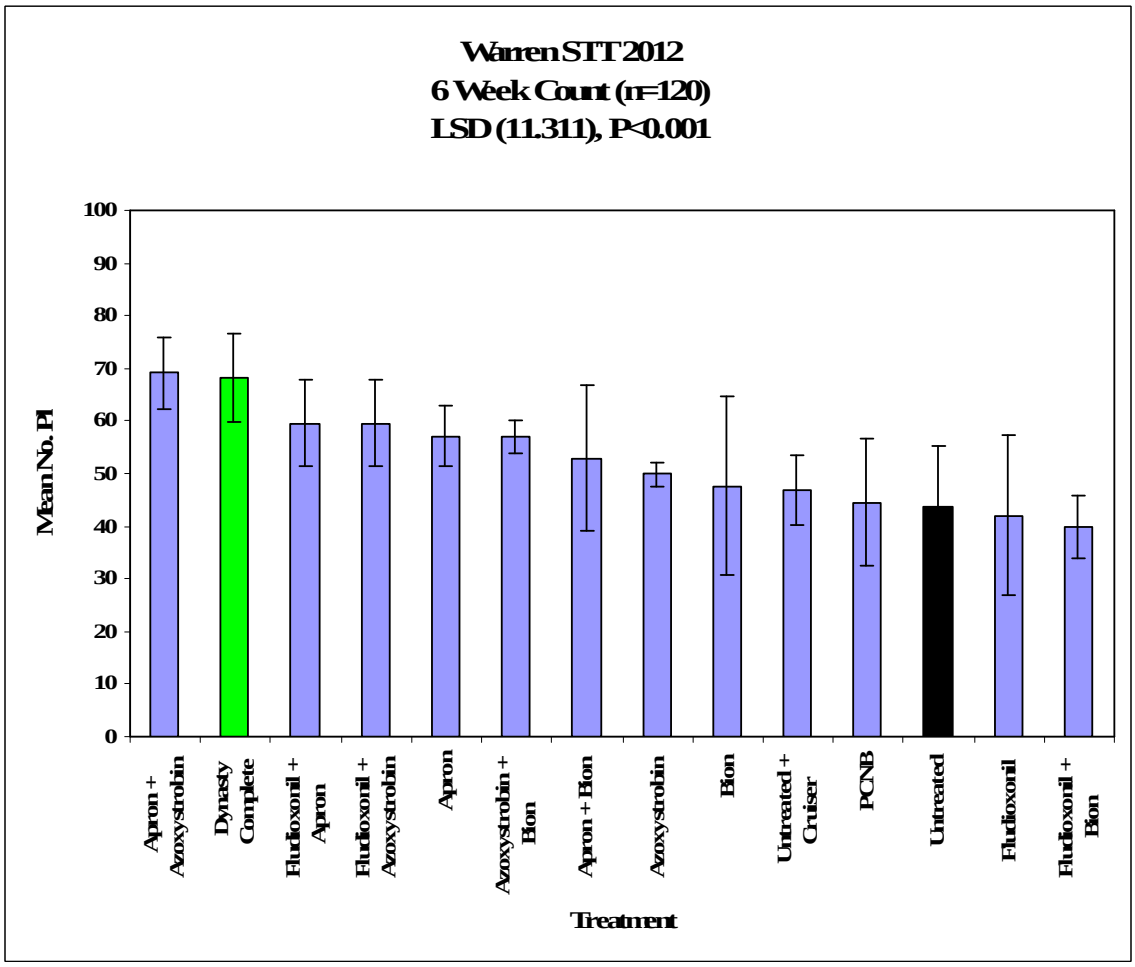


Figure 2.3.4 Mean stand counts six weeks following seed treatment with various fungicides at Warren.

Seedling mortality averaged 46% for the trial at Hillston. Compared to the untreated control, plots with seeds treated with Fludioxonil + Apron, Dynasty® Complete, Azoxystrobin and Apron + Azoxystrobin had significantly (P=0.041) higher stand counts (Figure 2.3.5).

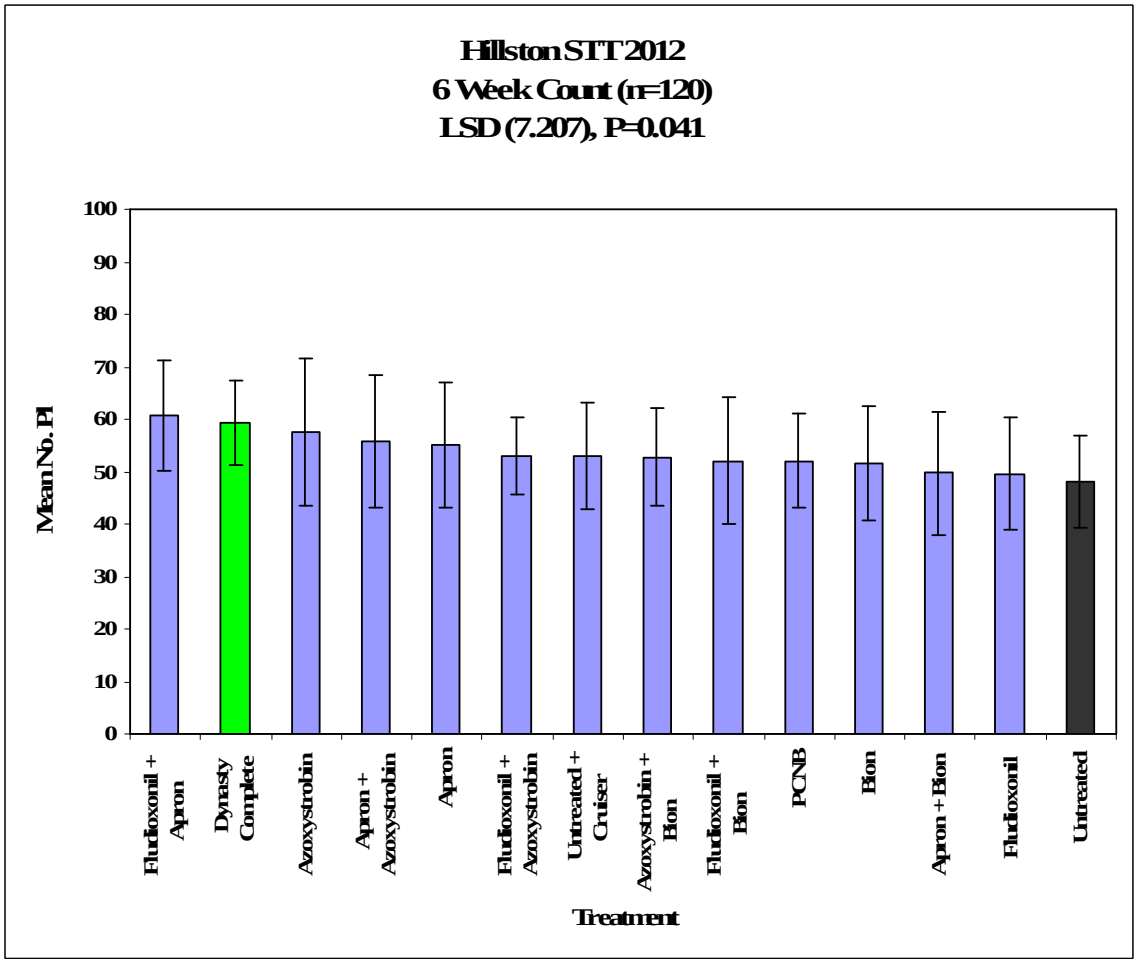


Figure 2.3.5 Mean stand counts six weeks following seed treatment with various fungicides at Hillston.

Mean stand counts were higher at Mungindi than any other site. Average mortality was 44%. Fludioxonil + Bion, Untreated + Cruiser, Apron, Azoxystrobin + Bion, Apron + Bion and Bion were all significantly lower than the untreated seed (Figure 2.3.6). These results suggest other abiotic factors such as moisture issues and or crusting may have contributed to mortality more than pathogen pressure.

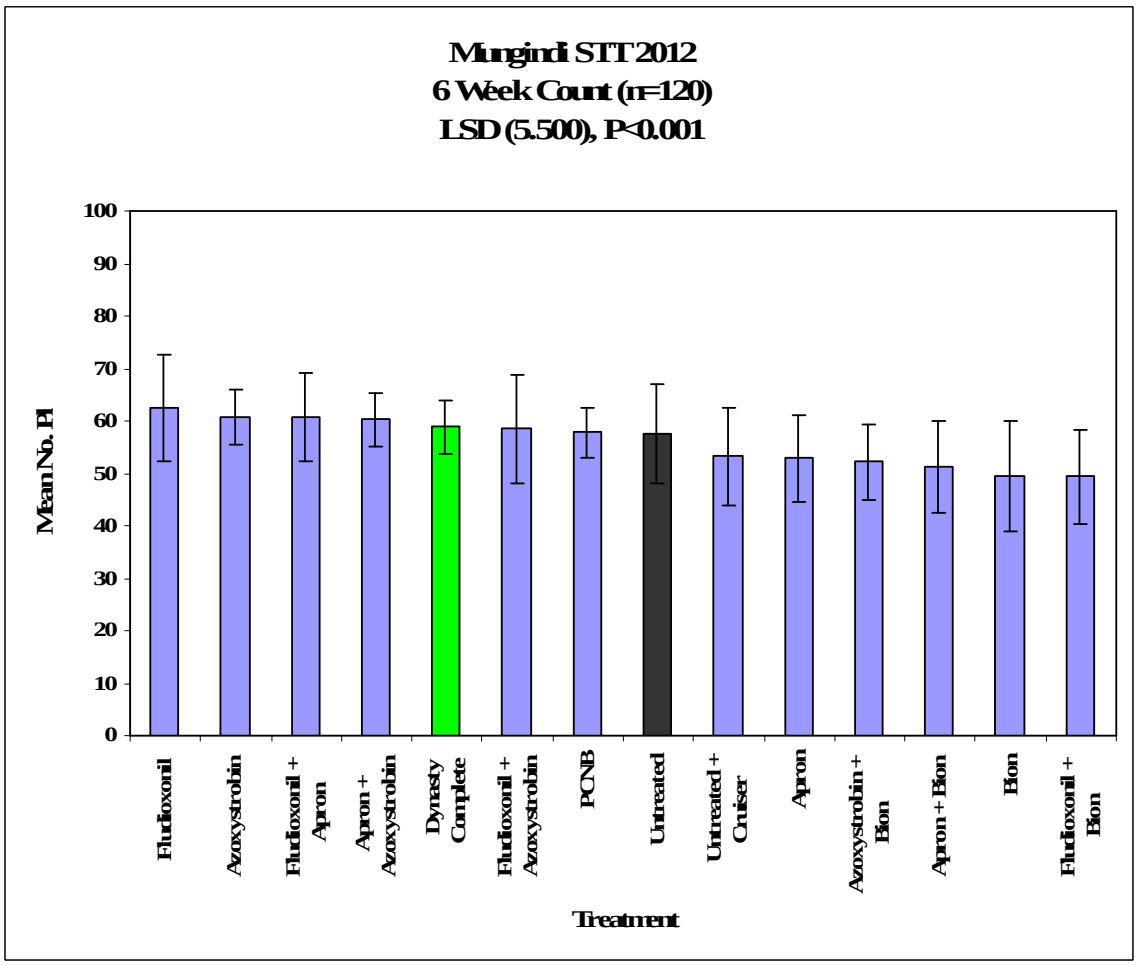


Figure 2.3.6 Mean stand counts six weeks following seed treatment with various fungicides at Mungindi.

Mean stand counts were highest at the Downs site compared to any other site. Average mortality was 37%. All rates of DC product + Apron, Apron, Apron + Azoxystrobin and Azoxystrobin + Bion were not significantly higher than the seed treated with the industry standard (Figure 2.3.7). Stand counts were significantly higher for seeds treated with Dynasty Complete than Fludioxonil + Bion and Fludioxonil. These results suggest other abiotic factors may have played a role in mortality.

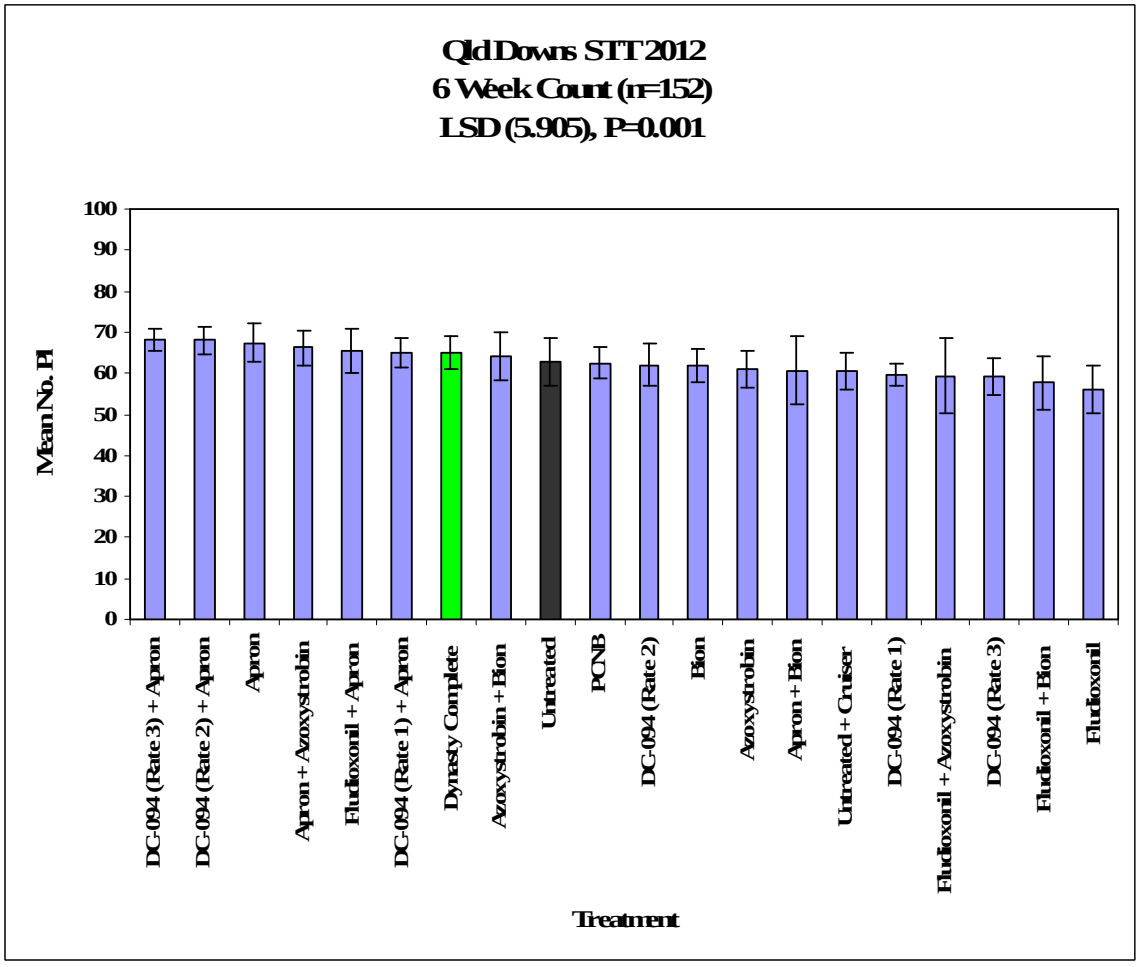


Figure 2.3.7 Mean stand counts six weeks after sowing following seed treatment with various fungicides at Downs.

Conclusions:

- Seed treatments reduced seedling mortality, although the results were varied across the different geographic locations in which the trials were conducted.
- Dynasty® CST and Dynasty® Plus consistently reduced seedling mortality in the early planting and normal planting trials at ACRI Narrabri and the Mungindi trial site.
- Seed treatment efficacy can vary depending on the time of planting, geographical area and local conditions in which seed are planted.

Sowing should be delayed as much as possible in order to minimize the effects of seedling diseases such as black root rot, *Pythium* spp. and *Rhizoctonia solani*. Growers should continue to treat seed with fungicides and insecticides in order to minimize the risk of stand loss and increased costs associated with replanting. Dynasty® CST was the most consistent seed treatment 2010/2011 season and Dynasty Complete in 2011/2012 season. These findings are consistent with 2009/2010 seed treatment results. In 2012/2013, stands counts in plots treated with the industry standard Dynasty Complete consistently were higher than the untreated control. Pressure from pathogens varied between trial sites and is strongly influenced by temperature and soil moisture. Use of seed treatments should be integrated with other management practices outlined in the Integrated Disease Management Guidelines produced by the Cotton CRC and Cotton R&D Corporation.

Objective 3 – Continue to evaluate IDM strategies for the control of black root rot including crop rotation and soil amendments.

3.1 Plant species screened in glasshouse studies for the effect on inoculum levels and disease development in cotton.

This milestone was carried out by the former cotton pathologist Alison Seyb. In summary a large glasshouse study was used to evaluate the effect of different crops: wheat, oats, canola, chickpea, faba beans, sunflower, vetch, onion, cotton and pigeon pea) on *T. basicola* inoculum in soil. Results indicated the treatment crops had a significant ($P < 0.001$) effect on inoculum levels in the soil (Figure 3.1.1). Treatments ordered highest to lowest were cotton, control, wheat, vetch, onion, sunflower, pigeon pea, canola, chickpea and oats. From this, canola, chickpea, oats, wheat, cotton and fallow were selected as treatments to be used in a field trial to be conducted at Old Field 2, ACRI. Faba beans were also included in the field trial, as this crop is used as a standard break crop in the local region.

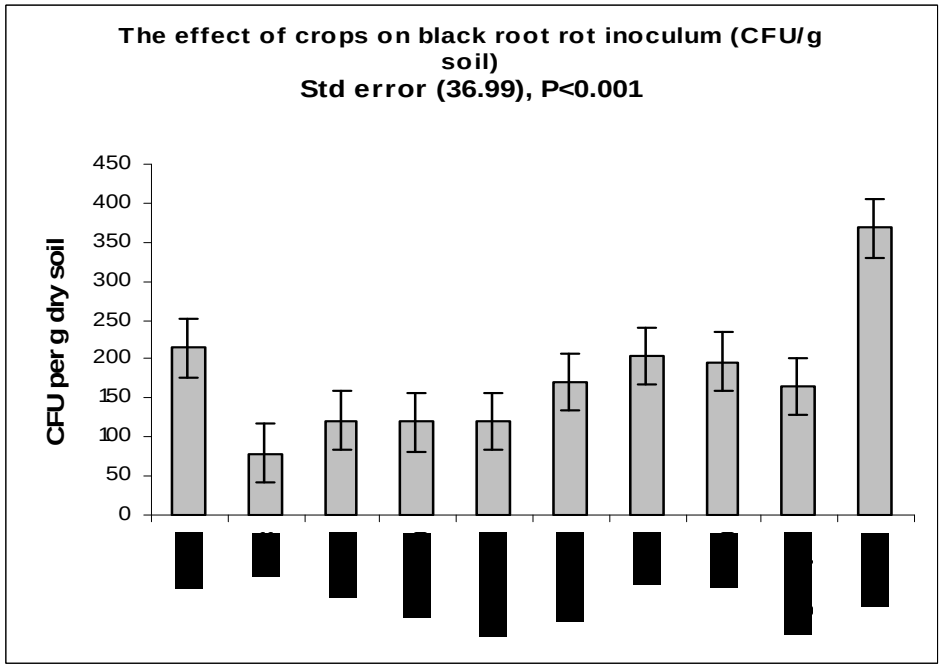


Figure 3.1.1 Black root rot inoculum levels in soil after growing different crops.

3.2 Laboratory studies completed of the potential of Thielaviopsis basicola chlamydospores to regerminate.

This work was carried out by the previous cotton pathologist Alison Seyb. Alison's report for this milestone was: laboratory studies on the potential of *T. basicola* chlamydospores to regerminate have been completed. Results showed chlamydospores germinate (56%) in water within 4 days. After being dried out for 7 days and then rewet, chlamydospores were unable to regerminate.

3.3 Possible soil amendments screened for the effect on chlamydospore and sclerotia germination completed using glasshouse studies.

The effect of different crop plants on *T. basicola* was undertaken with carrot as the chosen crop. The objective was to determine if macerated, germinated carrot seed would promote germination of chlamydospores in the absence of a host. Unfortunately, in the preliminary experiment conducted, there were difficulties with the culturing technique and as such no results have been obtained. Further work needs to be done on optimizing methods used in this experiment. No data is available for assays on *Verticillium dahliae* sclerotia germination due to problems with culturing technique. As the content of this project was varied, the value in completing this milestone as written was re-evaluated. Time constraints meant no further work was carried out.

3.4a Biofumigation Trial:

Results 2010 to 2011

Average Disease Severity:

There was no significant difference in the average disease severity of black root rot on cotton grown following the first year of growing biofumigation crops in winter 2009 (P=0.988), or 2010 (P=0.241). After a third year of biofumigation crops in winter 2011, the average disease severity was approaching significance (P=0.007). ADS was highest in the fallow treatment (6.442) followed by vetch (5.656), then chickpea (5.592) and finally canola (4.923) (Figure 3.4.2).

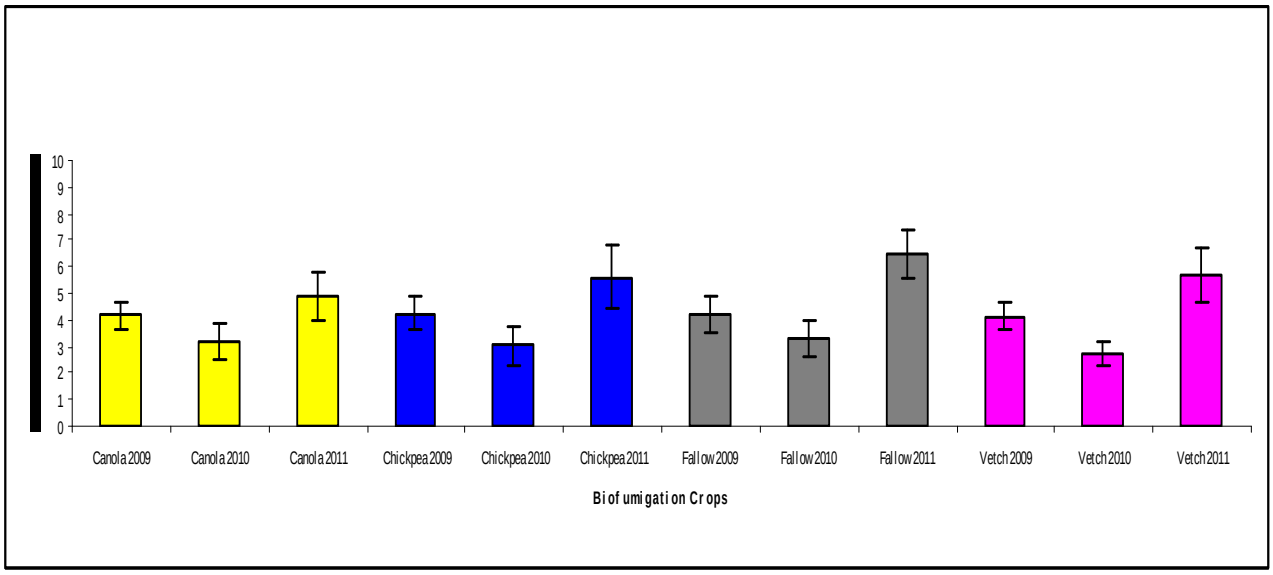


Figure 3.4.2 Effect of consecutive winters growing biofumigation crops: canola, chickpea, fallow and vetch on average disease severity of black root rot in cotton.

Biomass Assessment:

There was no significant difference in shoot dry weights between treatments (P=0.156) however there was a significant difference (P<0.001) in the shoot dry weight (g/plant) after three years of growing biofumigation crops. Figure 3.4.3 shows the increase in biomass per plant for each treatment after two rotations. This

increase was mainly attributed to greater water availability during winter. Reduced biomass after the third rotation was mainly due to less water availability and predation by birds. The dry matter cuts converted to tonnes per hectare were significantly (P=0.012) higher for canola (3.304) than both chickpea (2.035) and vetch (2.234).

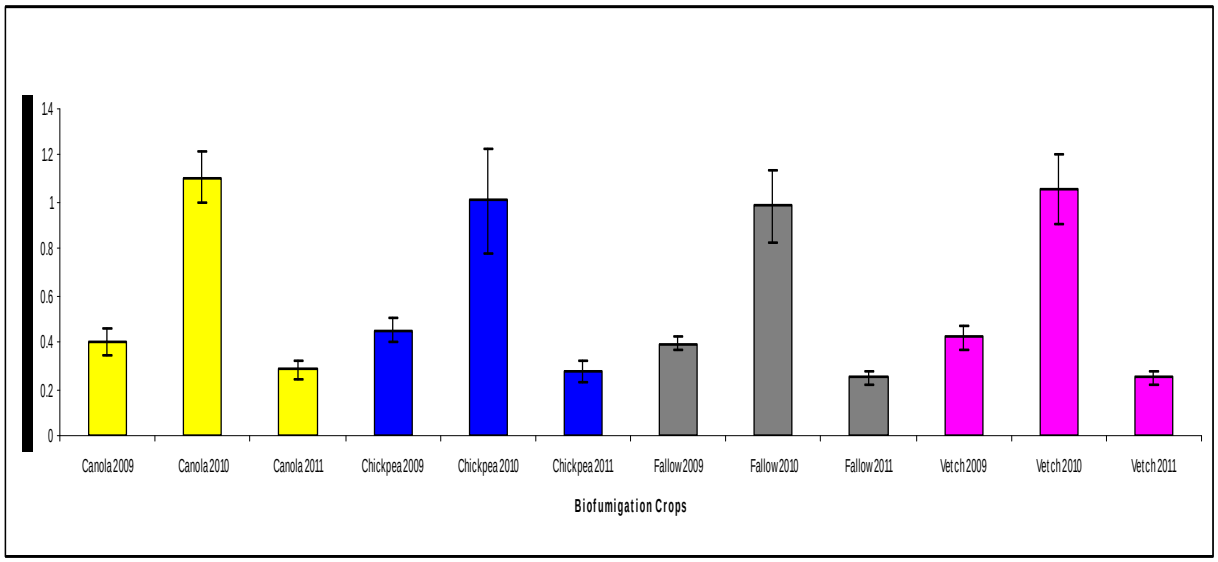


Figure 3.4.3 Average cotton biomass per plant (dry weight) after three winters growing biofumigation crops.

Stand Count

There was no significant difference in stand counts of cotton grown in any of the treatment plots (P=0.094) however there was a significant difference (P<0.001) between the first year and second year that biofumigation crops were grown (Figure 3.4.4).

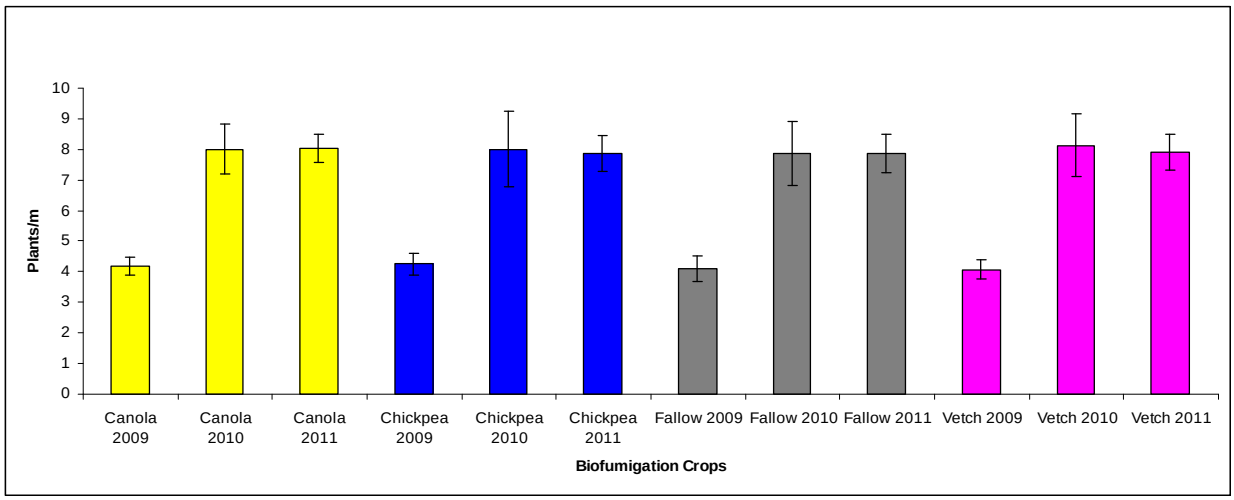


Figure 3.4.4 Effect of three winter biofumigation crops: canola, chickpea, fallow and vetch on the stand count per meter of cotton plants.

Results:
2012 to 2013
After the summer 2011/2012 cotton crop, the treatments were altered in winter 2012 from canola, chickpea, vetch and fallow to biofum blend (40% Doublet fodder radish, 50% *Carinata Brassica* and 10% Achilies White Mustard), Doublet fodder radish, vetch and fallow. There was no significant difference (P=0.061) in the dry mass (t/ha) between treatments (Table 3.4.2).

Table 3.4.2 Biofumigation crop biomass cuts were taken on the 28/8/12.

Crop	Dry Mass (t/ha)
Vetch (103 days after planting)	0.3209[a]
Biofum blend (88 days after planting)	0.6125[a]
Fodder radish (88 days after planting)	0.5718[a]

Growth room pot experiment: The glasshouse pot experiment was used to determine the base line levels of inoculum in the soil within each treatment. This was determined by rating disease severity (0 to 10) on seedling roots of cotton plants grown in soil collected from each treatment. Average disease severity was high under all treatment plots (9.4 across all treatments), however disease severity was significantly lower (P=0.028) under the fallow treatment (Table 3.4.3).

Table 3.4.3 Average disease severity on cotton roots planted into pots filled with soil collected from vetch, biofum blend, fodder radish and fallow treatment plots.

Crop	Logit ADS	Transformed Average Disease Severity
Vetch	2.798[a]	9.525677[a]
Biofum Blend	2.729[a]	9.487163[a]
Fodder radish	2.749[a]	9.498568[a]
Fallow	2.249[b]	9.145642[b]

Field trial results:
Average disease severity and stand counts:
Statistical analysis of the base line levels for average disease severity under the new treatments imposed in 2012 showed a significant effect, as did stand count (Table 3.4.4). Average disease severity was significantly lower (P=0.027) in plots that had been sown to vetch. The highest severity was in plots sown to the biofum blend 6.885, then bare fallow 6.873, then fodder radish 6.530 then vetch 6.092. Fodder radish had significantly lower (P=0.030) stand count per meter compared to fallow, biofum blend and vetch treatments. Treatments had no significant effect (P=0.153) on dry shoot weights.

Table 3.4.4 Average disease severity on cotton roots and stand counts per metre following vetch, biofum blend, fodder radish and fallow treatment.

Crop	Average Disease Severity	Stand Count/m
Biofum blend	6.885[a]	3.660[b]
Fallow	6.873[a]	3.449[b]
Fodder radish	6.530[a]	2.942[c]
Vetch	6.092[b]	4.399[a]

Conclusions:

Dry matter yield per hectare was very low. Yields of up to 4 to 5 tonnes per hectare should be strived for in order to get a biofumigation effect or significant effect on soil borne diseases. The plants flowered early due to water stress which added to the early cut out of reproductive growth.

The reduced stand count after the radish could be due to time between incorporating and planting cotton was too close, however the this is unlikely given the yields of biofumigation crops were poor and there would be little ITC in the soil.

This experiment has provided the opportunity to optimise the methods needed to apply a good long term trial. A similar biofumigation trial will be run in the new Diseases of Cotton XI project working in collaboration with Seed Force (www.seedforce.com) to achieve the optimum dry matter yield, fertiliser rates and seeding rates of biofumigation crops.

Seedforce is a supplier of biofumigation seed, imported from Jordan. Seedforce have agreed to provide the seed mixes for two treatments (Biofum blend and also a radish) as well as advice on crop requirements in order to produce the biomass needed to produce a biofumigant effect. An incomplete understanding of these factors may have contributed to the lack of results reported in past biofumigation trials.

3.4b Rotation Trial:

Field results: Initial results on commencement of trial showed no significant difference (P=0.303) in the average disease severity in plants assessed in the field for any treatment. The average disease severity of black root rot was not significantly different (P=0.051) between any of the rotation treatment crops (Table 3.4.5). There was no significant difference (P=0.496) in the incidence of Verticillium wilt following rotation crops (Table 3.4.6), although incidence was much lower following all crops in 2011/2012 season. Stand counts of cotton was significantly different (P<0.001) following rotation crops (Table 3.4.7).

Table 3.4.5 Average disease severity of black root rot after different rotation crops

Rotation Crop	2009/2010	2011/2012
Canola	3.696	3.821
Chickpea	3.055	4.006
Faba Beans	3.299	3.788
Fallow	3.132	3.434
Oats	3.281	3.525
Wheat	3.212	3.306
Standard errors of differences		
Average:	0.3203	0.2297
Maximum:	0.3231	0.2299
Minimum:	0.3173	0.2294
p=	0.463	0.051

Table 3.4.6 Incidence of Verticillium wilt after different rotation crops

Rotation Crop	2009/2010	2011/2012
Canola	11.34	4.09
Chickpea	23.02	3.426
Faba Beans	16.12	3.9
Fallow	14.93	8.281
Oats	12.38	3.692
Wheat	19.72	6.04
Standard errors of differences		
Average:	7.05	2.689
Maximum:	7.639	2.896
Minimum:	6.459	2.48
p=	0.675	0.496

Stand Counts:

Table 3.4.7 Stand counts (cotton) after different rotation crops

Rotation Crop	2009/2010	2011/2012
Canola	6.258	7.698
Chickpea	6.718	8.338
Faba Beans	6.479	8.152
Fallow	7.061	8.669
Oats	6.392	7.523
Wheat	6.341	7.922
Standard errors of differences		
Average:	0.5194	0.1989
Maximum:	0.5764	0.2144
Minimum:	0.4633	0.1833
p=	0.567	<0.001

No significant difference was found in the number of plants per meter (P=0.973) on commencement of the trial. The highest mean number of plants per meter was found in plots to be rotated with fallow (7.2), chickpea (6.8), faba bean (6.8), canola (6.7), wheat (6.7) and oats (6.7) with a standard error of difference of 0.6627. A significant difference was found in the number of plants per meter (P<0.001) after the rotation crops were grown in winter 2010 and bare fallow in winter 2011 (Figure 3.4.8). The highest mean number of plants per meter was recorded after the fallow rotation (8.7), followed by chickpea (8.4), faba bean (8.2), wheat (7.8), canola (7.6) and oat (7.5) rotations with a standard error of difference of 0.2495.

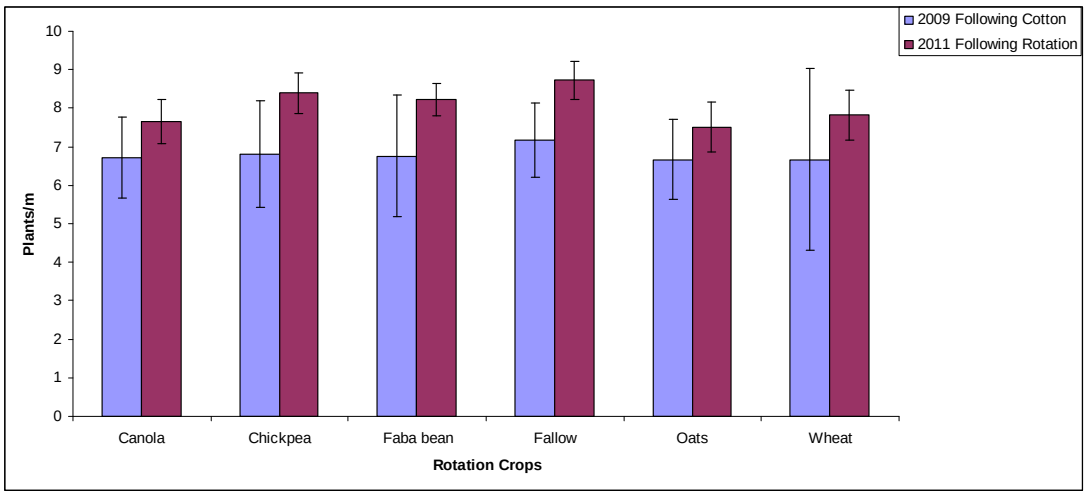


Figure 3.4.8 Average plants per meter before and after a winter rotation crop.

There was no significant difference (P=0.512) in the shoot dry weight per plant upon commencement of the trial. The highest shoot weights were recorded for the plots that were rotated with chickpea (0.3601), then fallow (0.3294), faba bean (0.3146), oats (0.3055), wheat (0.2964) and canola (0.2827) with a standard error of difference of 0.04157. There was a significant difference (P=0.038) in the shoot dry weight per plant after the rotation crops were grown in winter 2010 and bare fallow in winter 2011 (Figure 3.4.9). The highest shoot weight was recorded following rotations with fallow (0.3899), followed by chickpea (0.3374), wheat (0.3563), oats (0.3543), canola (0.3358) then faba bean (0.3294) with a standard error of 0.01952.

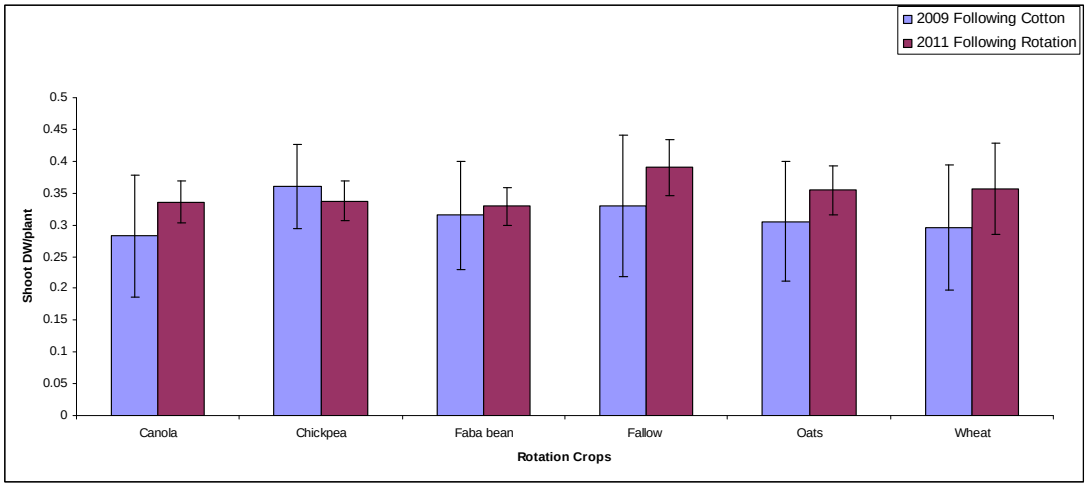


Figure 3.4.9 Average shoot weight per plant before and after a winter rotation crop.

Glasshouse results: There was no significant difference in the average disease severity of black root rot (P=0.762) of plants grown in the glasshouse from soil collected from the field on commencement of the trial. After the rotation crops were grown in winter 2010 and bare fallow in winter 2011, there was still no significant difference in the average disease severity of black root rot in plants grown from collected soil (P=0.276).

Initially the highest disease severity was recorded in pots from the treatment that was rotated with the canola (3.021) then oats (2.745), fallow (2.686), faba bean (2.583), wheat (2.540) and chickpea (2.537) rotations with a standard error of difference of 0.3623. After the rotation crops (Figure 3.4.10), the disease was most severe in soil that had the fallow rotation (6.970), followed by wheat (6.750), chickpea (6.170), canola (5.960), oats (5.590) and faba bean (5.035) with a standard error of difference of 0.8922.

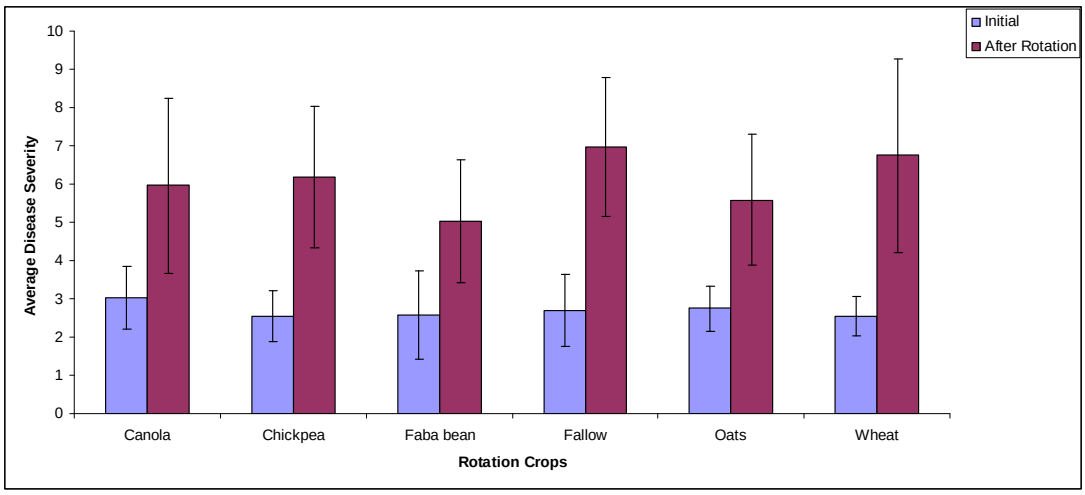


Figure 3.3.10 Average disease severity in cotton plants before and after a winter rotation crop.

There was no difference in the shoot dry weight (P=0.470) from the plants grown in soil collected initially from the field. Shoot dry weights for the different rotations were canola (0.1730), wheat (0.1487), oats (0.1478), fallow (0.1458), chickpea (0.1433) and faba bean (0.1350), with a standard error of difference of 0.01875. After rotation crops (Figure 3.4.11) the shoot dry weight were chickpea (0.1705), wheat (0.1696), canola (0.1671), faba bean (0.1586), fallow (0.1575) and oats (0.1456) with a standard error of difference of 0.01286.

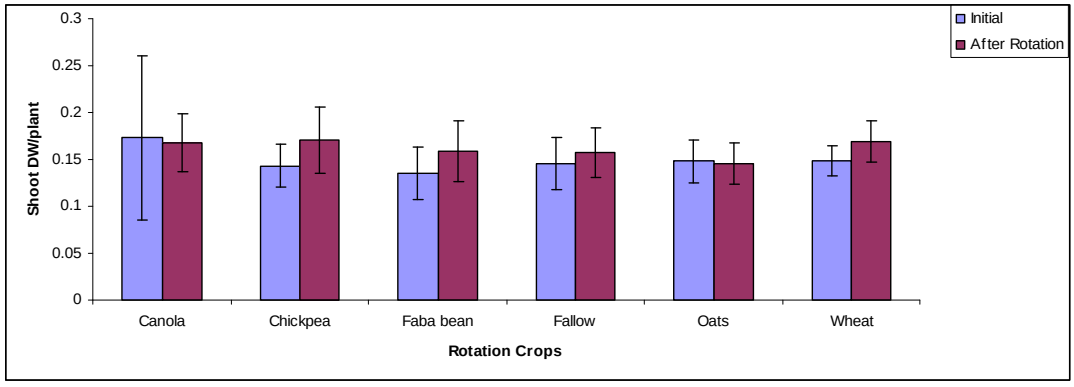


Figure 3.4.11 Average shoot weight per plant before and after a winter rotation crop.

There was no difference in the root dry weights of these plants grown in soil collected initially (P=0.677) or after rotation crops (P=0.316). Initially the highest root weight was recorded from soil collected from that were to be rotated with canola (0.02641), then oats (0.02303), chickpea (0.02087), fallow (0.02006), wheat (0.02000), and faba bean (0.01929), with a standard error of difference of 0.004778. After the rotation crops (Figure 3.4.12) the highest root weight recorded was in the chickpea rotation (0.03165), followed by canola (0.03025), wheat (0.02990), faba bean (0.02735), fallow (0.02659) and oats (0.02597), with a standard error of difference of 0.002942.

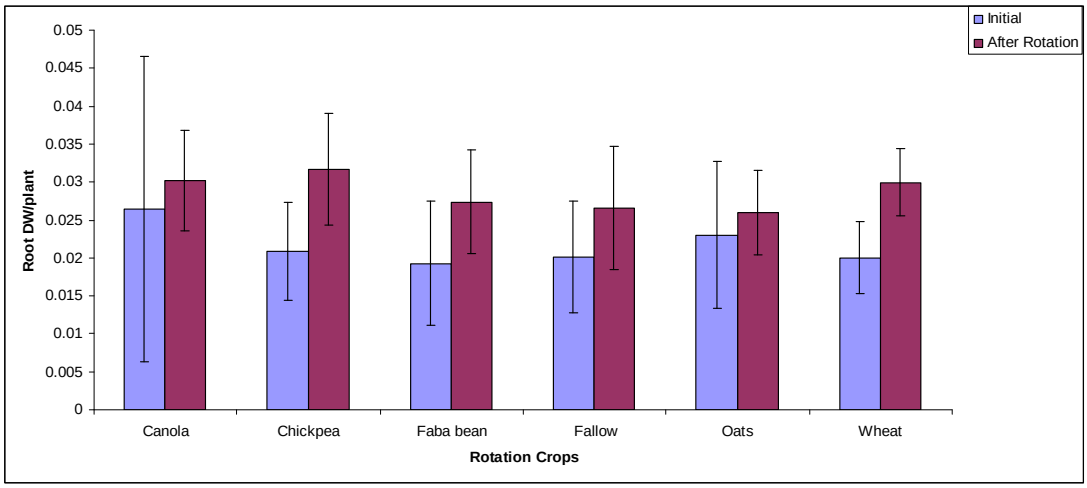


Figure 3.4.12 Average shoot weight per plant before and after a winter rotation crop.

Conclusions:

With no significant differences being detected between rotation crops on the average disease severity, CRDC felt no further evaluation of rotation crops was warranted. Instead the field will be maintained as a black root rot and Verticillium wilt nursery for future field trial and pot experiments. The field was laser levelled mid 2013. Continued effort will be made to lessen the weed seed bank within this field.

Objective 4 – Continue to investigate the effect of long bare fallows on mycorrhizal colonisation of cotton.

4.1 Long term enforced fallow experiment continued at ACRI.

VAM assessment:
The Diseases of Cotton IX by (Anderson, Lonergan, & Allen, 2010) reported winter 2009 assessments showed no significant difference in VAM levels between cropped and bare soil, indicating that three years of bare fallow was not associated with a reduction in colonisation of cotton roots by VAM fungi. These findings were consistent with those of Douds et al. (2011) who reported AM fungi to be remarkably persistent in soil in the absence of host plants, with viable spores present in field soil which had no plant cover for over three years.

Black root rot assessment:
The 2008/2009 and 2009/2010 cotton season saw no difference in disease severity in the cotton or bare fallow plots. There was a significant ($P < 0.001$) effect in 2010/2011 cotton season of treatment (cotton vs. fallow) on black root rot severity with the average disease severity on cotton grown in soil collected from cotton plots being 2.02 and fallow being 0.14.

4.2 Nurse crops investigated to restore mycorrhizal fungi in long bare fallow situations.
A literature search of the work done by Dr David Nehl found that this objective had already been carried out. Nehl examined the colonisation and growth of cotton after bare fallow for 18 months compared to rotation with winter nurse crops and also canola (a non-mycorrhizal plant). Nehl reported (Table 4.2.1 and 4.2.2) no significant difference in colonisation of cotton by AM fungi following winter nurse crops or summer nurse crops.

Table 4.2.1 Mycorrhizal colonisation and growth of cotton after bare fallow for 18 months or rotation with winter crops.

	1992–93 cotton crop		1994–95 cotton crop	
Previous crop	AM root (%)	Shoot mass (g/plant)	AM root (%)	Shoot mass (g/plant)
18 months BF uncultivated	30	1.6	49	0.74
18 months BF cultivated	38	1.6	40	0.67
Wheat	36	1.8	38	0.74
Chickpea	25	1.5	36	0.67
Linseed	31	1.7	39	0.63
Canola	30	1.3	35	0.68

AM = arbuscular mycorrhizal; BF = bare fallow

Table 4.2.2 Mycorrhizal colonisation and growth of cotton after bare fallow for 18 months or rotation with winter and summer cereals.

	Arbuscular mycorrhizal root (%)		
Treatment	1994–95	1996–97	1998–99
Bare fallow	65	71	56
Sorghum	62	54	68
Wheat*	64	55	55

* Barley used as the 1993 winter crop

Rotating crops each year with a cereal, legume or green manure crop before a cotton crop may restore sufficient mycorrhizal fungi for cotton. Natural soil communities contain a wide variety of AM fungal species. Planting different crops will favour different AM fungi ensures a combination of AM fungi present in the soil.

Soil disturbance can suppress and eventually cause AM fungi to die (in the absence of host plants). Minimising tillage aids in prolonging AM fungal networks in the soil profile. Use herbicides to control weeds and kill nurse crops instead of ploughing.

Commercial mycorrhizal inoculum is also available in powder, gels, liquid or granular forms; however these have not been tested in this project.

Conclusions:

This long term experiment to date has shown no significant difference in arbuscular mycorrhizal fungi (VAM) levels in plants growing in soil collected from long term bare fallow or cropped soil. These findings were consistent with Diseases of Cotton VIII final report (Nehl, 2007) in which VAM colonisation occurred in soils that had undergone bare fallows for up to four seasons or more. The bioassays of VAM inoculum in soil and studies of cotton root colonisation in crops within the field indicate arbuscular mycorrhizal fungi are capable of surviving in soils that undergo bare fallows for many seasons. Previous work done by Nehl et al (Nehl, Allen, Mondal, & Lonergan, 2004) on mycorrhizal fungi in long bare fallows reported after 18 months long bare fallow colonisation of cotton by AM fungi was no lower than with any other crop.

Nehl reported the mycorrhizal fungi that colonise cotton in Australia are undoubtable indigenous and have evolved to cope with long periods of drought. The AM fungi have a wide host range including weeds which aid in their preservation during fallows. Under current cotton farming systems, it is unlikely that there will be a suite of conditions such as periods of very long bare fallows with wetting and drying cycles and no weed growth that would result in declining populations in the soil.

Dr David Nehl's research has confirmed that – contrary to previously held views – long bare fallows such as this one, or a rotation with canola (a non-mycorrhizal plant) do not decrease mycorrhizal development in subsequent cotton crops

Objective 5 – Provide information on the survival of pathogen inoculum in soil.

5.1 Establishing the culture collection:

Maintaining the long term culture collection, 336 isolates, is important for future research on morphology, pathogenicity and genetic variation. Fifty two isolates were recovered from plates kept from 1983 to 2009 and a further 284 isolates sampled between 2009 and 2013. From these isolates, pathogens responsible for causing disease were identified before being subcultured to produce single strain isolates that were placed in the long term culture collection (Table 5.1.2).

Table 5.1.2 Single strain isolates in the long term culture collection.

Pathogens in long term storage	Total
Verticillium dahliae	67
Fusarium oxysporum vasinfectum	52
Alternaria	99
Thielaviopsis basicola	69
Drechslera	4
Rhizoctonia	2
Pythium	16
Alternaria macrospora	1
Curvularia	1
Sudden wilt	8
Sclerotinia	11
Mould	2
Unknown	4
Total	336

Conclusions:

The single strain culture collection is an important collection of the pathogens collected from commercial cotton farms in Australia. To date there are 336 isolates in long term storage, however this collection will continue to be built on each year of the disease surveys. This collection will be maintained to ensure that future studies on pathogenicity and morphological studies can be conducted. Future work includes re-isolating these strains and trialling several long term storage techniques.

5.2 The effects of environmental factors on survival of inoculum assessed in glasshouse studies.

Average disease severity almost doubled after the second experiment in the same soil in both the glasshouse and growth room pot experiments. Following the second planting the average disease severity was Dulla Dulla 1 (9.14), ACRI Old 2 (6.39), Dulla Dulla 3 (2.82) and Warilea Field 3 (2.10) after the second experiment in the glasshouse (Figure 5.2.1). Similar results were also recorded for the pot experiments in the growth room with average disease severity being: Dulla Dulla 1 (6.1), Dulla Dulla 3 (4.8), ACRI Old 2 (4.7) and Warilea Field 3 (2.7) (Figure 5.2.2). Average disease severity almost doubled after the second experiment for both the

second glasshouse and growth room trials. The increase in each case may be attributed to inoculum multiplying after the first round of plants were grown in the pots.

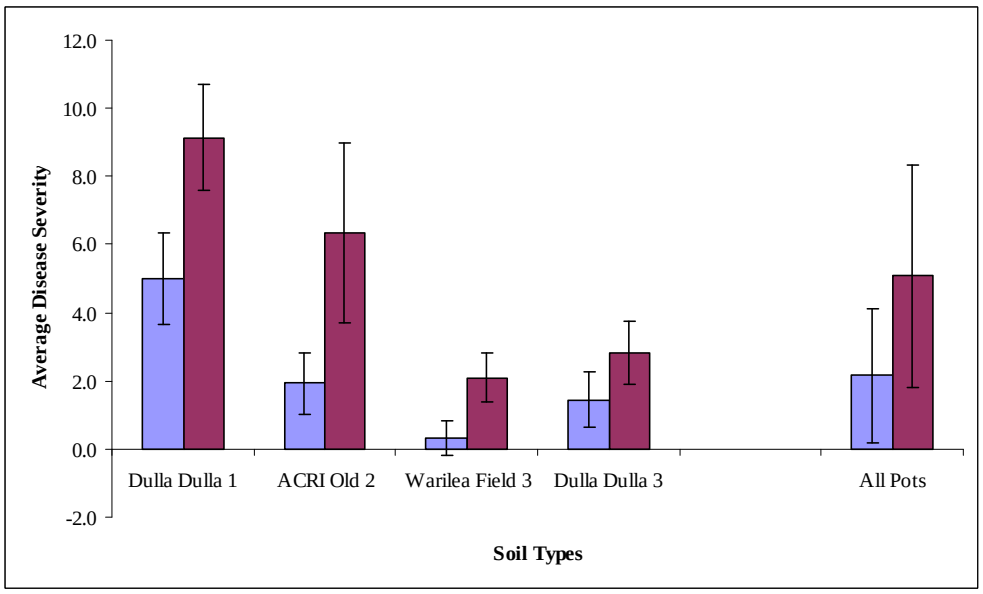


Figure 5.2.1 Average black root rot severity in glasshouse pot trials with naturally infected soil collected from different locations in NSW. Temperature range for the glasshouse was 15-25^{O}C. Results from first experiment (blue bars) and second experiment (red bars).

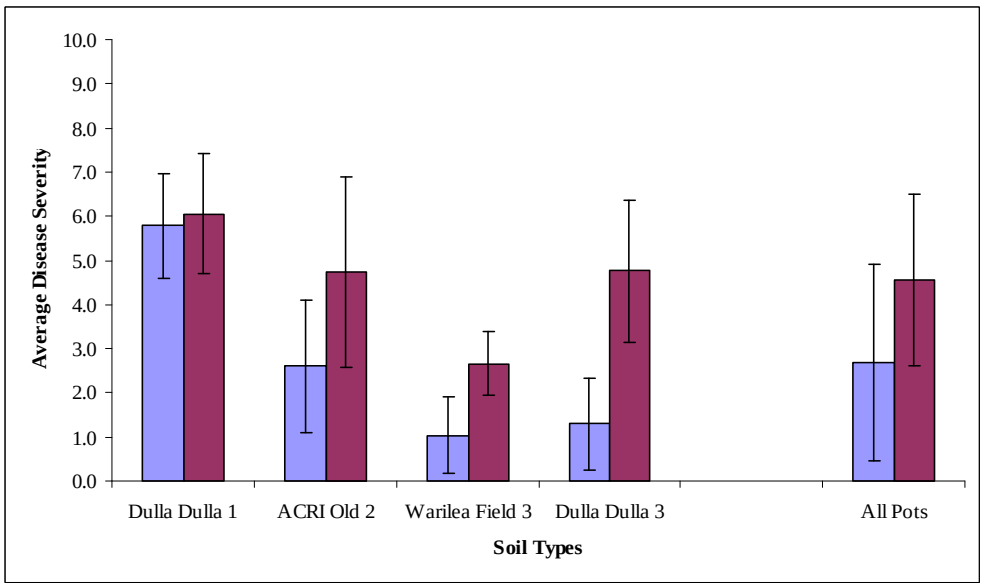


Figure 5.2.2 Average black root rot severity in growth room pot trials with naturally infected soil collected from different locations in NSW. Results from first experiment at 17-21^{O}C (blue bars) and second experiment 20-25^{O}C (red bars).

Conclusions:

Average disease severity varied between each soil type, indicating there was variation of inoculum levels within each soil type. These experiments were used as pilot studies for future glasshouse and growth room trials. The pot experiments showed high variation in the average disease severity. This may be a result of varying initial inoculum levels within each soil type. This highlighted the need be able to artificially inoculate pasteurised soil with a known level of inoculum for comparative studies. This will allow us to make comparisons of the pathogenicity of different isolates and evaluate if soil type has any effect on disease severity.

5.3 Evaluated the survival of the three pathogens in different soil types under a range of environmental conditions throughout New South Wales.

Soil type had a significant effect ($p<0.001$) on days to emergence (Figure 5.3.2). Seedlings grown in the sand soil required significantly more days to emergence then both the red and brown soil types. There was a significant interaction between soil type and inoculation. There were significant differences ($p<0.001$) between inoculated and uninoculated red and brown soil types but no difference between the inoculated and uninoculated sand soil. Seedlings grown in inoculated soils emerged faster than those grown in the uninoculated controls for each soil type. The average pH between soil types differed considerably: pH 4.98, 6.03 and 6.33 for the sand, red and brown soils respectively.

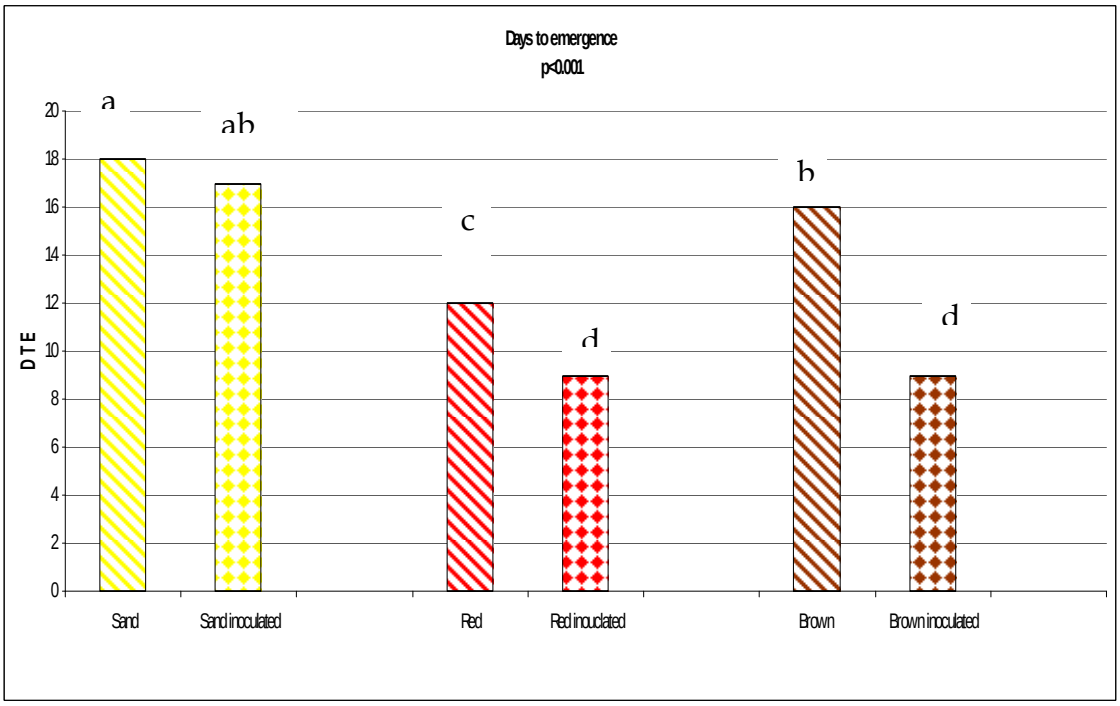


Figure 5.3.2 Significant difference in days to emergence between sand, red and brown soil types.

Soil type showed a marginally significant difference ($p=0.055$, sed=0.2005) in average disease severity of the sandy soil compared to red and brown soil (Figure 5.3.3). There was a significant difference ($p<0.001$, sed=0.2865) between inoculated and uninoculated plants with all controls having zero infection. Seedlings grown in the uninoculated sand, red and brown soils all had clean roots with scores of zero. The average disease severities for seedlings grown in the inoculated sand, red and brown soil were 7.4797, 8.3737 and 8.1726 respectively.

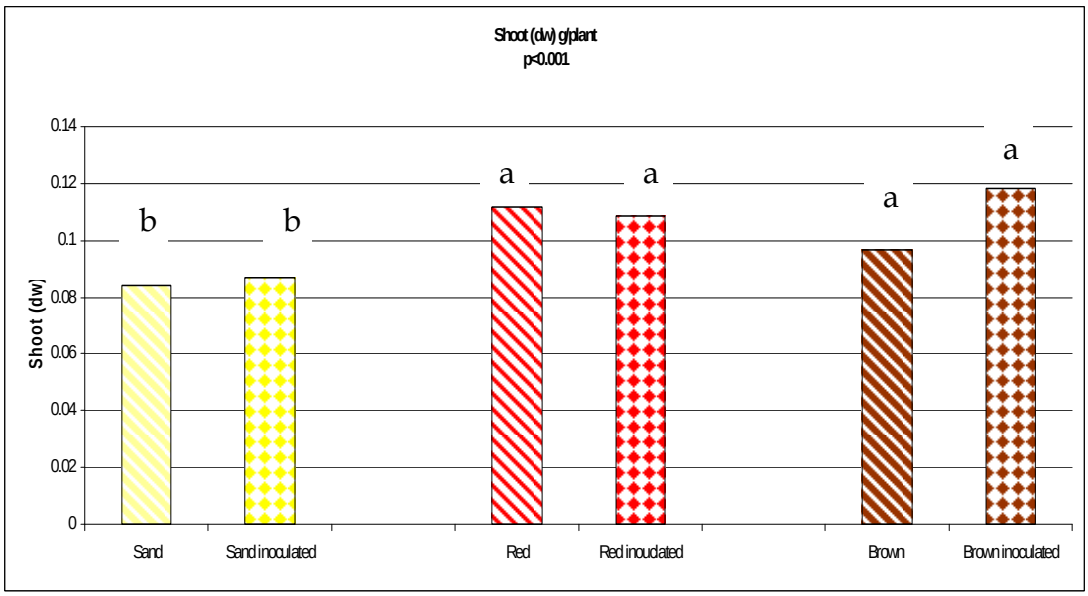


Figure 5.3.3 Average disease severity of seedlings grown for 21 days in inoculated and uninoculated sand, red and brown soil types. Note zero infection for uninoculated controls.

There was a significant interaction ($p<0.008$, sed=0.0422) between soil type and inoculation for ww shoot biomass (Figure 5.3.4), but no significant interaction for the dw shoot biomass.

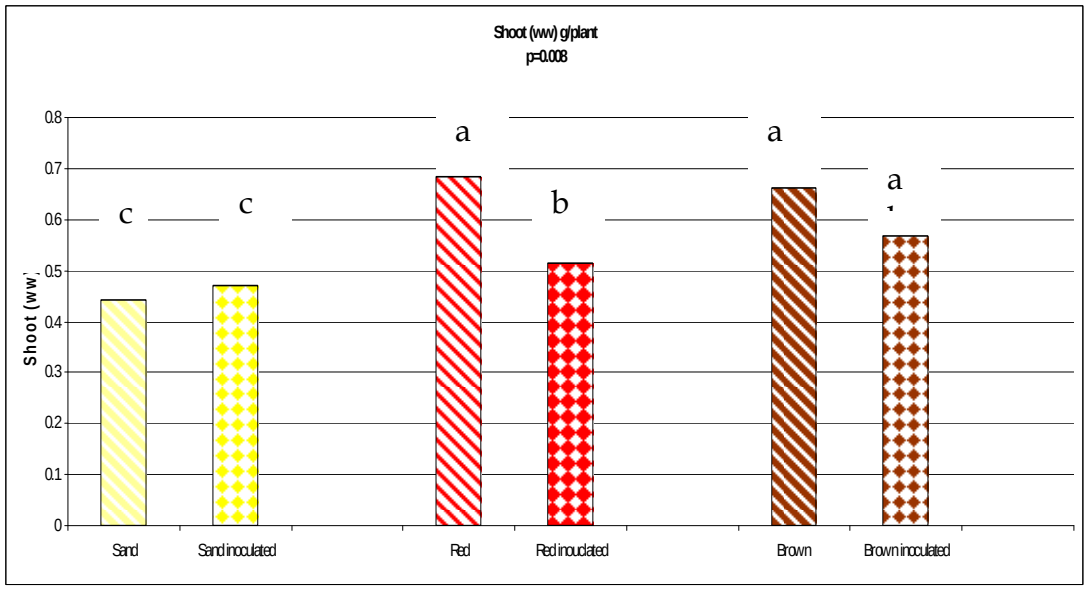


Figure 5.3.4 Shoot wet weights per plant grown for 21 days in inoculated and uninoculated sand, red and brown soil types.

Shoot dw biomass was significantly lower (p<0.001, sed=0.0065) for seedlings grown in the sand soil compared to those grown in red and brown soil types (Figure 5.3.5).

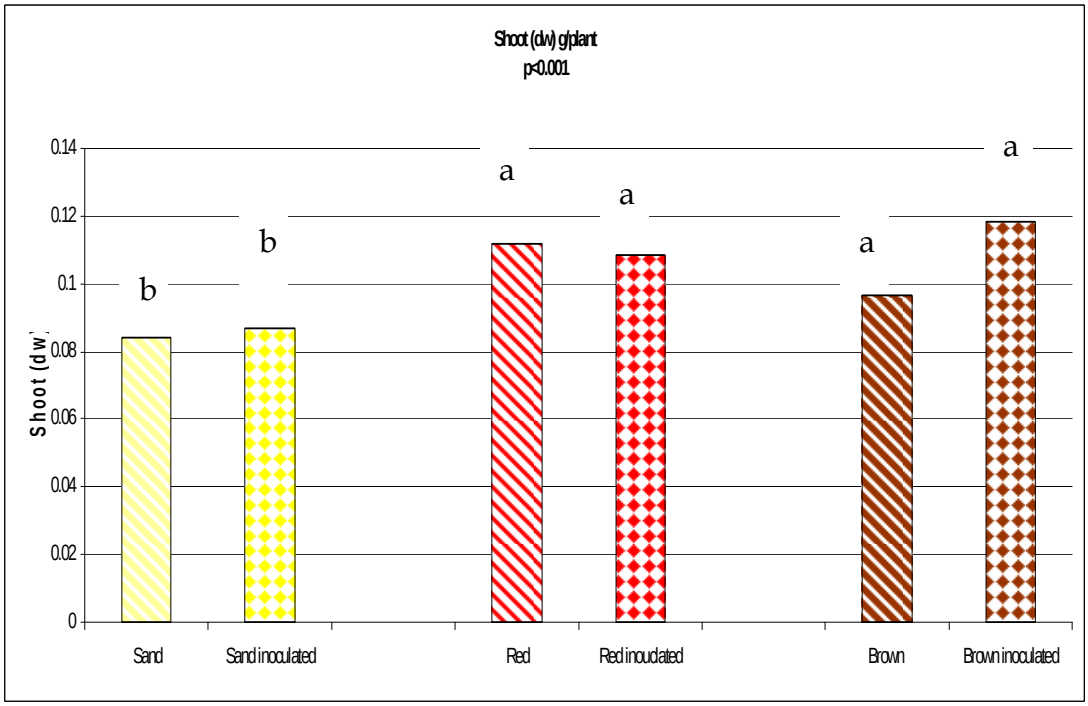


Figure 5.3.5 Shoot dry weights per plant grown for 21 days in inoculated and uninoculated sand, red and brown soil types.

a a

There was a significant interaction (p=0.005, sed=0.0237) between soil type and inoculation for ww root biomass (Figure 5.3.6). Root ww from seedlings grown in inoculated red and brown soil was significantly lower than those grown in uninoculated red and brown soil.

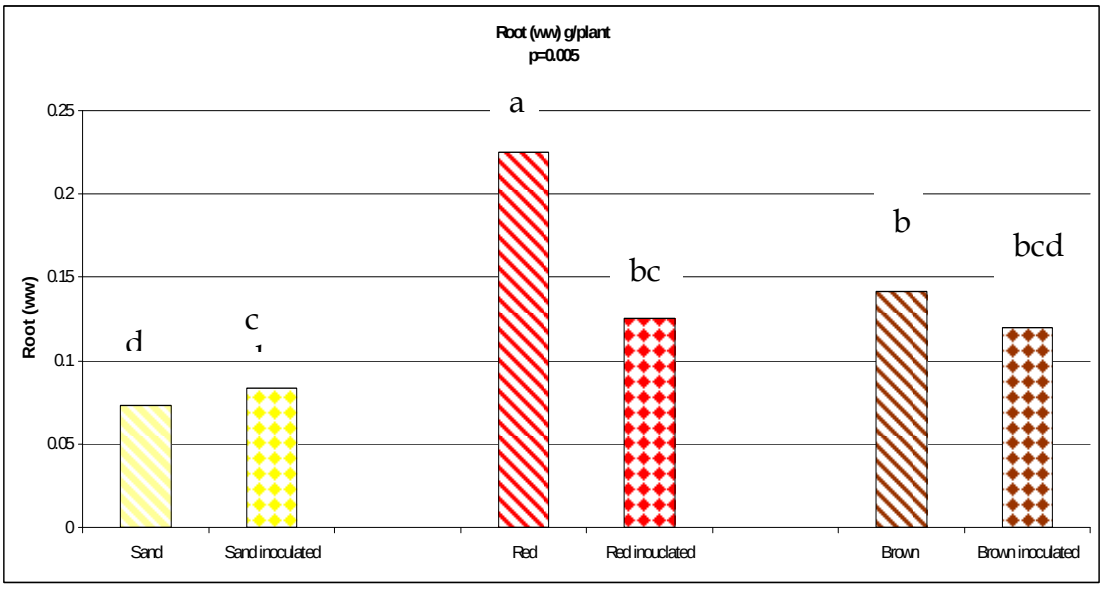


Figure 5.3.6 Root wet weights per plant grown for 21 days in inoculated and uninoculated sand, red and brown soil types.

There was a significant difference ($p<0.001$, sed=0.0001) in dw root biomass between the different soil types (Figure 5.3.7). Root (dw) weight was higher for all inoculated seedlings compared to uninoculated control seedlings and significantly higher for seedlings grown in sand and brown soil types.

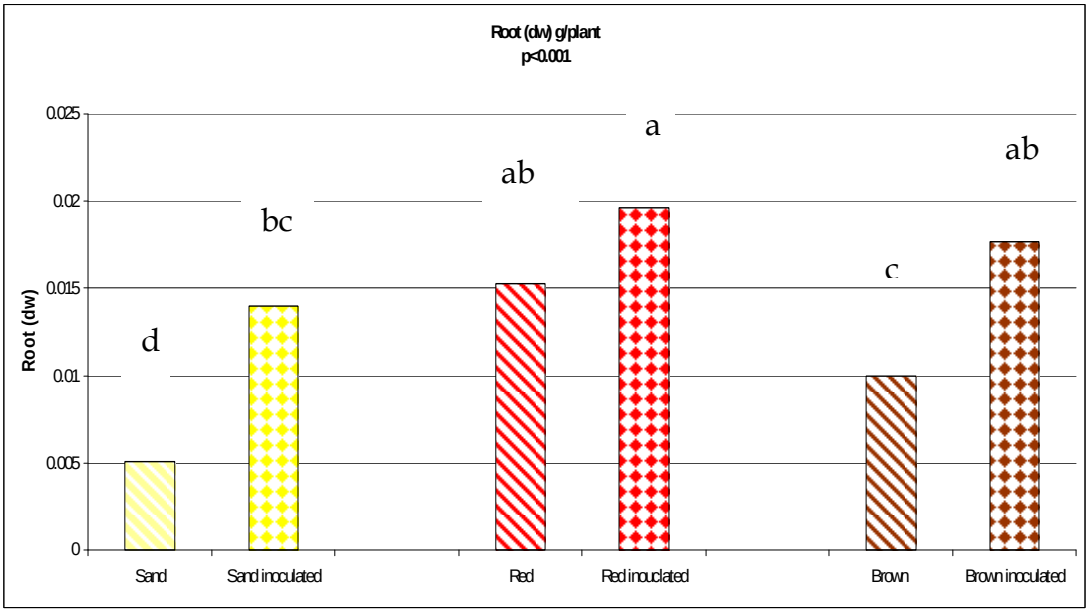


Figure 5.3.7 Root dry weights per plant grown for 21 days in inoculated and uninoculated sand, red and brown soil types.

Conclusions:

Soil type had a significant effect on days to emergence, particularly when grown in sand soil compared to red and brown soils. Days to emergence for seedlings were significantly higher when grown in the sand soil. Inoculating soil also seemed to effect seedling days to emergence with seedlings emerging faster in all inoculated soils compared to uninoculated soils. Disease severity was significantly higher in seedlings grown in both the red soil and brown soils compared to the sand soil.

Shoot biomass was significantly reduced for seedlings grown in inoculated red and brown soil compared to uninoculated red and brown soil. However there was no significant difference in the shoot biomass of seedlings grown in the inoculated or uninoculated sand soil.

There was a significant interaction between soil type and inoculation for ww root biomass. Although the ww root biomass was lower in all inoculated soils compared to uninoculated, it was only significantly lower in the red soil type. The dw root biomass was higher for all inoculated soil types, but only significantly higher when seedlings were grown in the sand and brown soil types. These findings are consistent with a study by Pearly Ly (2007). Ly reported significantly increased root branching for two cultivars (MCU-5 and Sicot-70) when inoculated with *T. basicola* compared to uninoculated seedlings.

Root architecture may play an important role in cottons ability to overcome disease. These results suggest further research should be done investigating cotton root architecture in the presence and absence of *T. basicola*.

5.4 Re-isolate 1995-2009 cultures stored in the long term storage collection.

A total of 52 isolates were recovered from plates kept from 1983 to 2009. See section 5.1 for methods. Isolates have been added to the long term collection and will be maintained for future research.

Objective 6 – Investigate potential *Verticillium dahliae* interaction with black root rot.

6.1 Identify fields with significant levels of both Verticillium wilt and black root rot.

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 (Table 6.1.1). Black root rot has been recorded in 2021 surveys since 1989/1990 and Verticillium wilt recorded in 2383 surveys since 1984/1985 (Table 6.1.2).

Black root rot has been recorded in 34.3% fields surveyed and of that 15.6% of the fields had an incidence of greater than or equal to 30% (Figure 6.1.1). Verticillium wilt was recorded in 54.5% of the fields and of that only 5% had incidence greater than or equal to 30%.

Table 6.1.1 Number of fields within each valley with greater than 30% black root rot and Verticillium wilt.

Valley	Greater than or equal to 30% black root rot	Greater than or equal to 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

Table 6.1.2 Number of fields with black root rot and Verticillium wilt.

	Since 1989-1990 till 2011-2012. Surveyed 315 fields ≥ 30% black root rot Incidence	Since 1984-1985 till 2011-2012. Surveyed 120 fields ≥ 30% Verticillium wilt Incidence
Fields with	693	1299
≥ 30%	315	120
Total Surveys	2021	2383

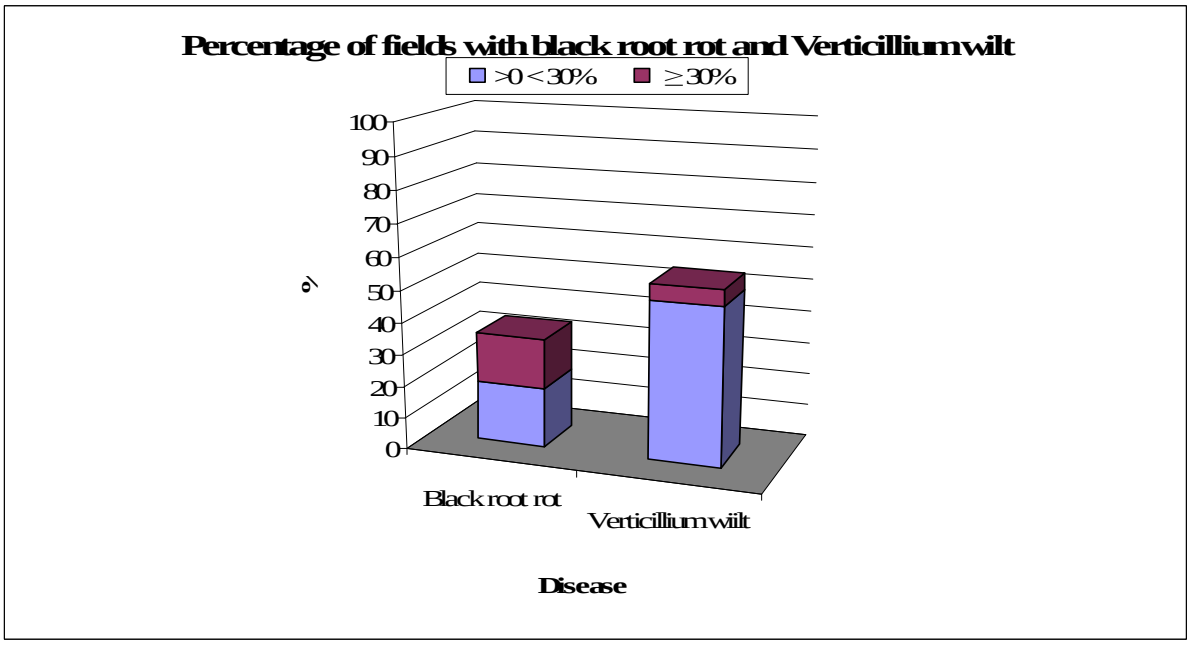


Figure 6.1.1 % fields with Verticillium wilt and black root rot.

6.2 Development of methods to determine the effects of *Thielaviopsis basicola* and *Verticillium dahliae* alone and together.

Methods for proposed glasshouse and field experimentation are currently being developed. One proposed experiment is taking soil cores to gather basic information on inoculum levels in the soil profile within cotton beds and furrows. Fields with known histories of black root rot and Verticillium wilt will be selected for sampling. Soil cores will be collected at different depths in the planting line and also at increments between beds. This will provide a profile on where the inoculum is in relation to the planting line on permanent beds. Soil cores will be taken from a field at bed preparation and 21 days after planting. This will be repeated at the end of the season.

Another proposed experiment is a completely randomised pot trial consisting of 4 treatments and 12 replicates was set up in a glasshouse. The treatments to be included will be plants growing in pasteurised soil that was inoculated with *T. basicola*, pasteurised soil inoculated with *V. dahliae*, pasteurised soil inoculated with both *T. basicola* and *Verticillium dahliae* and pasteurised soil as the control. Soil will be collected from Dulla Dulla to be pasteurised. The soil will be stored in a sterile bin for 4 days before 10" pots are filled. A total of 5 seeds will be planted into each pot. After emergence each pot will be stripped to 1 seedling per pot. The incidence of black root rot will be assessed on all plants by carefully removing from pots, roots washed under running water. Black root rot incidence will be calculated as the percentage of roots with distinctive blackened colour. The severity of the disease will be assessed on a scale of 0 to 10.

After the plants are assessed for black root rot they will be transplanted back into their original pots and maintained until maturity. The incidence of Verticillium wilt will then be assessed by cutting the stem of each plant and examined for the

distinctive brown discolouration of vascular tissue. Samples of the stem will be collected and isolated in the laboratory for confirmation of the disease.

Objective 7 – Investigate bacterial seed rots identifying the pathogen(s) and potential means of infection.

7.1 Laboratory and glasshouse experiments undertaken to identify the pathogen.

As a result of staff changes there was a substantial period where there was no project leader (pending my recruitment). In order to best meet this milestone a collaborative approach was negotiated with Dr Moazzem Khan (Research Scientist) with Department of Employment, Economic Development and Innovation (DEEDI) to collaborate on this milestone. The pathology unit collected boll samples during the 2011/2012 disease surveys and these were sent to Dr Khan pathogen identification and means of infection identified.

Objective 8 – Re-establish Australia's capacity to screen for exotic races of bacterial blight.

8.1 Obtain blight differentials from USA.

Dr Kirkby made several contacts in the USA prior to leaving for Texas on a DAFF scholarship. The objective of the trip was to gain firsthand experience with Hyper-virulent bacterial blight and Texas root rot in order to write the National Protocols should an incursion occur in Australia. Overseas contacts included Dr Peggy Thaxton, Fred Bourland, Richard Percy and James Frelichowski (curator of the US Cotton Germplasm Collection).

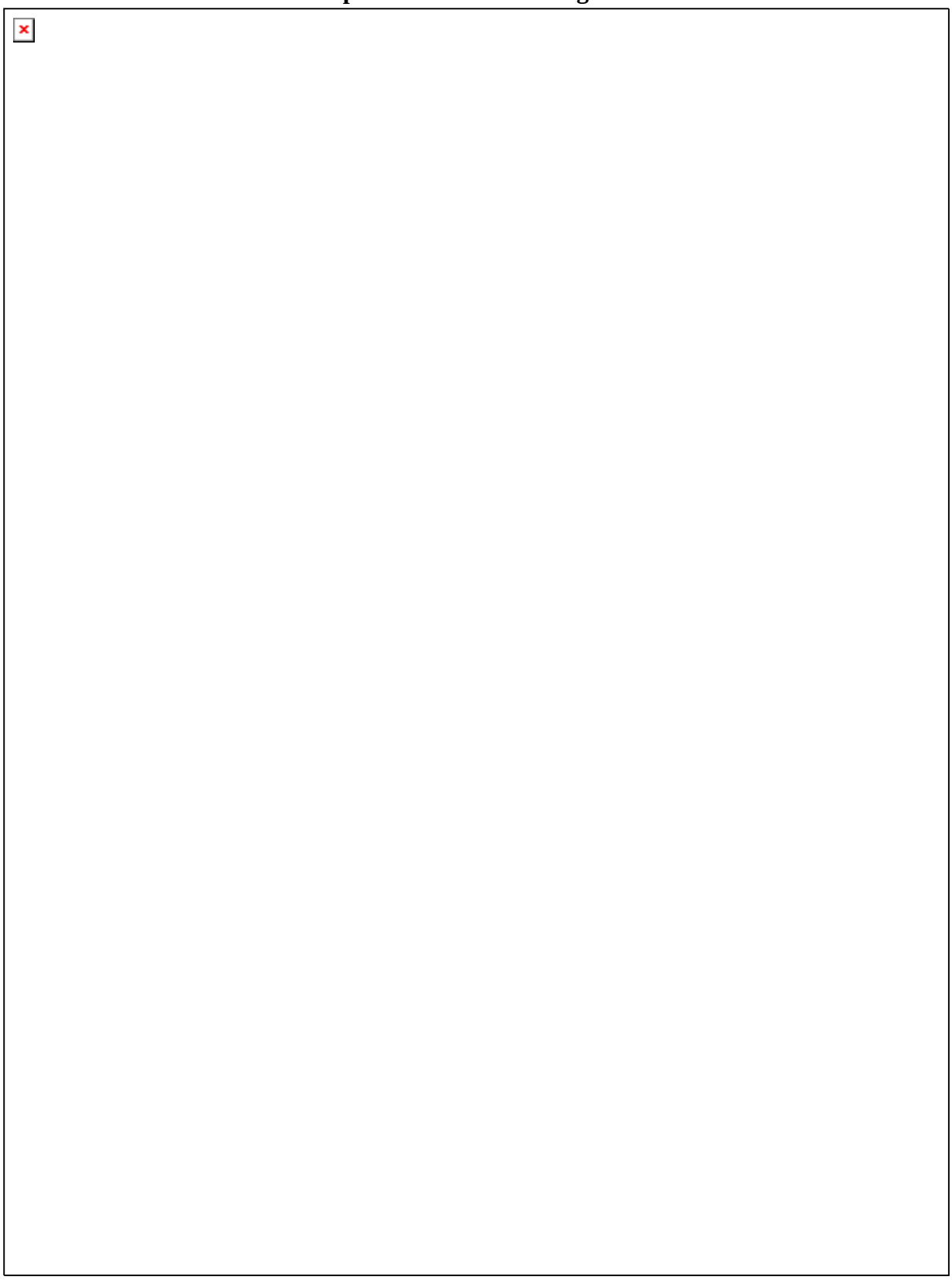
Dr Kirkby provided James with all the information on the 10 differential lines needed to be imported into Australia. Unfortunately USDA only had 5 of the 10 lines, those being Acala 44, Stoneville 20, Mebane B-1, Gregg and Empire B4. USDA-ARS National Plant Germplasm System has also been contacted about the missing lines. Dr Peggy Thaxton was able to supply the missing 5 lines (Stoneville 2B-S9, 1- 10B, 20-3, 101-102B and DPxP4) from Mississippi State University.

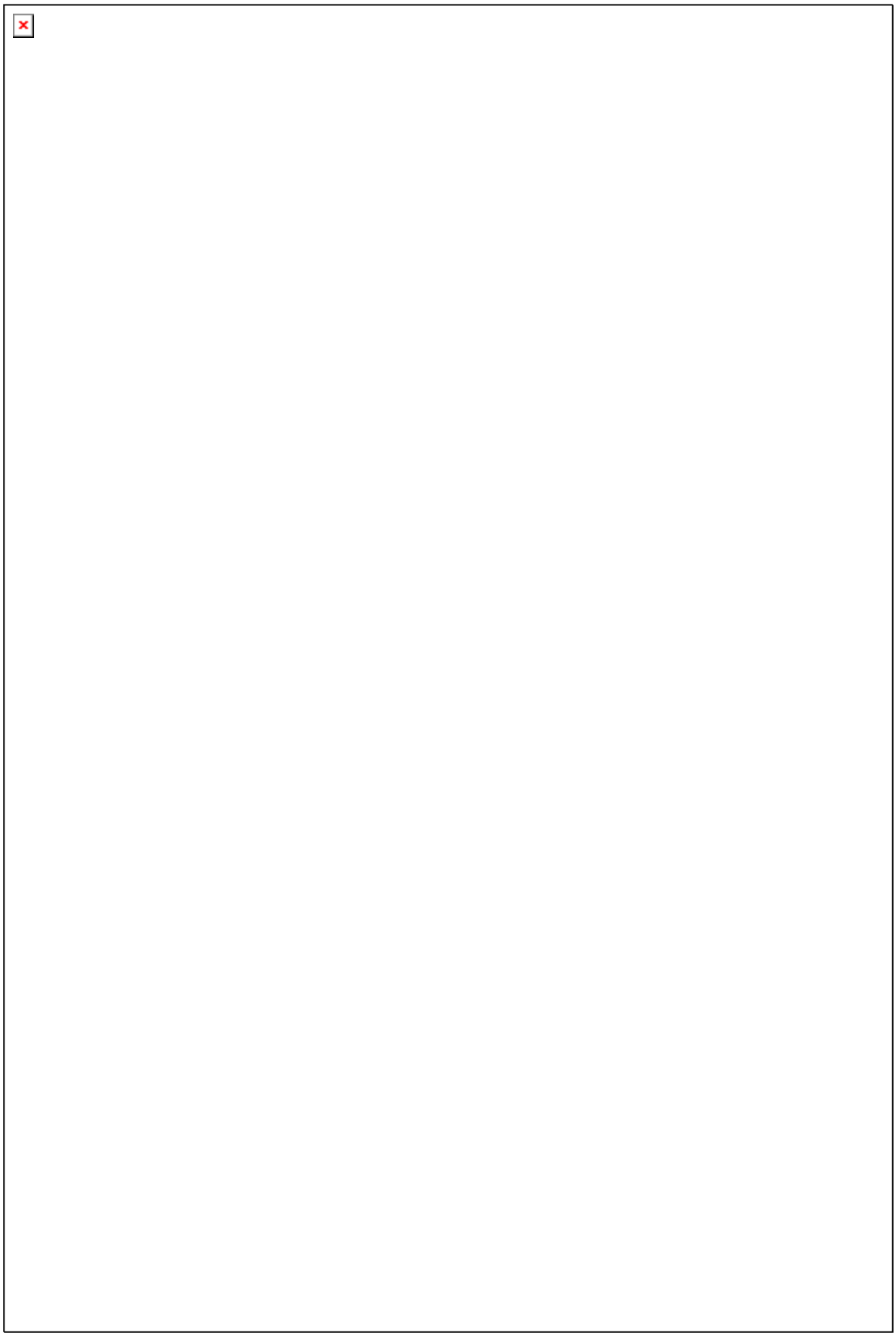
The seeds were imported (March 2012) by NSW Department Primary Industries under the import permit number IP 11015674. The differential lines were then grown for one generation in an AQIS approved facility for Post Entry Quarantine by Agri-Science Queensland, a service of the Department of Employment, Economic Development and Innovation.

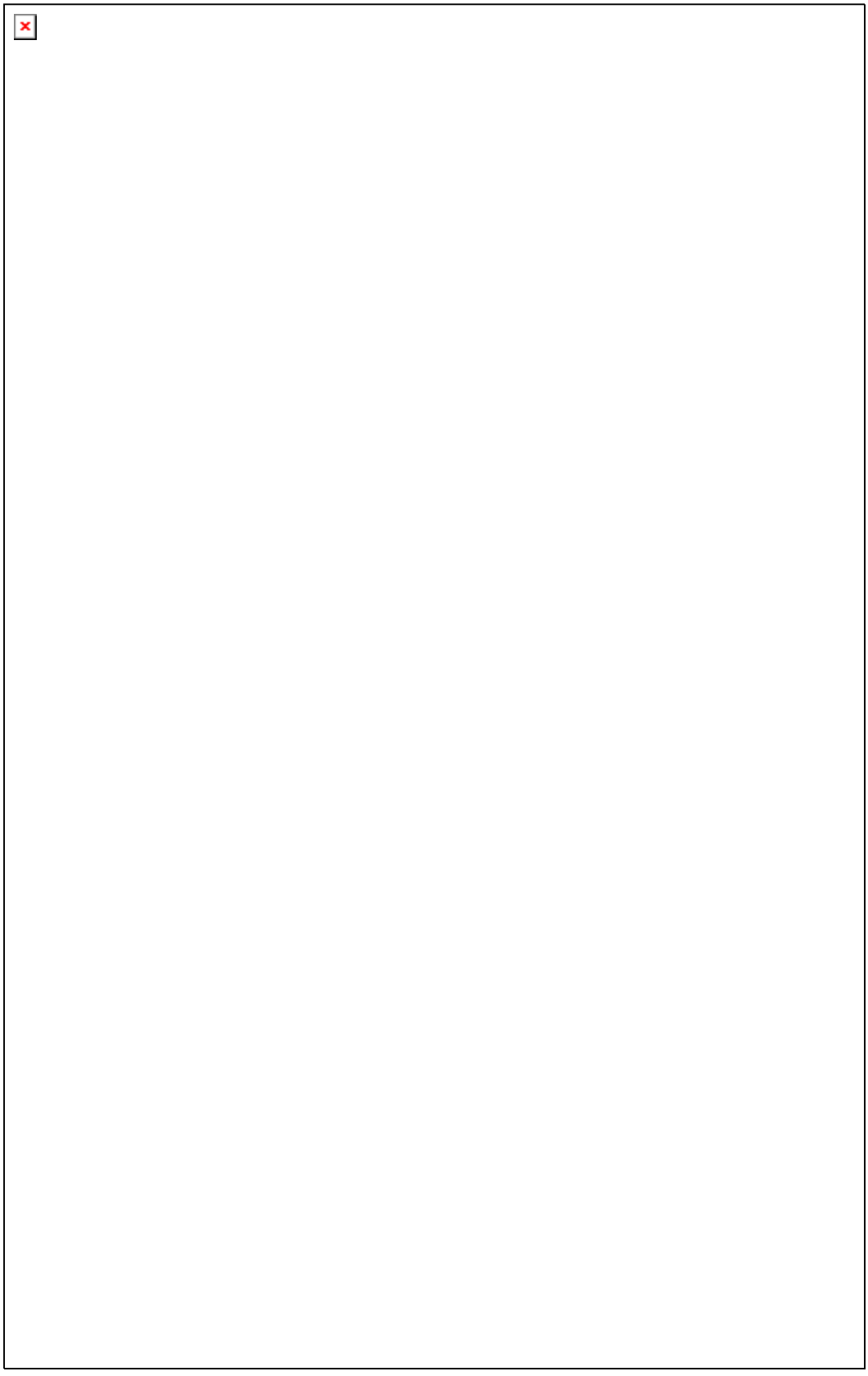
Cotton was harvested and ginned by Agri-Science Queensland before being sent to Narrabri. The original plants were then destroyed at the Post Entry Quarantine facility. Seeds from the 10 differential lines have been divided into two and stored in separate locations to minimise the risk of losing the lines due to circumstances out of our control.

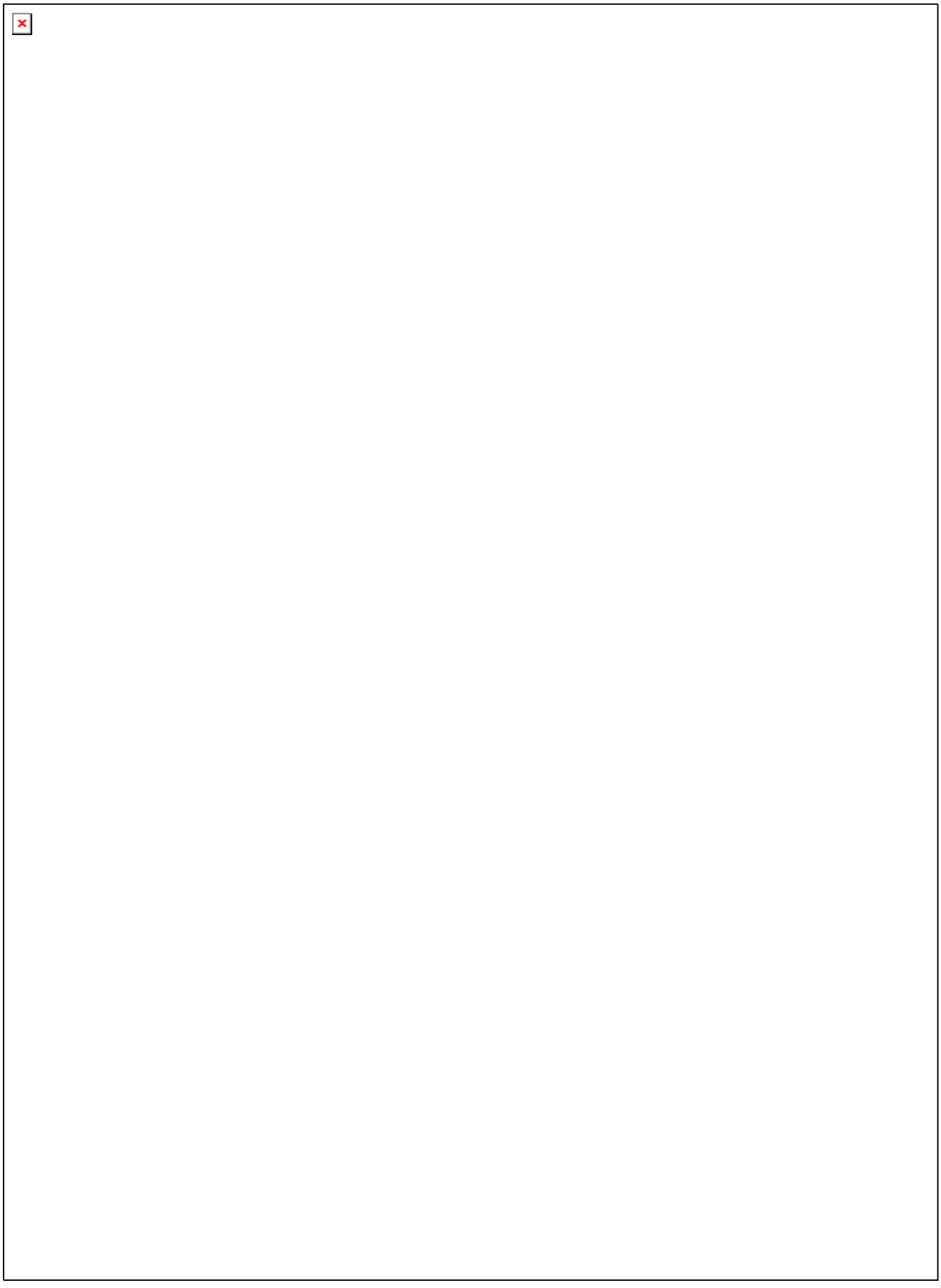
Objective 9 – Respond to industry disease issues as they arise

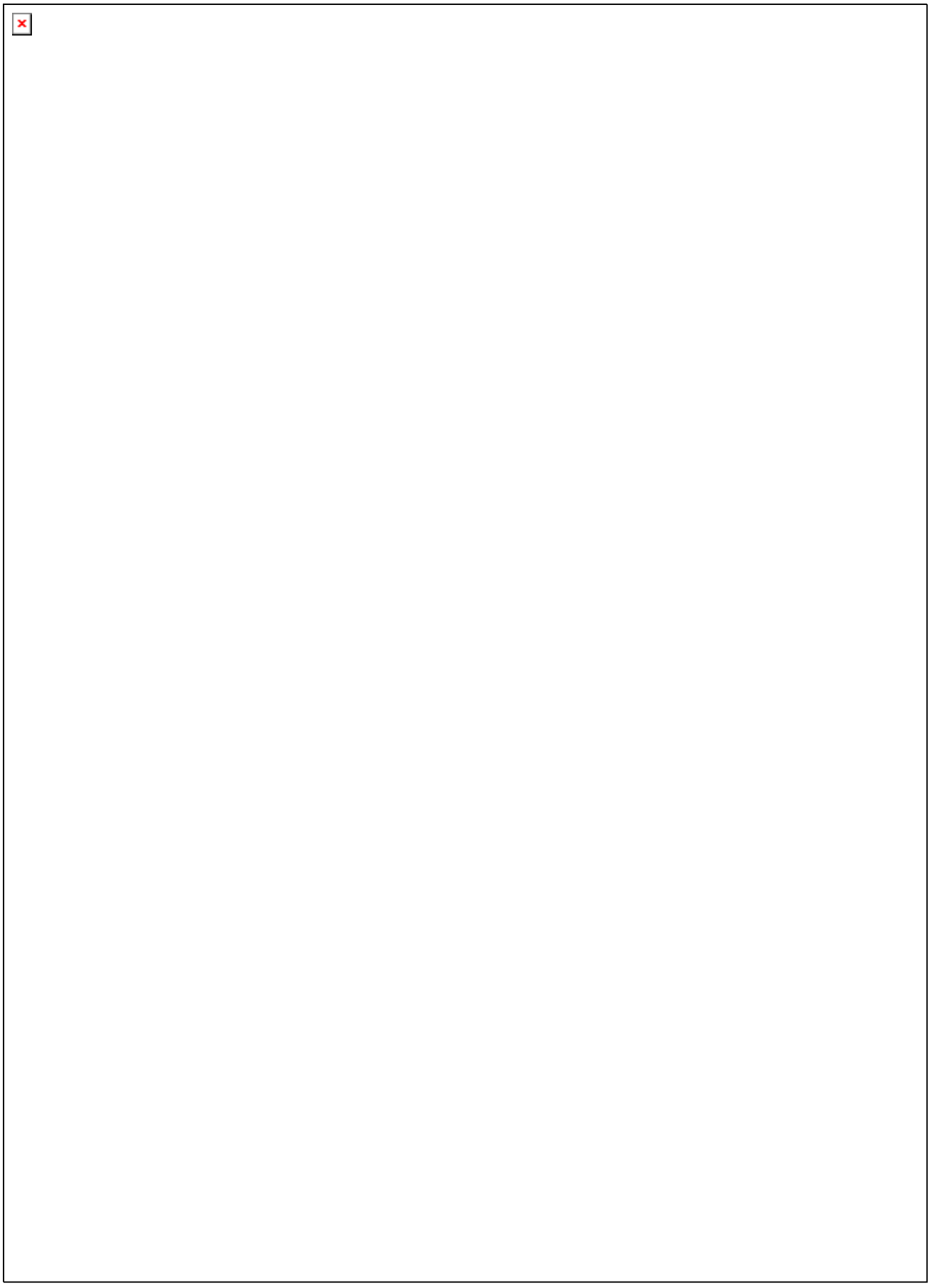
PathWAY- A new tool developed for communicating cotton disease issues

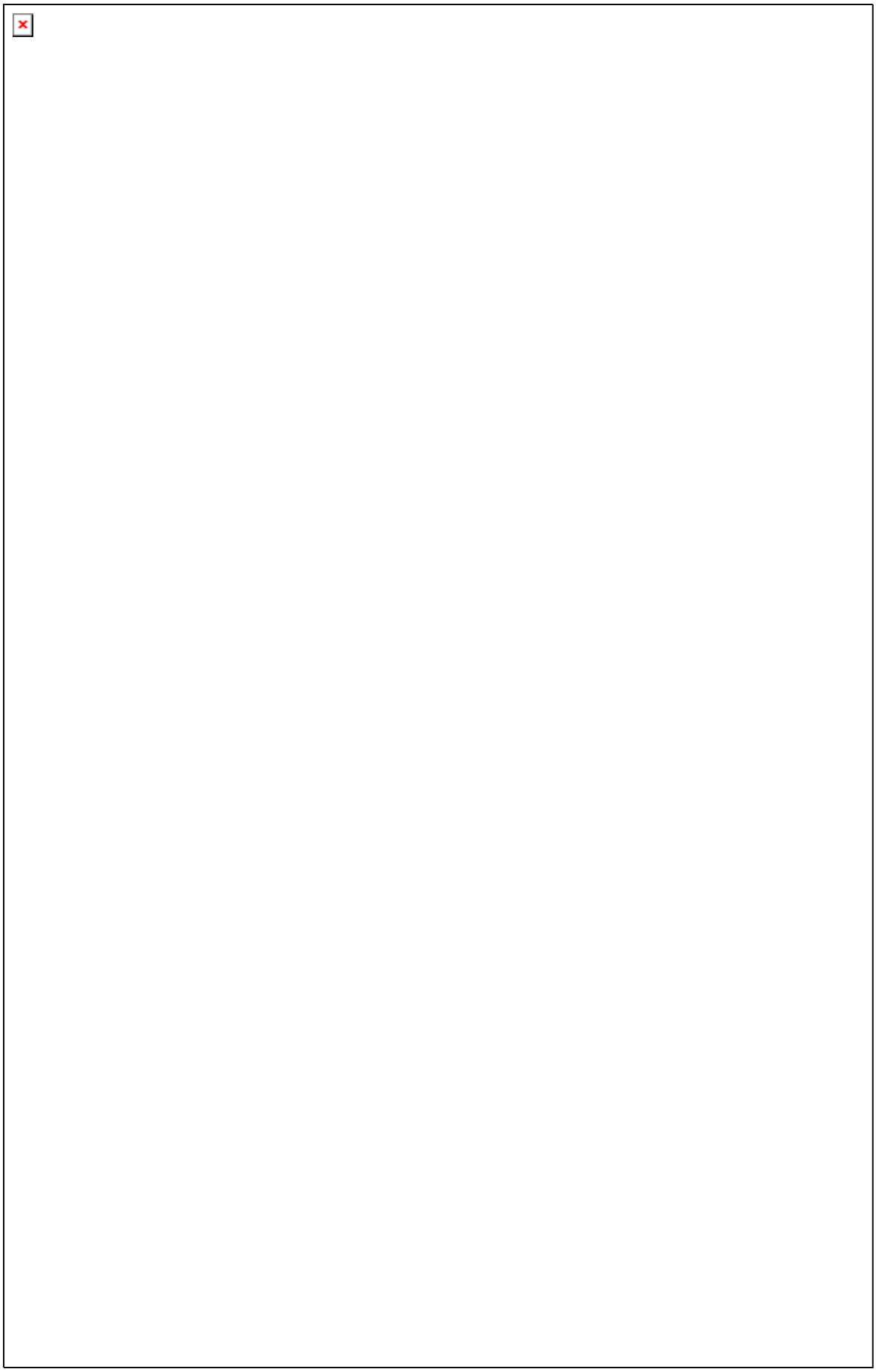












Outcomes

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.

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.)

National Diagnostic Protocol for Texas Root Rot Phymatotrichopsis omnivora

Diagnostic Scholarship Report


Prepared by:
Karen Kirkby
Dr Thomas Isakeit
Dr Terry Wheeler
Peter Lonergan

Address of agency:
Locked Bag 1000, Narrabri, NSW, 2390
www.industry.nsw.gov.au
Ph 0267 992454
Fax. 0267 991503

Australian Government
Department of Agriculture, Fisheries and Forestry
Office of the Australian Chief Plant Protection Officer

NSW GOVERNMENT Primary Industries

1. Introduction

Texas Root Rot (TRR) also known as Cotton Root Rot (CRR) is caused by the soil-borne ascomycete *Phymatotrichopsis omnivora* (Shear) Hennebert (Marek, Hansen, Romanish, & Thorn, 2009). TRR is native to south-western United States and Mexico where it is a devastating pathogen of cotton and alfalfa. The pathogen is found in Mexico, Venezuela, Brazil, and Libya and south-western states of USA including Arizona, Arkansas, California, Louisiana, Nervada, New Mexico, Oklahoma, Texas and Utah (CAB International, 2007) EPPO). This fungus does not cause disease in monocotyledonous plants; however it has potential to impact a huge range of dicotyledonous crops in Australia.

P. omnivora has a broad host range of over 2000 species of dicotyledonous plants including 107 economically important agricultural and horticultural crops, several tree species and ornamental plants (CAB International, 2007); (Blank, 1953). The major hosts and some other hosts are listed below. The major host is cotton including *Gossypium herbaceum, G. hirsutum*, and *G. barbadense*, Alfafa/Lucerne (*Medicago sativa*). TRR also affects nut crops including Almond (*Prunus dulcis*), Pecan (*Carya illinoinensis*), Walnut (*Juglans regia*); Apple (*Malus domestica*) and other *Rosaceae* including European Pear (*Pyrus communis*) and Peach (*Prunus persica*); Fig (*Ficus carica*); *Vitis vinifera* (grapevine); Sugarbeet (*Beta vulgaris var. saccharifera*); legumes including Peanut (*Arachis hypogaea*), Soyabean (*Glycine max*) and various beans (*Phaseolus* spp.); Okra (*Abelmoschus esculentus*) and other Malvaceae including Kennaf (*Hibiscus cannabinus*); Parsley (*Petroselinum crispum*) and other *Umbelliferae*; trees including Pine (*Pinus spp.*), Poplar (*Populus spp.*), Elm (*Ulmus spp.*), and Willow (*Salix spp.*) and Black Locust (*Robinia pseudoacacia*).

As of 2009, eight industry bodies (Apple and Pear Australia Ltd, AUSVEG, Citrus Australia Ltd, Cotton Australia Ltd, Grains Council of Australia, Nursery and Garden Industry Australia, Summer Fruit Australia Ltd, and Winemakers Federation of Australia) have agreed to cost sharing arrangements for *P. omnivora* under the Emergency Plant Pest Response Deed (EPPRD). Moreover, both the cotton and cherry industries consider *P. omnivora* to be a high priority pest (HPP) that could devastate production if it were to become established in Australia. Climate change is likely to increase the potential distribution of the fungus in Australia and thereby increase the risk posed by this fungus to Australian primary industries.

The rate of disease onset differs between plant species. Extensive root decay can often take up to two seasons in tree and fruit crops (CAB International). However, disease onset is sudden in vegetables and field crops. The fungus colonises the tap root, causing rapid decay and girdling of the outer periderm and cortical tissues and blocking the flow of water through the vascular cambium (Padil Toolbox). Plants consequently become chlorotic, wilt and then die.

Eradication options for the disease rely on the destruction of infected plant parts and extensive soil fumigation. The fungus will survive for long periods at depths of up to 2.4 m (Lyda & Kenerley, 1993). Rapid diagnosis is therefore important to avoid spread of the pathogen through the soil. The diagnosis of TRR is relatively straight forward due to the distinctive morphological characteristics of the pathogen. Diagnosis may be based on identification of symptoms *in planta* along with pathogen

morphology of isolated cultures. Diagnosis of TRR based on these techniques should take no longer than three weeks and should be confirmed by at least two laboratories.

1.1 Potential Distribution in Australia

The fungus will survive in alkaline (pH 7.2-8.0) calcareous (calcium carbonate >1%) non-sodic vertisols that occur in regions where the annual mean air temperature is greater than 15 ^{o}C (Percy, 1983). The average annual temperature across the cotton growing regions of Australia is > 15.6 ^{o}C. Cotton is usually grown in heavy cracking, mid to high pH vertisols that can be calcareous. Percy (1983) states that the fungus will not survive in soils where sodicity levels are greater than 2-3 meq/100g. The majority of irrigated soils have a sodium content of approximately 2.7 meq/100g soil. The conducive soil types coupled with transcontinental transport of plants and soil (Percy, 1983) indicate there is a distinct possibility for *P. omnivorum* to survive in the cotton growing regions of NSW and QLD, especially in areas of lower sodicity.

1.2 Transmission

The fungus proliferates in the field by the elongation of hyphal strands through the soil from plant to plant. Hyphal strands usually originate from a single focus of sclerotia or from a colonised plant, disseminating the infection from root to root. Thus patches of dead and dying plants appear to expand through a given season. Long range transmission can only occur when sclerotia and/or colonized plant parts are moved in soil and other media (eg. potting mix) or on contaminated machinery.

2. Taxonomic information and synonyms

Phymatotrichopsis omnivora (Shear) Hennebert was previously assumed to be a member of the Basidiomycota with possible teleomorphs *Sistotrema brinkmanii* and *Phanerochaete omnivora*. However, phylogenetic analyses of the nuclear small- and large-subunit ribosomal DNA and subunit 2 of RNA polymerase II from multiple isolates have shown this to be incorrect. *Phymatotrichopsis omnivora* is an anamorphic member of the Ascomycota for which no teleomorph has been described (Marek, et al., 2009). Synonyms for *Phymatotrichopsis omnivora* include *Phymatotrichum omnivorum* Duggar, *Ozonium omnivorum* Shear, *Ozonium auricomum* Link and *Hydnum omnivorum* Shear.

2.1 Classification

KINGDOM	Fungi
PHYLUM	Ascomycota
CLASS	Pezizomycetes
ORDER	Pezizales
FAMILY	Rhizinaceae
GENUS	*Phymatotrichopsis*
SPECIES	*omnivora*

3. Detection

Characteristic symptoms used for detection include wilting or dead plants, hyphae on the stem in the interface region between the above and below ground region of the stem.

3.1 Parts of the plant(s) on which it may be found

- Taproots
- Stem

3.2 Likely occurrence associated with the developmental stages of the host(s), climatic conditions and seasonality

P. omnivora has a low dispersal potential occurring particularly on heavy calcareous soils. The disease is not readily spread, persisting at certain locations where soil conditions are favourable. Depth of sclerotial placement is an important factor in symptom expression in the field; however the rate of fungal growth increases rapidly once soil temperatures exceed 22 ^{o}C (Rush, Gerik, & Lyda, 1984). Following a rain event mid-season, plants may begin to die in as little as 14 days.

3.3 Symptoms

The disease appears in patches in the field. The first symptoms of the disease are sudden wilting of plants with or without chlorosis of the leaves during the summer. The foliage droops, turns brown and may remain hanging on the branches for a few days before dropping off. At this stage the roots and lower section of the stem are covered with a network of yellow fungal strands visible to the naked eye. Discolouration can be seen inside the stem when cut in cross sections. Under high moisture conditions there may be sclerotia (brown to black in colour) on the surface of roots. Occasionally in cotton fields, on the ground near dying plants, the conidial stage develops as a creamy yellow spore mat.

3.4 Developmental stages

Mycelium strands formed on the roots of infected plants radiate outwards through the soil until it contacts new host roots. The hyphal strands grow intra- and intercellularly, penetrating the endodermis and xylem tissue. As the disease progresses the dead roots are extensively colonized by mycelium strands.

3.5 Sampling procedures critical for detection methods and diagnostic procedures

- Look for plants that are just starting to wilt. The leaves appear wilted but they are not yet dried up. Preferable to inspect fields in morning before the plants wilt from lack of water. Look in areas of the field where plants have been dead for some time
- Dig or pull up the wilted plants. Check to see if some or the entire top of the root is rotted by scraping the root with fingernail or knife. Roots of infected plants will be discoloured (brown, but not white). Check the lateral roots (also cut lower root in half, check for any discoloration (not white). Sample plants with rotted roots
- Using clippers, cut a sample that is 7-10 cm above the root. Cut off root 7 cm below soil line. Keep just the upper root-lower stem portion (about12 cm)
- If the plant has the symptoms described above record the field name and location (GPS coordinates), photograph the whole plant and place the sample in a labeled bag and seal
- Contact the NSW Primary Industries cotton pathologist (02) 67992474
- Bag the sample inside another labeled plastic bag and post to the Cotton Pathologist, Department of Primary Industries, Locked Bag 1000, Narrabri, NSW, 2390

4. Identification

P. omnivora can be easily identified by observation of its distinctive morphology. The species is characterized by distinctive branching acicular cruciform hyphal (200 µm in diameter) that is not known in any other species of fungus. Conidia may be unicellular, hyaline, globose or ovate (4.8 to 5.5 µm in diameter).

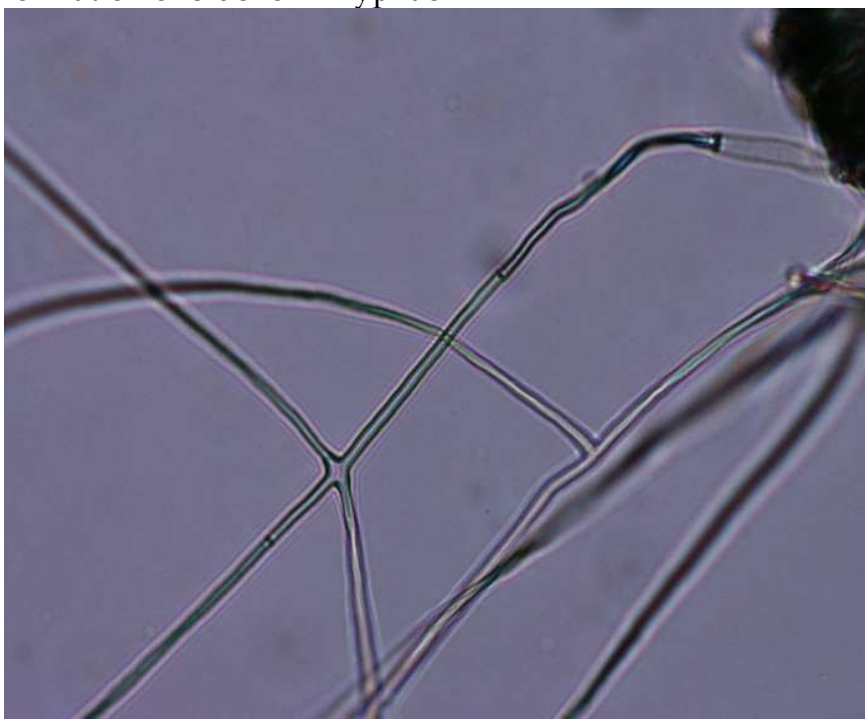
4.1 Methodology based on symptoms of plants infected with of P. omnivora

Infected plants may first show signs of sudden wilt. Plants have minimal root system. Brown to black vascular discoloration is seen in the stem.

4.2 Methodology based on morphology of *P. omnivora*

- Once at the lab place a drop of deionized water onto a microscope slide
- Using tweezers remove some of the brown/yellow mycelium from the root sample and place on the drop of water on the microscope slide
- Cover with a cover slip

- Examine under a compound microscope, looking for the characteristic hyphal formation of cruciform hyphae



4.3 Initial isolation of fungi from infected tissue

- Put on rubber gloves
- Remove lateral roots from the samples with clippers and discard
- Wash sample under running water. Remove soil with fingers or brush
- Copiously spray middle 10 cm of root/stem section with 70 % ethanol until it runs off
- Blot dry with clean paper towel and place on clean paper towel
- Hold stem at top end. Dip scalpel in alcohol and flame it. Use the scalpel to peel outer part of stem, peeling away from you
- Peel about 1/2 way around stem
- Look for browning. Peel more and deeper
- Peel close to root/stem interface OR look for where the root is browner than the rest
- Peel and cut out a piece (3 x 3 mm) at the brown area that is about 1 mm thick
- Place piece on Petri plate of 1/5 strength potato dextrose agar with streptomycin, about 60 mm from edge of the plate. Push the piece firmly onto the agar

- Repeat and put 3 additional pieces on plate. Take pieces from the same area of root or from nearby
- Cover with lid and place in a plastic bag
- Incubate at 30 ^{o}C for 4 days

5. Contact points for further information

The Cotton Pathology Group of NSW Primary Industries located at the Australian Cotton Research Institute (ACRI), Narrabri is the preferred diagnostic laboratory to process plants suspected of Texas or Cotton Root Rot. This laboratory has developed expertise in the detection and diagnosis of this disease through visiting Texas, USA where the disease is prevalent. Photos should be taken of suspected plants and sent as soon as possible to the laboratory, along with the location (GPS) of suspected infection and contact information.

- Karen Kirkby, Department Primary Industries, Locked Bag 1000, Narrabri, NSW, 2390, Australia. Phone 0267 992454, Fax 0267 991503, Email karen.kirkby@industry.nsw.gov.au
- Peter Lonergan, Department Primary Industries, Locked Bag 1000, Narrabri, NSW, 2390, Australia. Phone 0267 99 1531, Fax 0267 991503, Email perter.lonergan@industry.nsw.gov.au

– Dr. Terry Wheeler, Texas AgriLife Research, 1102 East FM 1294, Lubbock, 79403, Texas. Email ta-wheeler@tamu.edu
– Dr. Thomas Isakeit, Texas A&M University, College Station, Texas. Email
– t-isakeit@tamu.edu

All samples that test positive for an exotic disease will be sent to Dr Alison Seyb (Plant Pathologist) Private Bag 4008, Narellan, NSW, 2567 for independent confirmation.

6. Acknowledgements
The authors wish to thank Emilie Condon-Heck from Texas A&M University for independently reviewing this protocol before its publication.

7. Appendices (as appropriate)

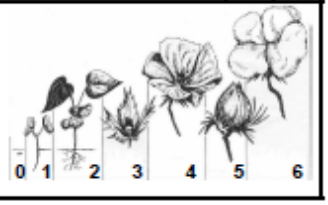
7.1 Sample form for detection of Texas root rot during early season cotton disease surveys

Code		Date		Deg	Min
				South	
Farm		Field		East	
				Surveyed by:	

	G/S	No True Leaves	Std	BRR (0-10)									
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													

CLCuVirus	Pr	Ab	Texas Root Rot	Pr	Ab
Blue Disease	Pr	Ab	Defoliating Vert Wilt	Pr	Ab
Exotic Fusarium Wilt	Pr	Ab	Hyperviralent Blight	Pr	Ab

Comments:



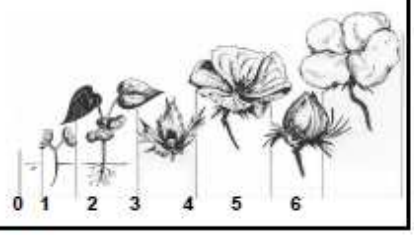
7.2 Sample form for detection of Texas root rot during late season cotton disease surveys

Code	Date		Degrees	Minutes
		South		
FarmField		East		
	G/S		Surveyed by:	

	Fov	Vd	Am%	PBR	OBR	SW	Hor	LM	Bchy	CBT
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
Total										

CLCuVirus	Pr	Ab	Texas Root Rot	Pr	Ab
Blue Disease	Pr	Ab	Defoliating Vert	Pr	Ab
Exotic Fov	Pr	Ab	Hyperviralent BBt	Pr	Ab

Comments:



National Diagnostic Protocol for Hypervirulent Bacterial Blight
Xanthomonas axonopodis pv. *malvacearum*

Diagnostic Scholarship Report

Prepared by:
Karen Kirkby
Dr. Terry Wheeler
Dr. Thomas Isakeit
Peter Lonergan

Address of agency:
Locked Bag 1000, Narrabri, NSW, 2390
www.industry.nsw.gov.au
Ph 0267 992454
Fax. 0267 991503

Australian Government
Department of Agriculture, Fisheries and Forestry
Office of the Australian Chief Plant Protection Officer

NSW GOVERNMENT Primary Industries

1. Introduction

Bacterial blight of cotton (*Gossypium hirsutum* and *G. barbadense*) is caused by the gram-negative aerobic non spore-forming rod-shaped bacterium *Xanthomonas axonopodis* pv. *malvacearum* (Wang, Brubaker, Summerell, Thrall, & Burdon, 2010). Bacterial blight is a major disease of cotton worldwide causing up to 70% yield losses during severe epidemics (Kirkpatrick & Rothrock, 2001). The pathogen is host-specific to cotton (*Gossypium hirsutum* (upland cotton), *G. barbadense* (pima cotton), *G. herbaceum*) although it can form endophytic infections in secondary hosts such as *Ceiba pentandra* (Kapok tree), *Jatropha curcas* (Barbados nut), *Thurberia thespesioides* (Wild cotton plant) and *Lochnera pusilla* (Tiny Periwinkle).

Bacterial blight has been found in all cotton growing regions of the world. Historically more than one race can be found in a given region. In order to minimise the effect of genotype-environment interactions, Hunter et al. (1968) developed a set of host differentials to determine races of bacterial blight. Nineteen races of the pathogen are recognised in the USA based on the response of these differentials to inoculation with the bacterium (Hillcocks, 1992). Each differential cultivar contains specific resistance genes so each race is a homogeneous group with the ability to overcome a specific set of resistance genes. Race 18 is the most widespread and aggressive among the races, being the predominant pathogen in the USA, and until recently, in Australia (Allen, 1991). Most modern cotton cultivars in Australia are now resistant to Race 18 and all other recognised races.

In Africa, several strains have evolved with the ability to overcome resistance in all 10 differential cotton cultivars. These isolates are herein designated as Hyper-virulent (HV) strains and can overcome the major resistance genes *B2*, *B3*, *B4*, *B6* and *B7* and combinations thereof found in USA cultivars. Several strains have been collected including the isolates HV1, HV3, HV7, Chad and the Sudan.

1.1 Potential Distribution in Australia

Bacterial blight of cotton was first recorded in Australia in 1923 with races 2 to 5, 7, 9, 10 and 18 reported between 1974 and 1983 (Allen, 1991). If HV strains were to arrive in Australia, the disease has potential to occur in all cotton growing regions. The HV1 strain is of particular concern to the Australian cotton industry since it is not known if the pathogen will overcome the resistance genes in Australian cotton cultivars. It is vital to ensure the capacity to differentiate between and correctly identify recognised races and HV strains should an outbreak of bacterial blight occur in Australia in the future.

1.2 Transmission

Bacterial blight is seed-borne, being carried over between generations in and on the seed. The pathogen may over-winter in the soil on un-decomposed plant debris including leaves, bracts and petioles (for up to 4 months) but more importantly it survives internally and externally on seed for up to 2 years (Kirkpatrick & Rothrock, 2001). The study by Alexander (2009) showed the pathogen survived the acid treatment used to delint seed.

2. Taxonomic information and synonyms

The bacterial blight pathogen was initially named *Pseudomonas malvacearum* by (Smith, 1901) who later referred to it as *Bacterium malvacearum* (Smith, 1920). Following that, Dowson (1939) reclassified it as *Xanthomonas malvaacearum* (E.F. Smith) Dow then *Xanthomonas campestris* pv. *malvacearum* (E.F. Smith) Dow (*Xcm*) (Dye et al., 1980). The name now generally accepted is *Xanthomonas axonopodis* pv. *malvacearum* (Kado, 2010).

2.1 Classification

KINGDOM	Bacteria
PHYLUM	Proteobacteria
CLASS	Gammaproteobacteria
ORDER	Xanthomonadales
FAMILY	Xanthomonadaceae
GENUS	*Xanthomonas*
SPECIES	*axonopodis*
SUBSPECIES	*malvacearum*

3. Detection

Symptoms of bacterial infection generally show 2 weeks after inoculation. Characteristic symptoms used for detection include water-soaked lesions on the underside of the leaves on the upper surface of leaves, bolls and bracts.

3.1 Parts of the plant(s) on which it may be found

- Cotyledons
- Leaves
- Stem
- Bracts
- Bolls
- Seed

3.2 Likely occurrence associated with developmental stages of the host(s), climatic conditions and seasonality

The disease is found in almost every country where cotton is grown today. The pathogen survives better in dry environments and in the USA this may be a more frequent problem due to overwinter survival. Rainfall with higher temperatures provide favourable conditions for disease development, so hot dry weather coupled with a few sporadic rain events can start epidemics. Consequently it is less important in countries that experience hot, dry weather for most of the cotton growing season (Hillcocks, 1992). Once a seedling is aboveground, it may become infected as a result of inoculum spread from other infected plants or debris through wind and/or rainfall or irrigation runoff.

3.3 Symptoms

Cotyledons: Small dark green 'water-soaked' lesions either circular or irregular in shape that penetrate all the way through the leaf, being visible on the under and upper surface of the leaf. Cotyledons may become distorted in susceptible cultivars. Under favourable conditions the infection spreads down the petiole to the stem, resulting in stunting or even death of the seedling.

Leaves: Angular leaf spots (ALS) usually 2-5mm appear water-soaked and penetrate all the way through the leaf, being more visible on the underside of leaves. Lesions that occur only on the upper surface are not bacterial blight. The ALS become dark brown to black as they dry out, becoming distorted and necrotic before finally shedding. Lesions extending along the main vein are known as vein blight and may occur with or without ALS. Vascular tissue may be invaded by the bacterium, indicated by roughly circular chlorotic patches about 1-3 cm in diameter surrounded by smaller angular spots.

Angular leaf spots that go all the way through the leaf on a cotton plant infected with bacterial blight (Image courtesy Jason Woodward, Texas).

Stem: In the most severe manifestation the infection moves down to the stem, indicated by sooty black lesions that may completely girdle the stem. This is referred to as blackarm and may lead to stems breaking under windy conditions or under the weight of developing bolls.
Bracts: flower buds and young bolls may shed once symptoms of bacterial blight appear.
Bolls: round 'water-soaked' spots 2-5mm in diameter and up to 10mm or more in susceptible hosts become dark brown with age. Lesions may be numerous and clustered close to the suture or the base of the boll under the epicalyx. In severe cases lesions penetrate the boll walls (usually where there has been insect damage) resulting in internal rot. Internal rot causes the lint to be stained yellow leading to surface contamination from the bacteria.


Water soaked lesion present on a cotton boll infected with bacterial blight.

Seeds: internal infection of the seed may enter through the micropyle or introduced by the stainer bug piercing the seed while feeding. The pathogen has been known to survive acid delinting.

3.4 Developmental stages:
Atkinson (1891) gave the names angular leaf spot (ALS), blackarm and bacterial boll rot to the various stages of bacterial blight disease, however during 1901-1905, E. F Smith demonstrated that these symptoms were caused by the same pathogen (Smith, 1920).

3.5 Sampling procedures critical for detection methods and diagnostic procedures:
It is important that growers, agronomists, consultants and pathologists continue to check the health of the plants in the field. These checks should be carried out throughout the growing season. Early detection of this exotic disease will allow control measures to be put in place.

- Look for plants that have lesions and or signs of defoliation
- Examine the under and upper surface of the leaves for the presence of angular water soaked lesions that go all the way through the leaf
- Examine the bolls for water soaked lesions
- If the plant has the lesions described above record the field name and location (GPS coordinates), photograph then remove the whole aerial part of the plant and place in a labeled bag and seal
- Contact the NSW Primary Industries cotton pathologist (02) 67992474
- Bag the sample inside another labeled plastic bag and post to the Cotton Pathologist, Department of Primary Industries, Locked Bag 1000, Narrabri, NSW, 2390

4. Identification

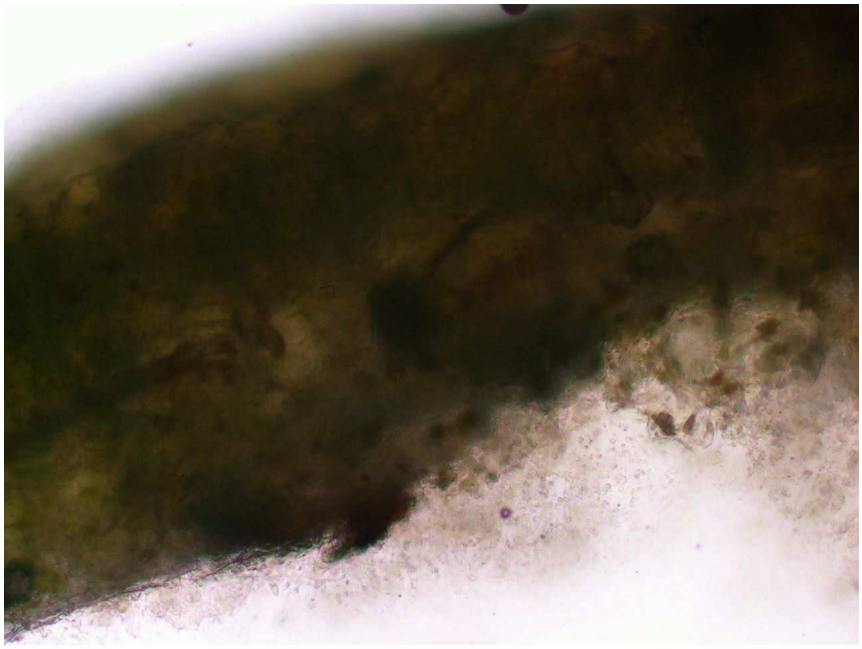
Bacterial blight may be identified on susceptible host plants when angular water soaked lesions that penetrate (visible on **both** bottom and top) of leaves are present. The race of the pathogen may be determined by the scratching the surface of each differential host line leaves with a sterile toothpick that has been dipped into an isolated culture of bacteria and examining the response of each host line (see 4.1 Methodology based on response to host differential set). The pathogen may be isolated on potato carrot dextrose agar (PCDA) media and identified by shiny yellow bacterial colonies (see 4.2 Methodology based on morphological and cultural characteristics).

4.1 Methodology based on morphological and cultural characteristics

Examine the whole plant and look for small angular and irregular shaped lesions on the under and upper side of leaves, making note that the pathogen causes lesions that go all the way through the leaf.

Bacterial stream test and initial isolation of bacteria from infected host tissue

- Fill a small beaker with 70% ethanol
- Place a ring spatula, spoon spatula, tweezers and a scalpel in the ethanol
- Surfaces sterilize the infected leaves by spraying with 70% ethanol and quickly blot the leaf dry with paper towel
- Place 1-5 µl of sterile water in a labeled Petri plate then cover with lid
- Cut out a small amount of diseased tissue (2mm x 4 mm) from the leaf using tweezers and a scalpel and place the tissue on a drop of water on a microscope slide. Cover with a coverslip and view under the microscope looking for bacteria streaming from the cut section of the tissue



Bacteria streaming from infected cotton tissue

• To isolate the bacteria, cut out another small amount of diseased tissue (2 mm x 4 mm) from the leaf using tweezers and a scalpel and place in the drop of water in Petri dish and cover with the lid. Leave the plate for 5-10 minutes

• Dip the spoon end of a spatula in a beaker of 70% ethanol then flame

• Using the back of the spoon, crush the tissue in the water drop

• Flame the inoculation loop and dip into water/sample

• Streak the bacteria onto potato carrot dextrose agar (PCDA). For agar recipe see Table 4.1.1. Ensure agar surface is dry before use as the bacterium is motile in surface moisture and discrete colonies will not form

• Incubate at 30°C for 24 to 48 hours (no light necessary). Colonies visible to the naked eye after only 24 hours are unlikely to be the pathogen. Bacterial blight colonies are slimy and yellow in color. To obtain pure cultures of the yellow bacterium, subculture these colonies onto the potato carrot dextrose agar with peptone and yeast extract (Bird, 1966) (Table 4.1.1) and incubate for 48-72 hours

Isolated bacterial colonies grown on potato carrot dextrose agar

Table 4.1.1 Potato carrot dextrose agar with peptone and yeast extract recipe from Bird (1966).

Ingredient	Per 1L
MgSo4	0.3 gms
CaCO3	0.2 gms
Agar	10 gms
Potato Dextrose Agar (Commercial)*	40 gms
Peptone	2.5 gms
Carrot Juice **	15 ml
Distilled H2O	1 L
Yeast extract	0.5 gms

* Difco

** Commercial canned carrot juice (everyday brand)

4.2 Methodology based on response of the scratch test to host differential set

- The minimum number of plants per differential line (Table 4.1.1) to be screened should be 10-20. A recent variety of cotton that is known to be resistant to bacterial blight race 18 should also be included in the screening process
- Fill tube pots with clean field soil
- Press seed onto the soil surface and top with sand. Water plants as needed
- Germinate plants in a temperature controlled growth room set at 27^{o}C. Maintain plants in growth room until 2 cotyledons have emerged

- With a sterile toothpick make a small cross on the under surface of the cotyledon

• On the same cotyledon take another sterile toothpick and immerse the tip in the cultured bacterial isolate before making another larger cross on the cotyledon

• Repeat this for all the lines to be screened

• Incubate the plants at approximately 100% relative humidity for 24 hours, preferably in a humidity chamber

• Return plants to the temperature controlled growth room

• Assess the plants 14 days after inoculation for the presence of necrotic tissue around the area of inoculation and/or water soaked lesions on the lower surface of the cotyledons. There should be no signs of infection around the area of the sterile scratch test

• To determine the race, record the pathogenicity differences between the differential lines and refer to Table 4.2.2

Table 4.2.1 Bacterial blight pathogen responses to host differentials adapted from Hunter et al (1968). *A recent variety that has shown resistance to bacterial blight should always be included in the screening.

Host Differentials	Race																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Acala 44	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stoneville 2B	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	-
Stoneville 20	-	+	-	-	-	-	-	+	+	+	-	+	-	+	+	+	+	+	-
Mebane B-1	-	-	-	-	-	+	+	+	-	+	-	-	-	-	-	+	-	+	-
1-10B	-	-	+	-	+	+	+	+	-	+	-	-	-	+	+	-	-	+	+
20-Mar	-	-	-	+	+	-	+	-	+	+	-	-	-	+	-	+	+	+	+
101-102B	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gregg	-	-				+	+	+		+	+	+		+				+	+
Empire B4	-	-				-	-	-		-	-	-		-				+	-
DPX P4 Resistant variety*	-	-				-	-	-		-	-	-		-				+	-

5. Contact points for further information

The Cotton Pathology Group of NSW Primary Industries located at Australian Cotton Research Institute (ACRI), Narrabri is the preferred diagnostic laboratory to process plants suspected of Hypervirulent Bacterial Blight. This laboratory has developed expertise in the detection and diagnosis of this disease through visiting Texas, USA where the disease is prevalent. Photos should be taken of suspected plants and sent as soon as possible to the laboratory, along with the location (GPS) of suspected infection and contact information.

- Karen Kirkby, Department Primary Industries, Locked Bag 1000, Narrabri, NSW, 2390, Australia. Phone 0267 992454, Fax 0267 991503, Email karen.kirkby@industry.nsw.gov.au
- Peter Lonergan, Department Primary Industries, Locked Bag 1000, Narrabri, NSW, 2390, Australia. Phone 0267 991531, Fax 0267 991503, Email peter.lonergan@industry.nsw.gov.au
- Dr. Terry Wheeler, Texas AgriLife Research, 1102 East FM 1294, Lubbock, 79403, Texas. Email ta-wheeler@tamu.edu
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- t-isakeit@tamu.edu

All samples that test positive for an exotic disease will be sent to Dr Alison Seyb (Plant Pathologist) Private Bag 4008, Narellan, NSW, 2567 for independent confirmation.

6. Acknowledgements

The authors wish to thank Dr. Jason Woodward from Texas AgriLife Extension and Dr. Robert Wright from Texas Tech and Texas AgriLife Research for their assistance with this protocol. The methods of identification used in this protocol have been used with success by staff at both AgriLife Research and Texas Tech for years.

7. Appendices (as appropriate)

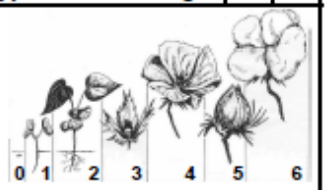
7.1 Sample form for detection of bacterial blight during early season cotton disease surveys.

Code	Date	Deg	Min
		South	
Farm	Field	East	
		Surveyed by:	

	G/S	No True Leaves	Std	BRR (0-10)									
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													

CLCuVirus	Pr	Ab	Texas Root Rot	Pr	Ab
Blue Disease	Pr	Ab	Defoliating Vert Wilt	Pr	Ab
Exotic Fusarium Wilt	Pr	Ab	Hyperviralent Blight	Pr	Ab

Comments:



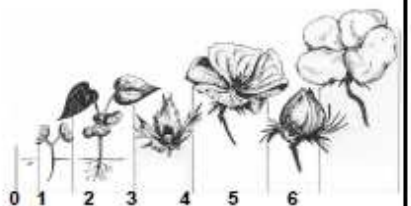
7.2 Sample form for detection of bacterial blight during late season cotton disease surveys

Code	Date		Degrees	Minutes
		South		
FarmField		East		
	G/S		Surveyed by:	

	Fov	Vd	Am%	PBR	OBR	SW	Hor	LM	Bchy	CBT
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
Total										

CLCuVirus	Pr	Ab	Texas Root Rot	Pr	Ab
Blue Disease	Pr	Ab	Defoliating Vert	Pr	Ab
Exotic Fov	Pr	Ab	Hyperviralent BBt	Pr	Ab

Comments:



c) required changes to the Intellectual Property register.

There are no changes required to the intellectual Property register.

Conclusion

8. 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?

Outcome 1: Disease Surveys

- The surveys provide a benchmark on incidence and distribution of disease in NSW each year and the indicate adoption of resistant varieties by farming systems. Information gathered from these surveys is added to the existing survey database and examined for changing trends of each disease. This coupled with data from experiments and observational studies has provided valuable strategies for integrated disease management aimed at decreasing the spread and severity of diseases.

- Direct management strategies that have resulted include good bed preparation, planting date in relation to soil temperature, pre-irrigation and variety selection. The incidence and severity of diseases have been shown to be largely impacted by the conditions in which the cotton seedlings are grown.

Outcome 2: Increased industry biosecurity preparedness

- ability to deal with biosecurity threats of exotic diseases through early detection from surveillance.
- The development of National Diagnostic Protocols for Texas root rot and Hypervirulent bacterial blight pathogens. These protocols will be implemented should an incursion occur within Australia. The biosecurity profile of Australian Cotton Research Institute (ACRI) and NSW DPI has been enhanced with the cotton industry and with the Australian Department Agriculture, Fisheries and Forestry (DAFF). This is of benefit for industry biosecurity preparedness.
- Australia's capacity to screen for Hypervirulent bacterial blight has been re-established with the importation of bacterial blight differentials in 2012. The differential set of cotton lines imported in 2012 for the screening of exotic races of bacterial blight included: Acala 44, Stoneville 20, Mebane B-1, Gregg, Empire B4, Stoneville 2B-S9, 1-10B, 20-3, 101-102B and DPxP4. This is of benefit for industry biosecurity preparedness.

- NSW ability to diagnose and differentiate between Fusarium species increased through the training of Technical Officer Peter Lonergan at the International Fusarium Laboratory Workshop in USA.

Outcome 3: Seed treatment efficacy trials

- Novel and existing fungal seed treatments were evaluated each year for effectiveness on reducing seedling mortality due to pathogens such as *Rhizoctonia* and *Pythium* spp.
- Performance of industry standard monitored each season for efficacy.

Outcome 4: PathWAY

- Industry disease issues are handled more efficiently
- Quantitative data collected each year on disease issues

Extension Opportunities

9. Detail a plan for the activities or other steps that may be taken:
   (a) to further develop or to exploit the project technology. (Not applicable)
   (b) for the future presentation and dissemination of the project outcomes.

Results from Diseases of Cotton X will continue to be presented at:

- Field and farm days
- Forums
- Upcoming Association of Australian Cotton Scientists Seminar

   (c) for future research.

Diseases of Cotton XI will continue with disease surveys, pathogen isolate collection, evaluating efficacy of fungicides and seed treatments. PathWAY will continue with improvements made where possible. Diseases of Cotton XI research will focus specifically on black root rot and Verticillium wilt with the view of producing fact sheets, posters and publications.

8. 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).

Allen, S. J., Anderson, C. M. T., Lehane, J., Lonergan, P. A., Scheikowski, L. J., & Smith, L. J. (2010). Cotton Pathology Survey 2009-10 *Cotton Pest Management Guide 2010-11* (pp. 122-125).

Allen, S. J., Kirkby, K. A., Lehane, J., Lonergan, P. A., Cooper, B. M., & Smith, L. J. (2011). Cotton Pathology Survey 2010-11 *Cotton Pest Management Guide 2011-12* (pp. 126-130).

Allen, S., Smith, L., Scheikowski, L., & Kirkby, K. (2011). Fusarium Wilt Update. *Disease*, 2011, from http://www.cottoncrc.org.au/content/Industry/Publications/Disease_Microbiology/Fusari um_Wilt_of_Cotton.aspx

Hulugalle, NR, Weaver, TB, Finlay, LA, Lonergan, P (2012) Soil properties, black root-rot incidence, yield, and greenhouse gas emissions in irrigated cotton cropping systems sown in a Vertosol with subsoil sodicity. *Soil Research* **50**, 278-292.

Kirkby, KA (2012) Diseases of Cotton X, In 'CRC Science Forum - Putting the pieces together.' Narrabri. Cotton Catchment Communities CRC.

Kirkby, KA, Allen, S, Lonergan, P (2012) An Update on Verticillium Wilt in Australia. In 'Proceedings of the Beltwide Cotton Conferences 2010-2012. Orlando, USA'.

Kirkby, KA, Smith, LJ, Lonergan, PA, Cooper, BM (2012) Response to Verticillium Wilt Outbreaks in 2011/12 Season. In 'Combined Fuscom and Crop Consultants Australia Cropping Solutions Seminar. Goondiwindi'. pp. 49-52. Crop Consultants Australia.

Kirkby, KA, Lonergan, PA, Smith, LJ, Scheikowski, L, Cooper, BR, Lehane, J (2012) Innovative Disease Management. In '16th Australian Cotton Conference. Broadbeach'. pp. 47-48. Cotton Research & Development Corporation.

Maas, S, Allen, S, Weir, D (Eds) (2012) 'Cotton Symptoms Guide.' The Australian Cotton Industry Development & Delivery Team

Smith, L. J., Lehane, J., Kirkby, K. A., Lonergan, P. A., Cooper, B. R., & Allen, S. J. (2012). Cotton Pathology 2011-12 *Cotton Pest Management Guide 2012-13* (pp. 128-132).

Poster presented at 16th Australian Cotton Conference – August 2012
Improving Communication Through Industry Partnerships – PathWAY

Media release 3rd September 2012 - Innovative disease management – reducing biosecurity threats of exotic cotton diseases

Media release led to 2 radio interviews:

- ABC Riverina Radio interview Cotton Diseases
- 2UE Radio Interview

Newspaper Articles:
The Courier- Future Cotton Leaders Program
The Land – Texas root rot
The Land – PathWAY
AgToday – Biosecurity

Magazine Articles:
Spotlight – How Would You Respond (Texas root rot)
Spotlight – Improving Communication through Industry Partnership - PathWAY

Publication submitted to Crop And Pasture Science
Kirkby, K. A., Lonergan, P. A., & Allen, S. J. (Submitted). Three decades of cotton disease surveys in NSW, Australia

Publication submitted for 2013 Cotton Production Guide
Kirkby, K. A., Lonergan, P. A., Cooper, B.R., Roser, S.E., Smith, L.J., Scheikowski, L. J., Bauer, B., Lehane, J, & Allen, S. J. (Submitted). Cotton Pathology 2012-2013.

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

Primefact sheets submitted 2013

- Vascular wilt disease findings 2003-2009
- Progression of Fusarium wilt

Part 4 – Final Report Executive Summary

Provide a one page Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

"Diseases of Cotton X" aimed to increase sustainability of the Australian cotton industry through comprehensive disease surveillance. Continued collaborative research, communication and education will enable better understanding of pathogen biology and ultimately improve integrated disease management strategies. The three year project resulted in several outcomes with direct consequences for the cotton industry.

Important outcomes for the Diseases of Cotton X project include: incorporating surveillance for exotic diseases into the biannual disease surveys has increased the industries preparedness to deal with biosecurity threats of exotic diseases through early detection. Evaluating existing and novel fungicide treatments each season ensures growers have access to the latest information on seed treatments available. This information helps the industry to make decisions on seed treatments in order to decrease seedling mortality.

National diagnostic protocols have been developed for Texas root rot and Hypervirulent bacterial blight and are currently being reviewed by DAFF. These protocols will aid the industry in rapid diagnosis using standard procedures should an incursion occur.

Australia capacity to screen for exotic races of bacterial blight has been re-established through the importation of 10 differential cotton lines in 2012. The lines included: Acala 44, Stoneville 20, Mebane B-1, Gregg, Empire B4, Stoneville 2B-S9, 1-10B, 20-3, 101-102B and DPxP4. The importation of these differential cotton lines has increased Australia's preparedness to diagnose exotic strains of bacterial blight in cotton.

The "Symptoms of diseases and disorders of cotton in Australia" was updated in 2012, titled "Cotton Symptoms Guide – the guide to symptoms of diseases and disorders in Australian cotton". The pathology unit worked in collaboration with Steve Allen (CSD) and the extension staff from Cotton Delivery and Development Team, particularly Susan Maas. Updated images were supplied and many drafts of the book were reviewed. The industry benefits from having an excellent resource full of images, a symptoms key, looks like section and information on how to send a sample for confirmation.

The network known as PathWAY was developed by the principal researcher to enable disease enquiries to industry staff from growers and consultants to be captured and quantified as they occur. In the twelve months it has been running, PathWAY has documented over 50 enquiries. This collaborative network links cotton professionals from across agencies and borders and consists of pathologists, virologists, researchers, extension, consultants and funding body representatives. The network has facilitated a co-ordinated approach to enquiries resulting in faster responses to disease issues.

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