FINAL REPORT

TEMPLATE

CRDC ID: MLAB1901

Project Title: Ready to use soil test to manage black root rot risks

Confidential or for public release? For Public Release

Part 1 – Contact Details & Submission Checklist

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Submission checklist.

Please ensure all documentation has been completed and included with this final report:

☒ Final report template (this document)

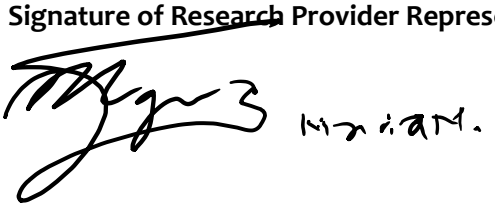
☐ Final Technical Report (see Part 3)

☐ Final Schedule 2: IP register

☐ Final financial report

☐ PDF of all journal articles (for CRDC's records)

Signature of Research Provider Representative:



Date submitted: 03/11/2020

Part 2 - Monitoring & Evaluation

This data forms part of CRDC's M&E data collection. Please complete all fields and add additional rows into each table if required.

Achievement against milestones in the Full Research Proposal

Milestone	Achieved/ Partially Achieved/ Not Achieved	Explanation
1.1 Can the predictive test quantify levels of T. basicola in artificially infected soils?	*Achieved*	*Soils from different farms were "diluted" with sterile sand to artificially increase/decrease the amount of T. basicola infective propagules. T. basicola was then quantified after cotton was planted in these soils. The ready to use test was capable of detecting intermediate, very high or no levels of BRR and these levels were significantly correlated with shoot dry weights under controlled conditions. A linear regression showed that even at 30 BRR counts, the disease was already reducing dry weights.*
1.2 Is there a variation in the quantification of T. basicola due to soil characteristics/crop history?	*Achieved*	*The ready to use test can detect, with reasonable accuracy, BRR from soils with different chemical and physical properties and or crop histories. In this project we used soils from different cotton production areas and these soils varied in the levels of BRR detected, from below detectable level to very high levels (in the thousands/g of soil)*
2.1 Is there a relationship between artificially infected soils with T. basicola and "naturally" (field soils) infected soils?	Partially Achieved	*It was not possible to correlate (not significantly) root dry weights of cotton seedlings growing in soils diluted at different rates or "artificially" infective soils with BRR levels after 6 or 10 weeks under controlled conditions. Data showed that BRR was not statistically different when the soil dilution rates were analysed, except for low (20%) against high levels (50% dilution). The intermediate levels were not correlated at all with seedlings dry weighs (plant biomass).*
3.1 Is there such a thing as BRR disease complex?	Partially Achieved	*In addition to T. basicola other fungi were observed to be involved in the health of cotton seedlings. However, the ready to use test reported data did not correlate significantly with dry weights of cotton seedlings in the soils showing no BRR (below detectable level).*
3.2 What other fungi are predominantly involved in causing BRR?	Achieved	*Predominantly, the ready to use test was able to detect other pathogenic fungi such as Fusarium and Verticillium*. However, the detection *of Verticillium took twice as long as the T. basicola incubation, which in practical terms is too long if management practices need to be implemented.*

Outputs produced *(Please refer to examples document to assist in completing this section).*

Output	Description
1.1 Quantification of the levels of T. basicola from soil VS amount of inoculum added	*Field, infected soils were "diluted" with sterile sand to create "artificial" levels of BRR. Soils were diluted 50, 40, 30 and 20%. These soils were then planted with cotton seedlings for 6 and 10 weeks under controlled conditions (growth room). The soils were then collected for BRR analysis using the ready to use test. Biomass of the cotton seedlings were also determined to find statistical correlations with BRR levels.*
1.2 Quantification of T. basicola will vary according to soil type, history of the disease, etc. and the predictive test is capable to detect the differences	*Field soils from farms/sites with different physical and chemical characteristics and crop histories were analysed using the ready to use test. Soils from Hillston, Carrathool, St George, Wee Waa, Hay, Griffith, Narrabri, Gwydir and Moree were used in some of the experimental setup. Approximately, 50 soils samples were analysed throughout the duration of the project. Different approaches were used to maximise experimental results, these included using trap plants such as peas, beans and pansy with the selected soils in vitro or in pots. The best approach was to use diluted filed soils, as above, to grow cotton seedlings. These seedlings were infected by BRR at different degrees or levels depending on the dilution. Cotton grew for 6 and 10 weeks and results varied depending to the farm/site but also depending on the dilution (low against high infected soil). Significant correlations were found.*
2.1 Quantification of T. basicola from field soils or "naturally" infected soils using previous quantification from artificially infected soils as threshold (spores/g soil)	*After the experiments described here, the ready to use test was calibrated to be able to correlate better the decrease in biomass of cotton seedlings with the levels of T. basicola. We infer that the statistical correlation will keep improving as more data is input into the mathematical model thus reducing the risks associated with the disease.*
3.1 The correlation between T. basicola and disease severity is not there thus why BRR levels symptoms are high? Could BRR disease severity be misinterpreted due to more than just T. basicola causing the disease?	*Concurrent with the quantification of T. basicola by the ready to use test, other soil fungi were detected during the experiments. The interaction with other pathogenic fungi could not explain reduction of the seedling's biomass when BRR levels were below detectable levels. These other fungi were recorded in units/g of soil.*

Outcomes from project outputs *(Refer to examples document).*

Outcome	Description
1.1 and *1.2 Successful quantification of T. basicola by the ready to use test in soils with different chemical and physical properties and managements practices.*	*The ready to use test was able to detect with reasonable accuracy different levels of T. basicola in field soils diluted with sterile sand. The diluted soils mimic different levels of BRR for the stablished experiments. There were always consistent, repeatable detection levels by the test for very low or very high T. basicola levels. In addition, statistical*

	correlation showed that as low as 30 colonies of T. basicola could reduce dry weights of cotton seedlings.
2.1 Testing the robustness of the method to detect several levels of T. basicola in different soils/sites	*The ready to use test was calibrated to better detect the levels of T. basicola in different soils. The percentage of reduction of biomass was used as an indicator for the low and high levels of the disease.*
3.1 Finding a Correlation between BRR and other soil borne fungi	*Apart from T. basicola, Fusarium and Verticillium were the main pathogenic fungi detected by the ready to use test. Whenever there was a reduction in the biomass of cotton seedlings, more than just T. basicola was found in intermediate to high levels in the soils analysed.*

Part 3 – Technical Report

Projects may require different approaches to the structure of the Technical report. A detailed technical report should normally include the following items:

I. Table of contents

Contents

II. Executive summary

Approximately 50 soils samples from farms/sites with different physical and chemical characteristics and crop histories were analysed using the ready to use test to elucidate if the soil test could be used to reduce the risks associated with BRR. Soils from Hillston, Carrathool, St George, Wee Waa, Hay, Griffith, Narrabri, Gwydir and Moree were used in some of the experiments completed throughout the duration of this project. Different approaches were used to maximise experimental results, these included using trap plants such as peas, beans and pansy with the selected soils in vitro or in pots. The best approach was to use diluted filed soils, with sterile sand, to create "artificial" levels of BRR. Soils were diluted 50, 40, 30 and 20% where cotton seedlings were grown for 6 and 10 weeks under controlled conditions (growth room).

Results showed that T. basicola levels varied depending on the site/farm the soil was sampled from, but BRR also varied depending on the percentage the soil was diluted. Results showed that 8 samples had below detectable level or zero colonies, 6 sites had less than 10 BRR/g soil, 8 had 10-30 BRR/g soil, 11 samples had 30-100 colonies and 9 samples had more than 100 colonies (up to 2000)/g soil after the soils were planted with cotton for 6-10 weeks under controlled conditions.
The correlation between shoot dry weights of the cotton seedlings with T. basicola levels was not significant in Part a of the experiment after 6 weeks under controlled conditions. However, the correlation was highly significant when the seedlings were left for 10 weeks instead of 6 (P= 0.0003). This correlation showed that as low as 30 BRR colonies/g soil negatively impacted dry weights, with a more pronounced effect after 100 BRR colonies/g soil.

The severity of the disease measured as browning of the tap root in Part a of the experiment, showed that severity was always higher when the soil dilution contained more infected soil at 50% when compared to 20% dilution (Table 4). However, severity measured as browning of the tap roots did not correlate with the levels of BRR (P=0.1545, R squared= 0.0324). On Part b of the experiment, as roots were more lignified after 10 weeks, browning of the root was not a reliable way to measure the effect of BRR. Thus, root dry weights were measured and data analysis showed a decrease in root dry weights with the increase of infected soil. However, the differences were not always significant and varied from site to site, but root dry weights were always higher at 20%. The correlation of the two variables was not significant (P= 0.3174; R squared= 0.01333).

Other pathogenic fungi were also detected by the ready to use test, these fungi mainly included Fusarium and Verticillium. However, the effect of these other fungi on cotton biomass was not positively correlated in any of the experiments, indicating that other factors may be involved in the biomass reduction.

To conclude, the ready to use test provided a repeatable, quantitative detection, with reasonable levels of accuracy, of T. basicola at very low, intermediate and very high levels including other pathogenic fungi such as Fusarium and Verticillium.

III. Introduction and background

Based on data presented at FUSCOM 2020, black root rot (BRR) was detected in 47% of the fields surveyed in the Darling downs. However, the disease severity was low, with an average of 5% (black necrosis in tap roots). In the Border Rivers area, all the fields surveyed had BRR, but again only one of the fields was found to be severely infected by the pathogen. Beyond this region, in areas where BRR has not been a problem in the past due to climate conditions, the disease has become more prevalent, with damages fluctuating greatly. If BRR in the soil could be quantified and correlated with plant biomass and severity, then a pre plant or ready to use test to manage the risks associated with the disease could be implemented to improve management decisions by growers and consultants.

From a small CRDC project conducted in 2016, it was concluded that a soil test developed in that project was capable of detecting two different levels (with or without) of T. basicola in soils from a single farm under different crop rotations (management practices). However, only two soils were tested and more were needed to confirm the usefulness of the test and to stablish safe thresholds. The soils used in this project came from sites/farms with different physical and chemical properties and different histories of incidence/severity of the disease to better predict the disease in cotton.

This project aimed to

1. *Quantify the levels of T. basicola in field soils and "artificially" infected soils by the ready to use test so they can be correlated with decrease in plant biomass and disease severity.*
2. *test the robustness of the ready to use test to detect several levels of T. basicola in soils from farms/sites with different chemical and physical properties.*
3. *Determine if other fungi than T. basicola are present when levels of BRR are low but plant disease is severe.*

IV. General materials and methods

After receiving ±50 soil samples with different BRR histories, collected by the National disease Survey team from different cotton producing regions, samples were dried at room temperature for a week, were then grinded and sieved through a 1 mm mesh and separated in 4 equal parts to account for variability during sampling. The soils were stored at room temperature for the duration of this project. See **Error! Reference source not found.** *for the complete list of samples. Before setting up any experiment, the soils were plated in modified carrot medium and incubated for 2-3 weeks at room temperature to quantify the initial levels of T. basicola (and other fungi). Single reports as per the one in Appendix 1 were sent to each of the farms sampled.*

Table 1 Soil samples included in the "ready to use test" project to manage BRR 2018-2020

MLABS Lab ID	*Number of samples*	*Sent by*	*Region- sent from*
1302	*1*	*Kieran Okeeffe- CottonInfo*	*Whitton*
1469	*1*	*Kieran Okeeffe- CottonInfo*	*Whitton*
1968	*10*	*Charles Morgan- CFM*	*Hay & Hillston*
2024	*2*	*Kieran Okeeffe- CottonInfo*	*Griffith*
2070	*9*	*Amanda Thomas- CottonInfo*	*Macquarie, Lachlan*
2083	*13*	*Kieran Okeeffe- CottonInfo*	*Hillston, Canargo, Coleambally, Griffith*
2084	*6*	*Elsie Hudson- CottonInfo*	*Narrabri, Glencoe, Drayton*
2099	*5*	*Annabel Twine- CottonInfo*	*Darling Downs- Dalby*
2211	*6*	*Charles Morgan/R. Malone- CFM*	*Hay & Hillston*
2102	*13*	*Janelle Montgomery- CottonInfo*	*Moree*

Due to the nature of the project, the experimental setup was divided in 3 parts (not in order of completion):

1. *Part 1 Inoculum density and disease severity correlation using soils from different farms to trap T. basicola in 3 trap seedlings: beans, soybean and pansy*
2. *Part 2a: Recovery of BRR from artificially infected soil and the effect of the pathogen on cotton seedlings at 6 weeks*
3. *Part 2b Recovery of BRR from artificially infected soil and the effect of the pathogen on cotton seedlings at 10 weeks*

4.1. Part 1 Inoculum density and disease severity correlation using soils from different farms to trap T. basicola in 3 trap seedlings: beans, soybean and pansy

The aims of this experiment were:

1. *phenotyping the disease levels in trap plants/seedling using field soils against the levels quantified by the ready to use test so then a correlation between severity and the in vitro method could be stablished and;*
2. *finding plant species, other than cotton, to trap T. basicola for easier and quicker quantification to be used in further experiments during the life of the project.*

4.1.1 Materials and Methods, Part 1

1. *For each soil sample, a 50g subsample was incubated for 4 weeks at 18 Celsius to "activate" T. basicola so then when a seed was placed in contact with the soil it would infect the seedling quickly. Experimental set up is shown in* Figure 1 *and* Figure 2.
2. *There were 3 replicates for each sample. Sterile 50mL conical centrifuge tubes were sown with either pansy, peas or soybean as trap plants for T. basicola. Seedlings were left in a growth room at 23 Celsius for 3-4 weeks so then symptoms could be observed. Figure 2 shows an overview of the seedling in the growth room.*
3. *Seedlings were harvested by cutting off the shoots to determine dry weighs. As the soils were used as they came from the farms, it was impossible to separate complete root systems to determine the damage. Most soils were heavy and the roots were stuck in the soil particles. However, the soils were used to quantify T. basicola/g soil by the ready to use test to try to correlate the variables.*
4. *Sterile Petri dishes were set up with 10g of soils, incubated for 4 weeks at 21 Celsius and sown with green bean or pea seeds as an alternative method to the one described above. Petri dishes were easier to handle and less labour intensive and the results were quicker to obtain. However, after just 7 days of incubation, the results showed that neither green beans or peas were good for trapping T. basicola as more than 80% of the seedlings died after been in contact with the farm soils. See* Figure 3.

Figure 1 After soils were incubated for 4 weeks to activate T. basicola, either green beans, pansy or peas were sown into the soil to trap T. basicola. Seedlings were kept for 3-4 weeks until the symptoms were observed.

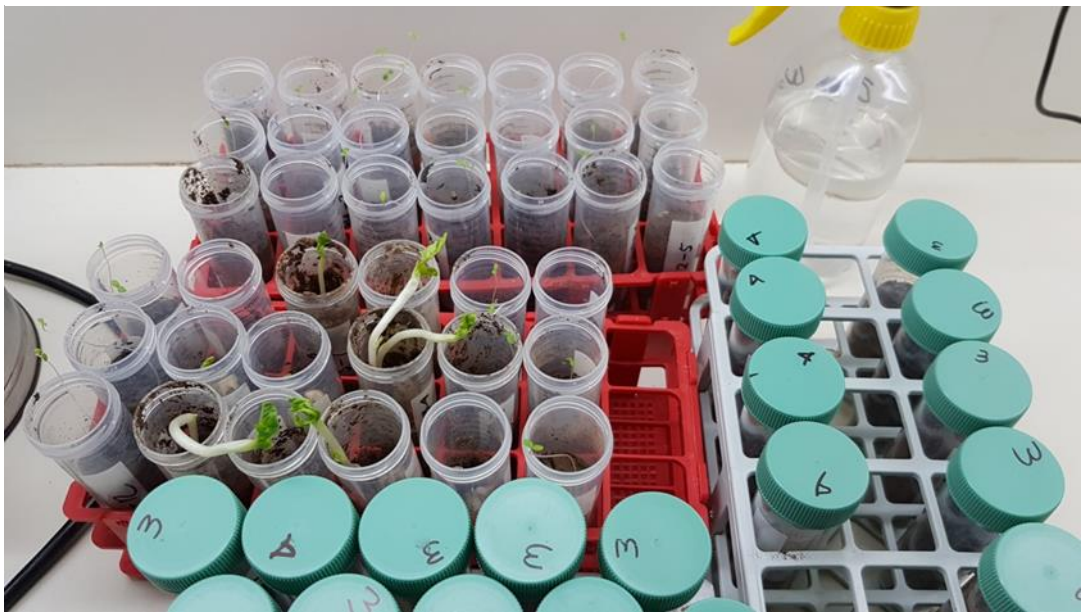
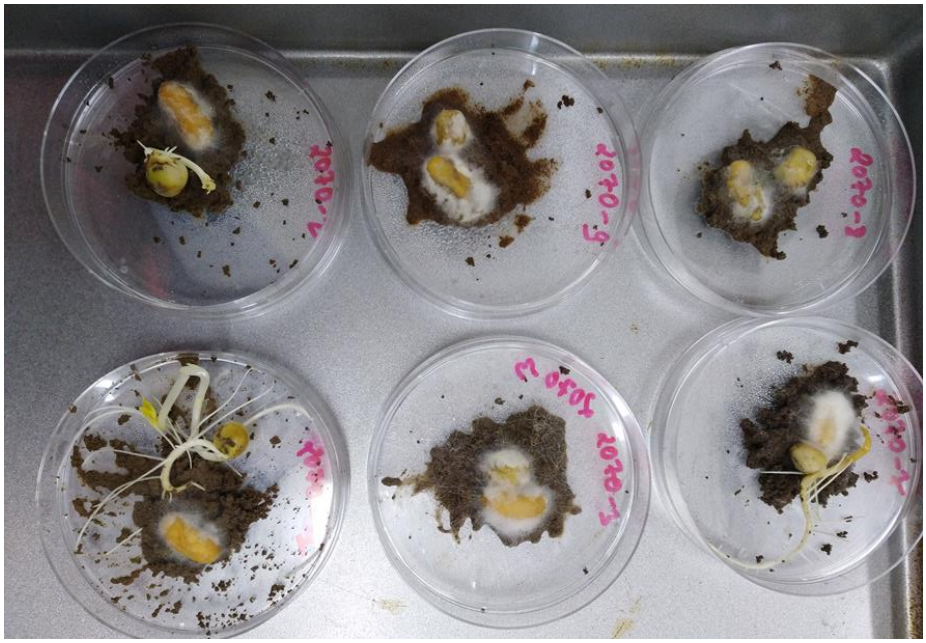
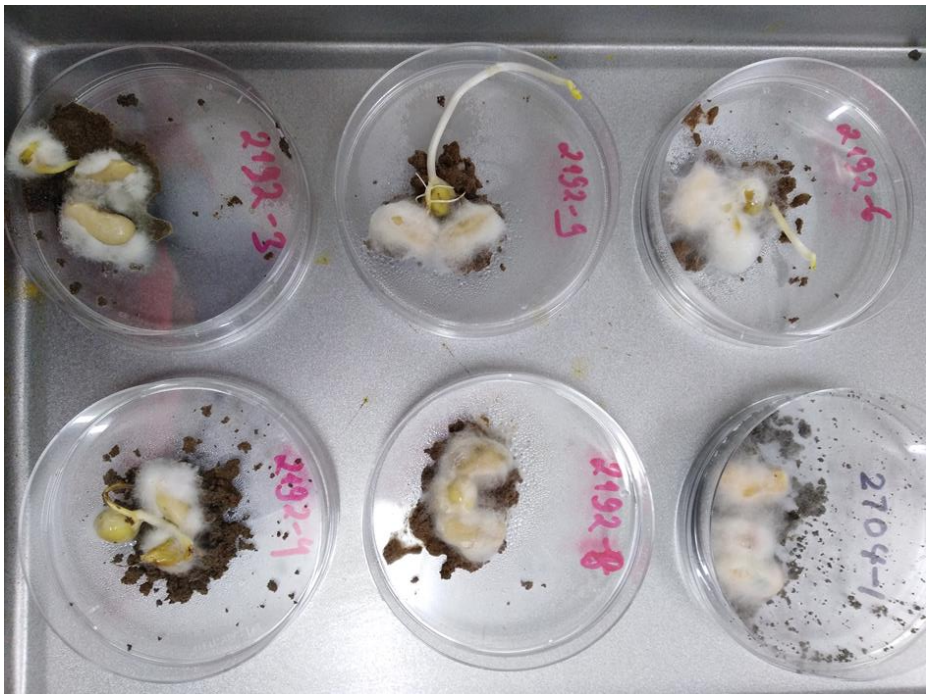


Figure 2 An overview of the green bean, peas or pansy seedlings in the growth room in soils from farms with different levels of black root rot. Seedlings were harvested 3-4 weeks after sowing. Seedlings were used to trap T. basicola. 3 replicates per sample.

Figure 3 Soils from farms with different levels of black root rot were incubated for 4 weeks at 21 Celsius in Petri dishes to "activate" T. basicola so then green beans or pea seedlings could be used as trap plants. All seeds were attacked by soil borne fungi after 7 days of Incubation and were not able to grow.





V. Results

5.1. Results Part 1: Inoculum density and disease severity correlation using soils from different farms to trap T. basicola in peas, green beans and pansies

The results showed that none of the trap plants was simulating the severity of the disease observed in the field after 3-4 weeks in the growth room. The aim of the experiments using the trap plants was to minimise the time to achieve results related to severity and to make it easier to quantify the damage in the roots (less suberized roots) compared to using cotton seedlings. There was no correlation between the grow of either peas or pansies with severity of BRR (observed) when using this experimental setup (data not shown).

To elaborate, data showed that in a few cases only the levels of BRR quantified in the soil (very low, low, moderate or high) matched the symptoms in the trap plant (Table 2). For example, when pea was growth in a soil with high counts of BRR (W3-cotton/rice rotation, 180 colonies/g soil), the plant developed poorly after 4 weeks in the growth room, which was according to the predicted response. On the other hand, when the levels of BRR were around 400 colonies per gram of soil (WGN-F5, WGS-F5), peas and or pansies showed "excellent" or "moderate" plant growth when the expected result was very poor growth. Due to the high variation in the results when using the trap plants, it was concluded that using cotton seedlings was the best way to move forward and arrangements were made to set up the new experiments using cotton seeds.

In addition to the variability in the response to the disease in peas and pansies, an additional result from the trap plants experiment showed that when results indicated "moderate" levels with the number of colonies varying from 20-60/g of soil, the response seen in the trap plant was mainly a decrease in growth. The response expected was in the "excellent growth" range but that was not observed, which meant that either the test levels in the ready to use test should be adjusted to classify the response as high, or that the levels of infective fungi in the soil is not being detected accordingly. To try to adjust the sensitivity of the current test, a new experiment was set up to measure T. basicola infective propagules using a modification of the Most Probable Number (MPN) method. The concept uses soil dilutions to achieve quantification of propagules in low levels with a plant as indicator, in this case a cotton plant. The experiment set up and design are shown in Figure 4 *and* Figure 5. *According to Table 2 the samples used for the experiment were mainly classified as high and moderate BRR. The aim was to achieve a better classification for the intermediate level of response in the field against the current test.*

Figure 4 Experimental overview using a modification of the Most Probable Number (MPN) method to calibrate better the levels of BRR detected by the ready to test with the response of the plant in the field. 4 replicates per soil dilutions. Details of treatments are in Table 3 below. Soils were diluted by 50, 40, 30 and 20% with sterile sand.

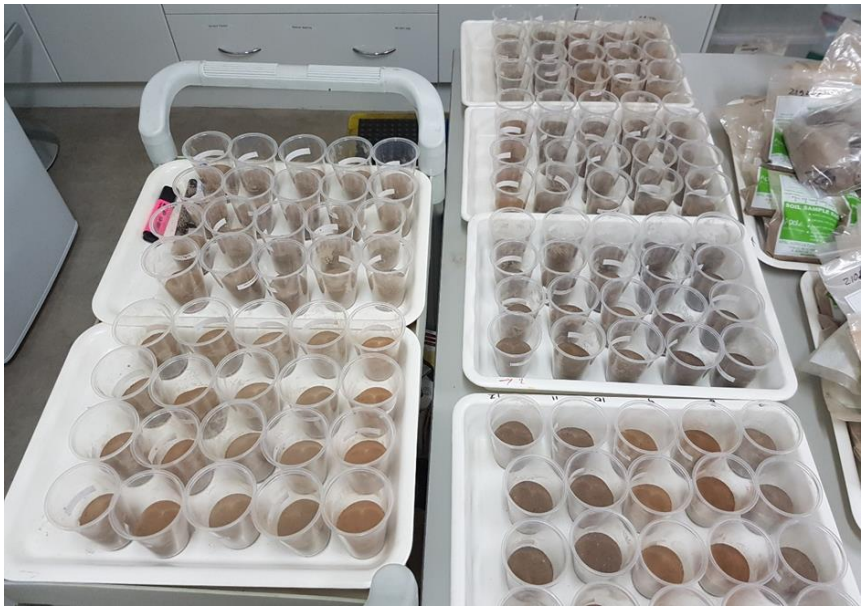


Table 2 Response of peas and pansies as trap plants for BRR (T. basicola) under controlled conditions. Trap plants were growth in a growth room for 3-4 weeks in field soils with different levels of the disease. When plants were classified as (+) or (-) they showed a decrease in growth when compared to plants classified as showing excellent growth. NA= not used in this experiment as not enough soil was available.

			Mean		Mean	Mean	Other Fungi	
Sample Number	**Lab ID**	**Sample Name/description**	**Counts-BRR in media**	**Level expressed in Reports**	**Response of PEA in growth room**	**Response of PANSY in growth room**	**Fus**	**Vert**
1	1302-1	W3-rice before cotton	180	High	Dead plant	Excellent growth	NA	NQ
2	1469-1	W 1st May-Rice after cotton	65	Moderate	(+) or (-)	Excellent growth	NQ	NQ
3	1720-1	BRR levels	460	High	2-Excellent growth, 2-(+) or (-)	Excellent growth	140	NQ
4	1801-1	Lrd C3-C5 BRR	302	High	2-(+) or (-)	Excellent growth	NQ	NQ
5	1801-2	WGS F5	483	High	2-(+) or (-)	(+) or (-)	NQ	NQ
6	1801-3	WGN F5	395	High	Excellent growth	(+) or (-)	NQ	NQ
7	1823-1	Mtm-F6 (a) top	3	Very low	Excellent growth	Excellent growth	170	NQ
8	1823-2	Mtm-F6 (b) bottom	2	Very low	(+) or (-)	Excellent growth	155	NQ
9	1823-3	Mtm-F7 (a) top	20	Low	Excellent growth	Excellent growth	222	NQ
10	1823-4	Mtm-F7 (b) bottom	3	Very low	(+) or (-)	Excellent growth	107	NQ
11	1823-5	Mtm-F8 (a) top	3	Very low	Dead plant	(+) or (-)	138	NQ
12	1823-6	Mtm-F8 (b) bottom	32.5	Low	Excellent growth	Excellent growth	138	NQ
13	1968-1	B1	35	Moderate	(+) or (-)	Excellent growth	260	NQ
14	1968-2	B4	225	High	NQ- not enough soil	NA- not enough soil	177	NQ
15	1968-3	B7	85	High	NA- not enough soil	NA- not enough soil	222	NQ
16	1968-4	C4	40	Moderate	NA- not enough soil	NA- not enough soil	227	NQ
17	1968-5	D4	12.5	Very low	NA- not enough soil	NA- not enough soil	240	NQ
18	1968-6	B5	0	BDL	NA- not enough soil	NA- not enough soil	177	NQ
19	1968-7	B20	0	BDL	NA- not enough soil	NA- not enough soil	68	NQ
20	1968-8	B28	0	BDL	NA- not enough soil	NA- not enough soil	62	NQ
21	1968-9	G11	0	BDL	NA- not enough soil	NA- not enough soil	100	NQ
22	1968-10	H22	222	High	NA- not enough soil	NA- not enough soil	92	NQ

23	2024-1	W3	100	High	Excellent growth	Excellent growth	230	NQ
24	2024-2	F1	1	Very low	Dead plant	Excellent growth	312	NQ
25	2070-1	M1-Field 6-Mtm	35	Low	Excellent growth	Excellent growth	198	NQ
26	2070-2	M2-Field 15	40	Moderate	Dead plant	(+) or (-)	205	NQ
27	2070-3	M3 Field 5-W	40	Moderate	(+) or (-)	Dead plant	228	NQ
28	2070-4	M4-Field 41-MF	10	Low	Excellent growth	Excellent growth	425	NQ
29	2070-5	M5-Field 21c WB	30	Low	(+) or (-)	Dead plant	193	NQ
30	2070-6	L1 Field Y1	5	Very low	Excellent growth	(+) or (-)	335	NQ
31	2070-7	L2 Field WW	0	BDL	(+) or (-)	Dead plant	375	NQ
32	2070-8	L3-Field-WW	0	BDL	Excellent growth	Excellent growth	26	NQ
33	2070-9	M6-Field 2-Mtm	40	Moderate	(+) or (-)	(+) or (-)	237	NQ
34	2074-1	Paddock W8-RW	3	Very low	Dead plant	(+) or (-)	230	NQ
35	2075-1	ChA Paddock 1 Cut Area	10	Very low	Excellent growth	(+) or (-)	342	125
36	2075-2	ChA Paddock 1 Fill Area	1	Very low	Excellent growth	(+) or (-)	268	168
37	2075-3	ChA Paddock 6 Fill Area	1	Very low	(+) or (-)	(+) or (-)	228	38
38	2083-1	Sth-K Hill P10	23	Low	Excellent growth	Excellent growth	295	88
39	2083-2	Sth-K Can	0	BDL	Excellent growth	Excellent growth	205	0
40	2083-3	Sth-K Col Field 15	0	BDL	(+) or (-)	Excellent growth	145	0
41	2083-4	Sth-K H F75	0	BDL	Excellent growth	Excellent growth	193	0
42	2083-5	Sth-K H Co	40	Moderate	Excellent growth	Excellent growth	187	0
43	2083-6	Sth-K Hill R11	160	High	Excellent growth	Dead plant	213	50
44	2083-7	Sth-K Grth CRd	0	BDL	(+) or (-)	Excellent growth	105	0
45	2083-8	Sth-K Hill P9	175	High	Dead plant	Excellent growth	205	20
46	2083-10	Sth-K Hill NP	195	High	Excellent growth	Dead plant	203	0
47	2083-11	Sth-K Wtt W3 Top bay	0	BDL	Excellent growth	Excellent growth	245	0
48	2083-12	Sth-K Wtt IREC Middle 2	0	BDL	Excellent growth	Dead plant	300	0
49	2083-13	Sth-K Grth CRd CA	0	BDL	Excellent growth	Dead plant	175	285
50	2084-1	SM Unya Field 1	0	BDL	Excellent growth	Dead	143	NQ
51	2084-2	TS G Field 5	0	BDL	Excellent growth	Dead	248	NQ

52	2084-3	Au Nari Field 62	0	BDL	Excellent growth	Good	300	NQ
53	2084-4	Au Nari Field 46	8	Low	(+) or (-)	Good	463	NQ
54	2084-5	Au Nari Field 69	2.5	Very low	Excellent growth	(+) or (-)	177.5	NQ
55	2084-6	JH Dry Field 4	55	Moderate	Dead plant	(+) or (-)	230	NQ
56	2099-1	LG L1	5	Very low	Good	Dead	273	NQ
57	2099-2	Rlea Field 6	0	BDL	(+) or (-)	Good	258	NQ
58	2099-3	Olans F2	0	BDL	Good	(+) or (-)	440	NQ
59	2099-4	Fvw 6	0	BDL	Good	Good	470	NQ
60	2099-5	Fre M2	28	Low	(+) or (-)	Good	198	NQ
61	2102-1	Gir-Moe-Bnt 1	0	BDL	Good	Good	305	NQ
62	2102-2	Gir-Moe-Bnt 2	0	BDL	(+) or (-)	Good	208	NQ
63	2102-3	Gir-Moe-Crl 2	25	Low	Good	Good	165	NQ
64	2102-4	Gir-Moe-DPark 4	35	Moderate	(+) or (-)	Dead	360	NQ
65	2102-5	Gir-Moe-Frfd 16	10	Very low	(+) or (-)	(+) or (-)	193	NQ
66	2102-6	Gir-Moe-Frfd 15	0	BDL	Good	Good	180	NQ
67	2102-7	Gir-Moe-Mkn 5	0	BDL	Good	(+) or (-)	398	NQ
68	2102-8	Gir-Moe-Mkn 7	2.5	Very low	Good	Good	348	NQ
69	2102-9	Gir-Moe-RMll 18	250	High	Dead	Dead		NQ
70	2102-10	Gir-Moe-Sth-K Cmle 2	10	Low	(+) or (-)	Good	460	NQ
71	2102-11	Gir-Moe-Tllerg 34	5	Very low	Dead	Good	355	NQ
72	2102-12	Gir-Moe-Yba 61	0	BDL	(+) or (-)	(+) or (-)	397	NQ
73	2102-13	Gir-Moe-Yrow 2	28	Moderate	(+) or (-)	Dead	258	NQ
74	2192-1	NO BRR-1	NA-used for MPN Experiment		Dead	Good	NQ	NQ
75	2192-2	NO BRR-2	NA-used for MPN Experiment		Dead	Dead	NQ	NQ
76	2192-3	NO BRR-3	NA-used for MPN Experiment		Dead	(+) or (-)	NQ	NQ
77	2192-4	NO BRR-4	NA-used for MPN Experiment		Dead	Good	NQ	NQ
78	2192-5	NO BRR-5	NA-used for MPN Experiment		Good	(+) or (-)	NQ	NQ
79	2192-6	High BRR-1	NA-used for MPN Experiment		Good	(+) or (-)	NQ	NQ
80	2192-7	High BRR-2	NA-used for MPN Experiment		Good	Good	NQ	NQ

81	*2192-8*	*High BRR-3*	*NA-used for MPN Experiment*		*Good*	*Dead*	*NQ*	*NQ*
82	*2192-9*	*High BRR-4*	*NA-used for MPN Experiment*		*(+) or (-)*	*Dead*	*NQ*	*NQ*
83	*2192-10*	*High BRR-5*	*NA-used for MPN Experiment*		*(+) or (-)*	*Dead*	*NQ*	*NQ*
84	*2211-1*	*C4-Cotton removed-flooded-wheat*	*15*	*Low*	*NA- not enough soil*	*NA- not enough soil*	*55*	*NQ*
85	*2211-2*	*C4#1-Cotton removed-flooded-wheat*	*112.5*	*High*	*NA- not enough soil*	*NA- not enough soil*	*37.5*	*NQ*
86	*2211-3*	*B4B-Wheat-Cotton removed-biofumigant*	*17.5*	*Low*	*NA- not enough soil*	*NA- not enough soil*	*47.5*	*NQ*
87	*2211-4*	*C4A-Cotton removed-flooded-wheat*	*117.5*	*High*	*NA- not enough soil*	*NA- not enough soil*	*50*	*NQ*
88	*2211-5*	*C4B-Cotton removed-flooded-wheat*	*0*	*BDL*	*NA- not enough soil*	*NA- not enough soil*	*55*	*NQ*
89	*2211-6*	*C4#2-Cotton removed-flooded-wheat*	*117.5*	*High*	*NA- not enough soil*	*NA- not enough soil*	*50*	*NQ*
90	*2211-7*	*C3-wheat-cotton-wheat*	*247.5*	*High*	*NA- not enough soil*	*NA- not enough soil*	*52.5*	*NQ*
91	*2211-8*	*C2-cotton-faba-fallow-cotton*	*272.5*	*High*	*NA- not enough soil*	*NA- not enough soil*	*80*	*NQ*
92	*2211-9*	*C1-cotton-faba-fallow-cotton*	*0*	*BDL*	*NA- not enough soil*	*NA- not enough soil*	*155*	*NQ*
93	*2211-10*	*B4A-Wheat-Cotton removed-biofumigant*	*25*	*Low*	*NA- not enough soil*	*NA- not enough soil*	*15*	*NQ*
94	*2211-11*	*B4#2-Wheat-Cotton removed-biofumigant*	*40*	*Moderate*	*NA- not enough soil*	*NA- not enough soil*	*32.5*	*NQ*
95	*2211-12*	*B2-cotton-faba-fallow-cotton*	*72.5*	*High*	*NA- not enough soil*	*NA- not enough soil*	*85*	*NQ*
96	*2211-13*	*A1-cotton-faba-fallow-cotton*	*0*	*BDL*	*NA- not enough soil*	*NA- not enough soil*	*130*	*NQ*
97	*2211-14*	*D2-wheat-cotton-cotton*	*180*	*High*	*NA- not enough soil*	*NA- not enough soil*	*22.5*	*NQ*
98	*2211-15*	*B4#1-Wheat-Cotton removed-biofumigant*	*0*	*BDL*	*NA- not enough soil*	*NA- not enough soil*	*6.5*	*NQ*
99	*2211-16*	*B1-cotton-faba-fallow-cotton*	*15*	*Low*	*NA- not enough soil*	*NA- not enough soil*	*105*	*NQ*

5.2. *Part 2 (a and b): Recovery of BRR from artificially infected soil and the effect of the pathogen on cotton seedlings*

The aim of this experiment was to test the robustness of the ready to use test in diluted field soils (artificially infected) to improve the levels of detection of BRR in soils labelled as low, moderate or high. Due to soil shortages from different farms, the approach initially proposed was slightly changed to achieve the aims. The experimental setup was divided into two small experiments rather than a big one to be able to modify the setup if needed. However, the methods described below were applied to both experiments with a brief description when steps were modified.

5.2.1 *Materials and Methods, Part 2*

1. *Each soil sample was diluted with dried sterile sand up to 200g/pot (*Table 3*). The table shows one example of how the field soils were diluted to achieve different rates of BRR. Other samples included in Part a of the experiment were 1469-1 (Whitton), 1823-6 (Macquarie) and 2102-5 (Moree). For the second part of the experiment or Part b, the samples included were 1302-1 (Whitton), 1469-1 (Whitton), 2102-3 (Moree), 2102-13 (Moree) and 2211-16 (Hay). Each diluted soil was replicated 4 times (n=4). An overview of both experimental setups is shown in* Figure 4*a and Figure 4b.*
2. *After diluting the soils, they were weighed and potted. Pots were then watered to achieve 50% water pore space and were incubated at 16-18 Celsius for 4 weeks to activate T. basicola.*
3. *After 4 weeks, 3 pre-germinated cotton seeds were placed in each pot; after emergence seedlings were trimmed off to keep 2 plants per pot. Cotton seeds were provided by Cotton Seed Distributors (CSD), the variety used in both experiments was Sicot 620 (non-GMO variety). Briefly, cotton seeds were prepared for germination as follows: seeds were sterilised by sonication for 60 s, followed by 2 washes with 70% ethanol, one wash with 2% bleach and 5 washes with sterile zero water. Seeds were let to dry in paper towels to eliminate any excess of water and ethanol or bleach residues. Seeds were then placed in new humidified paper towels and were incubated at 25 Celsius for several days until germination.*
4. *For Part a of the experiment, pots with cotton seedlings were kept in the growth room for 6 weeks at 25 Celsius with 14h light/10h dark (*Figure 5*). For Part b of the experiment, seedlings were left for 10 weeks after germination as results from Part a of the experiments gave an indication of a longer period was needed (Figure 5).*
5. *Cotton seedlings were harvested by separating the shoots from the roots. Shoots were cut off and kept clean from soil and were dried at 85 Celsius for 48h to determine dry weighs. Roots were carefully separated from soil and washed with tap water trying to avoid losing fine roots. Pictures of the roots were taken to help with scoring for BRR damage (*Figure 6*).*
6. *The roots were scored for root damage (or root health) based on a 4-level scoring system: 1 for healthy, creamish coloured roots, to 4 for dark browned roots.*
7. *Soils were kept in the fridge at 4 Celsius for quantification of T. basicola by the ready to use test. The ready to use test used a two-step process. Briefly, harvested soils were dried, grinded and sieved to a particle size of 0. 5mm. 1g of each soil were weighed into sterile 50 mL plastic centrifuge tubes. After adding modified carrot medium up to the 50 mL mark, tubes were mixed quickly with the soil and 25 mL were dispensed into Petri dishes. Dishes were incubated for 2 weeks at room temperature and inspected under the microscope (light microscopy) to count T. basicola colonies (BRR). Petri dishes were incubated for a second term for a further 2 weeks to account for any other fungi developing in the dishes. These other fungi were also inspected under the microscope before quantification.*
8. *T. basicola and the most frequent fungi growing from the soils were sent to Microgenetix, Melbourne for DNA sequencing to allow for confirmation of the counted colonies.*

Table 3 Experimental setup using a modified Most Probable Number (MPN) method to change the levels of BRR in soils. 4 replicates per soil dilutions. Dilutions were achieved using sterile sand.

Sample ID-	% dilution	Soil: sand ratio
1302-1 (Whitton)	20	20: 80
	30	30:70
	40	40:60
	50	50:50

Figure 5 A modification of the Most Probable Number (MPN) method was used to change the levels of BRR in field soils for this experiment. On the left, cotton seedlings variety SICOT 620 growing under controlled conditions as part a of the experiment. On the right, Part b of the experiment with the aim to test more soils. 4 replicates per sample.

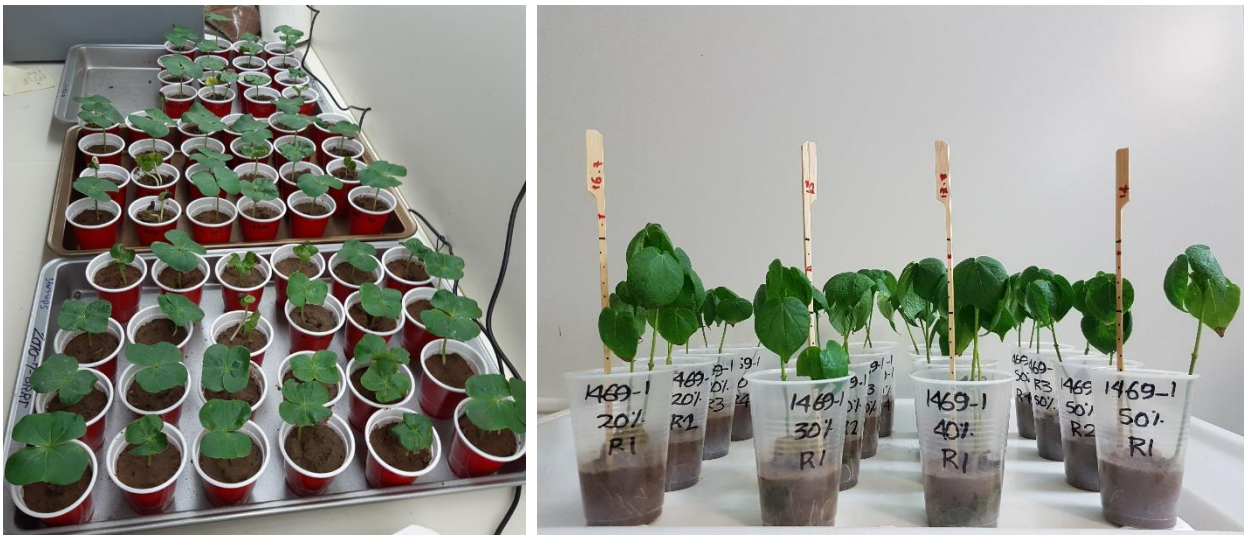


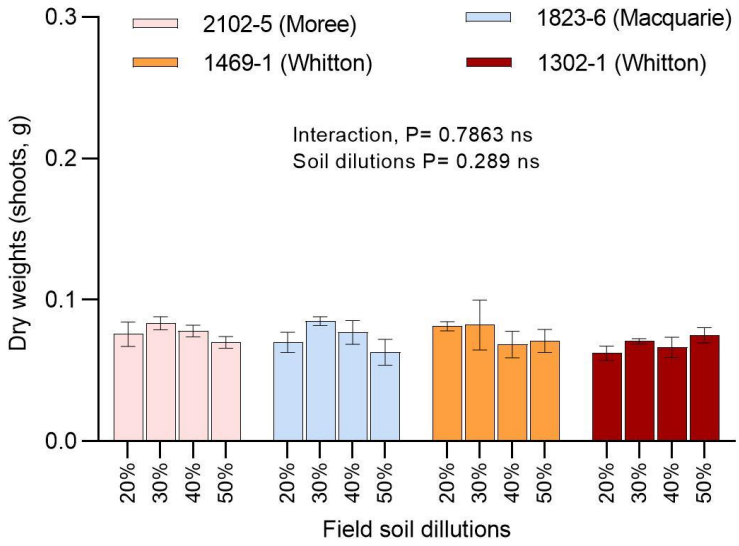
Figure 6 Roots were washed after harvest and were prepared for scoring the damage caused by BRR. Pictures were also taken to help with the scoring. On the left, roots were harvested and scored after 6 weeks of development (Part a of Experiment) and on the right roots were harvested and weighed after 12 weeks in the growth room (Part b).

5.2.2 *Results Part 2a: Effect of BRR from artificially infected soils on cotton seedlings*

5.2.2.1 Shoot dry weights, Part a

The results indicated that shoot dry weights were not significantly different when comparing the diluted soils from the field sites/farms (Figure 7). However, there was a slight trend were soils with higher levels of infected soil (40-50%) had lower shoot biomass, except for sample 1302-1 from Whitton where this trend was not observed and soils diluted 30, 40 and 50% had all higher shoot dry weights than dilution 20% with less infected soil. Even though the effect was not significant, the reduction of dry weights when a soil with more infected soil (50%) was compared to one with less infected soil (20%) varied from around 7.6% for soils from 2102-5 (Moree), 10% for soils from sample 1823-6 (Macquarie) and up to 12.5% in soils from sample 1469-1 (Whitton).

Figure 7 Shoot dry weights of cotton seedlings grown for 6 weeks in soils from different farms/sites, which were diluted at different rates (20%, 30%, etc) to achieve different levels of T. basicola (BRR). There were no significant differences between treatments (P= 7863, n=4).



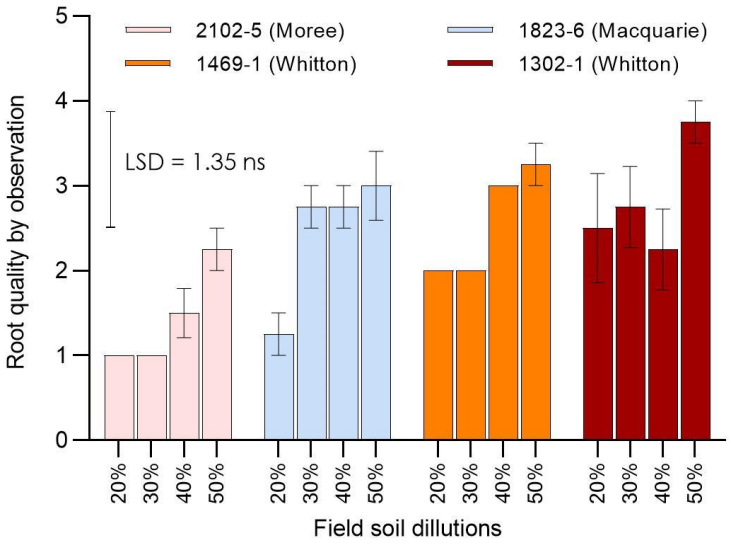
5.2.2.2. *Root quality (disease severity), Part a*

Root quality was assessed by ranking the colour of the root from 1 to 4 (from good to very poor). In general, the results showed that the more diluted the infected soil was (20%) the less brown coloured the roots of the cotton seedlings were (Figure 8). When analysing each field sample by one-way ANOVA (Table 4), results showed significant differences among the levels of dilution of the samples, with more damaged, browned roots whenever the infected soil in the dilution was higher. For example, sample 2102-5 from Moree, had significantly "better" root system at 20 and 30% compared to 50% dilution (P= 0.0439). In addition, sample 1823-6 from Macquarie had similar root health at 30, 40 and 50%, but at 20% dilution, the root was observed to be healthier (Figure 8 and Table 4). A similar root colour or ranking was observed in sample 1302-1 from Whitton, in which the root system was statistically the same at 20, 30 and 40% dilutions. However, at 50%, roots were ranked 4 (dark browned roots) more frequently.

Table 4 The effect of T. basicola (BRR) on root quality (disease severity) of cotton seedlings (visual ranking). Probability was obtained after one-way ANOVA. n=4

Sample	*Probability*	*Significance at 0.05*
2102-5 (Moree)	*0.0017*	*yes*
1823-6 (Macquarie)	*0.0050*	*yes*
1469-1 (Whitton)	*0.0001*	*yes*
1302-1 (Whitton)	*0.1929*	*no*

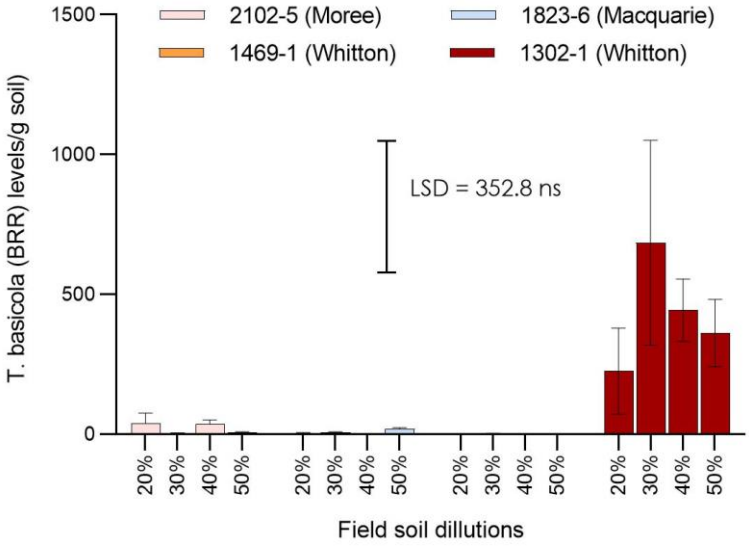
Figure 8 Root quality of 6 weeks old cotton seedlings grown in soils from different sites/farms, which were diluted to achieve different levels of T. basicola (BRR). There were significant differences between the soil dilutions (P= 0.001, n=4).



5.2.2.3. Levels of T. basicola (BRR) in the diluted soils, Part a

*The levels of BRR varied from below detectable levels for sample 1469-1 (Whitton) to more than 600 colonies/g soil for sample 1302-1 (Whitton) (*Figure 9*). Variation was also observed among the different soil dilutions in each field sample, which resulted in non-significant differences between any of the soils tested (P= 0.5557). Two samples from Whitton gave very contrasting results, sample 1302-1 was the only sample with very high levels of T. basicola. In contrast, sample 1469-1 had very low levels of BRR (less than 1 count per g of soil). The results indicated that the ready to use test was able to detect differences in the inoculum levels of BRR in soils with different properties.*

Figure 9 T. basicola (BRR) colonies in soils from different sites/farms after growing cotton for 12 weeks under controlled conditions. Field soils were diluted at different rates (20%, 30%, etc) to achieve different levels of BRR. n=4



5.2.2.4 Quantification of other pathogenic fungi in the diluted soil samples, Part a

Apart from T. basicola (BRR), other pathogenic fungi, mainly Fusarium and Verticillium, were recorded in the field samples. These fungi were mainly quantified in the 3 samples with low levels of BRR, Figure 9 and Figure 10. *The one-way ANOVA for each of the field samples with their corresponding dilutions showed no significant differences among the diluted soils. Except for sample 1823-6 from Macquarie where the field soil diluted at 30% showed the lowest levels of these other pathogenic fungi compared to for example, 50%. Sample 1302-1 from Whitton had the lowest levels of other pathogenic fungi but this sample recorded the highest levels of BRR, Figures 9 and 10. In addition, when data was analysed for any correlation between BRR and other pathogenic fungi, the results showed a statistically significant correlation (P=0.0021, n=64) indicating that whenever BRR was found in high levels, other pathogenic fungi were low* (Figure 11).

Figure 10 Other pathogenic fungi from soils sampled from different farms/sites, which were diluted to different percentages to achieve different levels of Inoculum. n=4 per sample. No statistical differences were found in any of the dilutions, except for 1823-1 (Whitton).

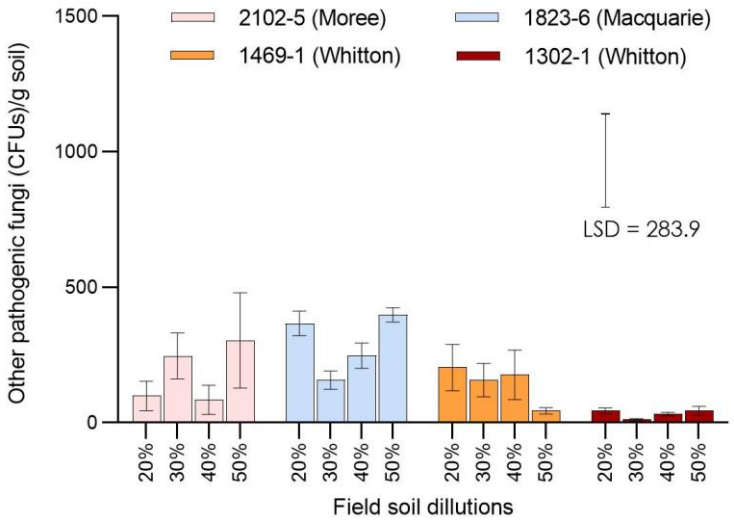
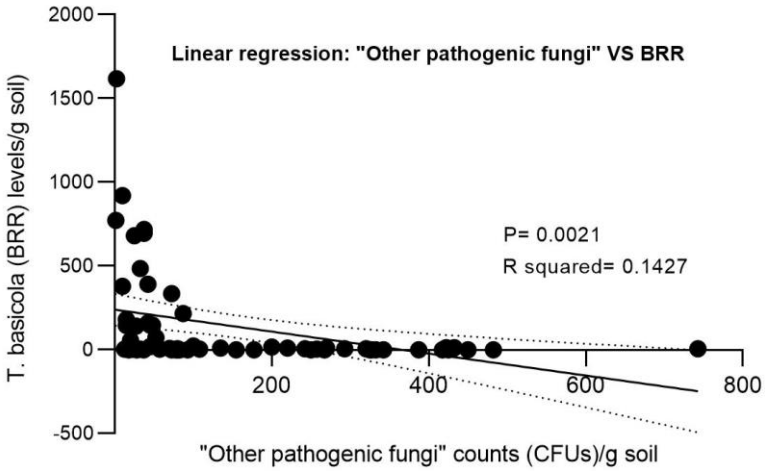


Figure 11 Linear regression showing the Interaction between T. basicola (BRR) and other pathogenic fungi levels. The correlation was statistically significant (P= 0.0021, n=64).

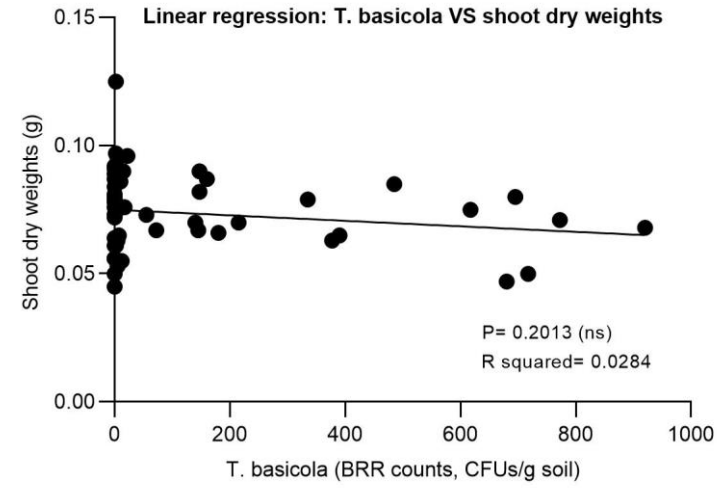


5.2.2.5 *Effect of T. basicola (BRR) levels and other pathogenic fungi on the reduction of cotton seedlings biomass, Part a*

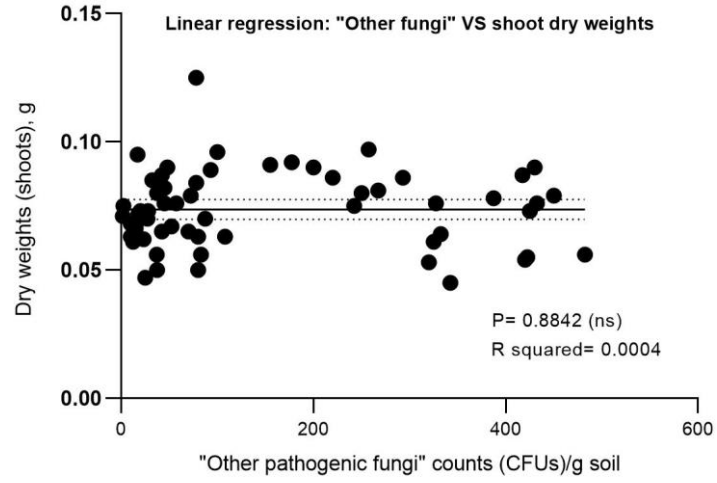
The reduction in biomass in cotton seedlings due to T. basicola was not significant when the data were statistically correlated (P=0.3364, n=64) (Figure 12*a*)*, indicating no effect of BRR on the biomass of cotton seedlings or on the quality of the root seedlings (P=0.1545, data not shown). In addition, there was no statistical effect of other pathogenic fungi on either dry weight of shoots (P=8842) or root quality (P=0.3041) (Figure 12b). The result could indicate: (1) that the seedlings were not affected at all by BRR and other pathogenic fungi at the time the experiment was harvested (even though the roots were brown coloured). (2) That cotton seedlings needed to be left longer in the diluted soils to achieve clearer results. Thus, with the aim to obtain better correlations, Part b of the experiment, which will be described in the following section, was left for longer under similar experimental conditions.*

Figure 12 Linear regression showing the effect of (A) T. basicola (BRR) and (B) other pathogenic fungi on shoot dry weights of cotton seedlings. The correlation was not statistically significant for any of the variables measured (P=0.213 and 0.8842).

A)



B)

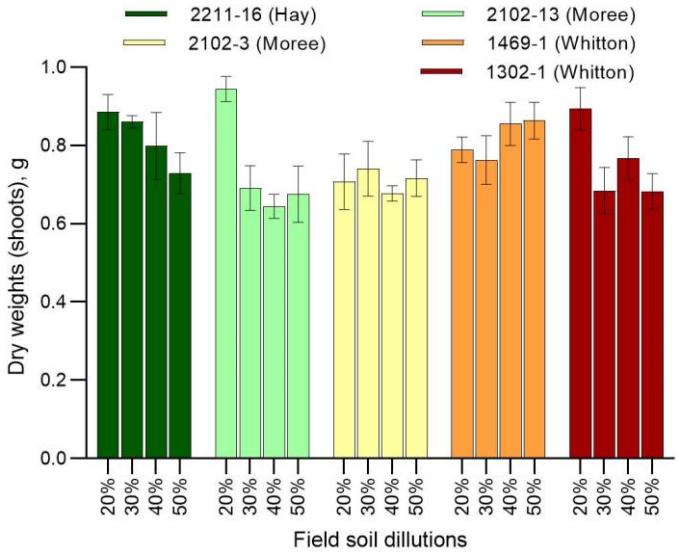


5.2.3. *Results Part 2b: Recovery of BRR from artificially infected soil and the effect on cotton seedlings*

5.2.3.1. *Shoot dry weights, Part b*

*As expected, the shoot dry weighs of cotton plants showed high variability when planted in the soils from different farms and also among the diluted samples (*Figure 13*). Some clear trends were observed as follows: (1) Statistically significant reduction in biomass in samples 2211-16 from Hay, 2102-13 from Moree and 1302-1 from Whitton when the infected soil was increased from 20 to 50%. The results confirmed what has been already published in terms of a decrease in biomass with the increase of BRR. (2) Samples 2102-3 from Moree and 1469-1 from Whitton did not show a reduction in shoot dry weights with the increase of infected soil. (3) The sample from Hay (2211-16) was the only sample that showed a constant decrease of dry weights with the increase of infected soil.*

Figure 13 Shoot dry weights of cotton seedlings grown for 12 weeks in soils from different farms/sites, which were diluted at different rates (20%, 30%, etc) to achieve different levels of T. basicola (BRR). Significant differences were observed for treatments (n=4).

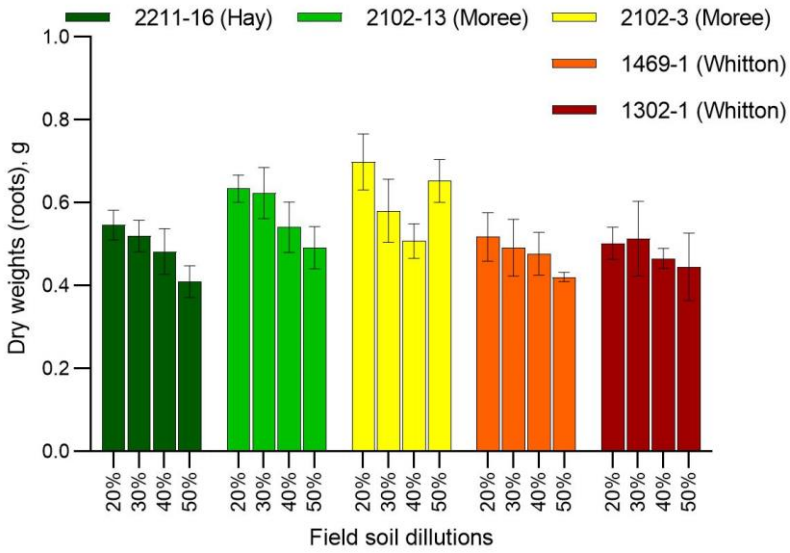


5.2.3.2. *Root dry weights, Part b*

*The root dry weights had similar trends to shoot dry weights: the less infected the soil in the pot, the more roots weighed (*Figure 14*). In some cases, such as for sample 2102-13 from Moree, root dry weights were significantly higher when comparing 20% dilution against 40%, but not when compared to 50%.*

When differences were statistically the same, some trends were clearly observed. For example, sample 2211-16 from Hay had a reduction of root dry weights of 9.7% when the soil increased the percentage of infected field soil from 20 to 40% and up to 17.6% when infected soil increased from 20 to 50%. Other indication of this reduction can be seen in sample 1469-1 from Whitton where root dry weights decreased from up to 18.6% when the infected field soil increased from 20% to 50% in the pot, Figure 14

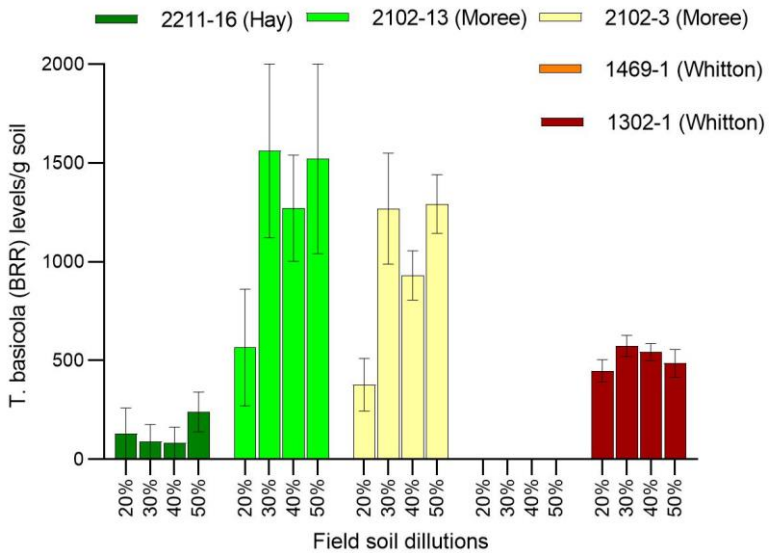
Figure 14 Root dry weights of cotton seedlings grown for 12 weeks in soils from different farms/sites, which were diluted at different rates (20%, 30%, etc) to achieve different levels of T. basicola (BRR). Significant differences were observed for treatments (n=4).



5.2.3.3. Levels of T. basicola (BRR) in the diluted soils, Part b

*The levels of T. basicola (BRR) varied between the field samples and also between the different soil dilutions. The ready to use test was able to detect T. basicola at very low (0.0 counts), intermediate (around 100) and very high levels (more than 1500 counts) (*Figure 15*). Furthermore, some important trends were observed, for example the dilution with 20% infected soil always showed lower levels of BRR compared to any of the other dilutions. However, for most of the samples, no significant differences were observed when comparing 30, 40 and 50% dilutions. Another important finding was seen for sample 1469-1 (Whitton), which did not show any indication of T. basicola infection after the experiment, this was also described above in Part a. Furthermore, sample 1301-1 (Whitton) showed similar levels of the disease in both parts of this experiment (Part a and b), which in both cases indicates that the ready to use test is currently giving repeatable results (*Figure 15*).*

Figure 15 T. basicola (BRR colonies) levels in soils from different sites/farms after growing cotton for 12 weeks under controlled conditions. Field soils were diluted at different rates (20%, 30%, etc). n=4

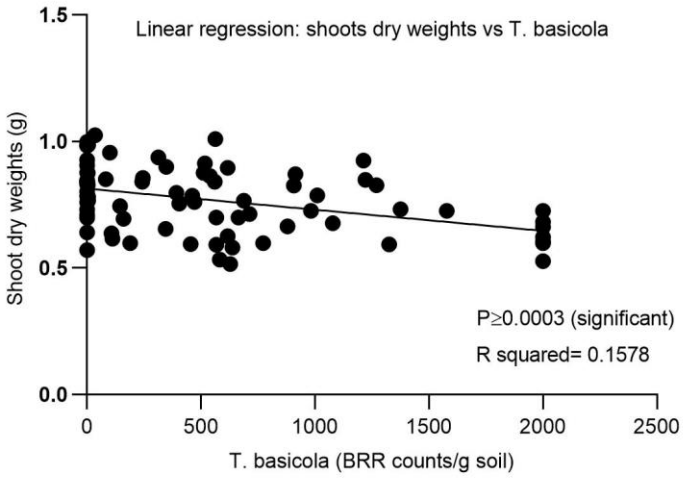


5.2.3.4. T. basicola (BRR) levels and the interaction with reduction of biomass, Part b

There was a significant interaction when the data for shoot dry weights and levels of BRR were analysed. From Figure 16*a it can be seen that there was a significant correlation between the reduction of shoot dry weights with the increase of BRR counts (P= 0.0003), which gives some clarity on the usefulness of the test. Root dry weights and BRR levels did not have a significant correlation when the data for all the sites was analysed (P= 0.3174; R squared= 0.01333),* Figure 16*b. In addition, correlation analysis for single farms/sites showed significance for sample 2102-13 from Moree only (P= 0.0289, R squared= 0.2975), all the other samples did not correlate significantly (Figure 18).*

Figure 16 Linear regression showing the Interaction between shoot dry weights and T. basicola levels (BRR counts). The correlation was statistically significant (P= 0.0003, n=80).

A)



B)

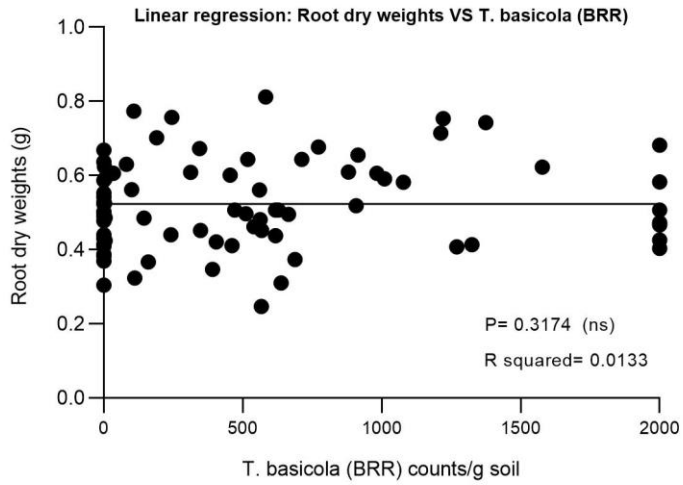
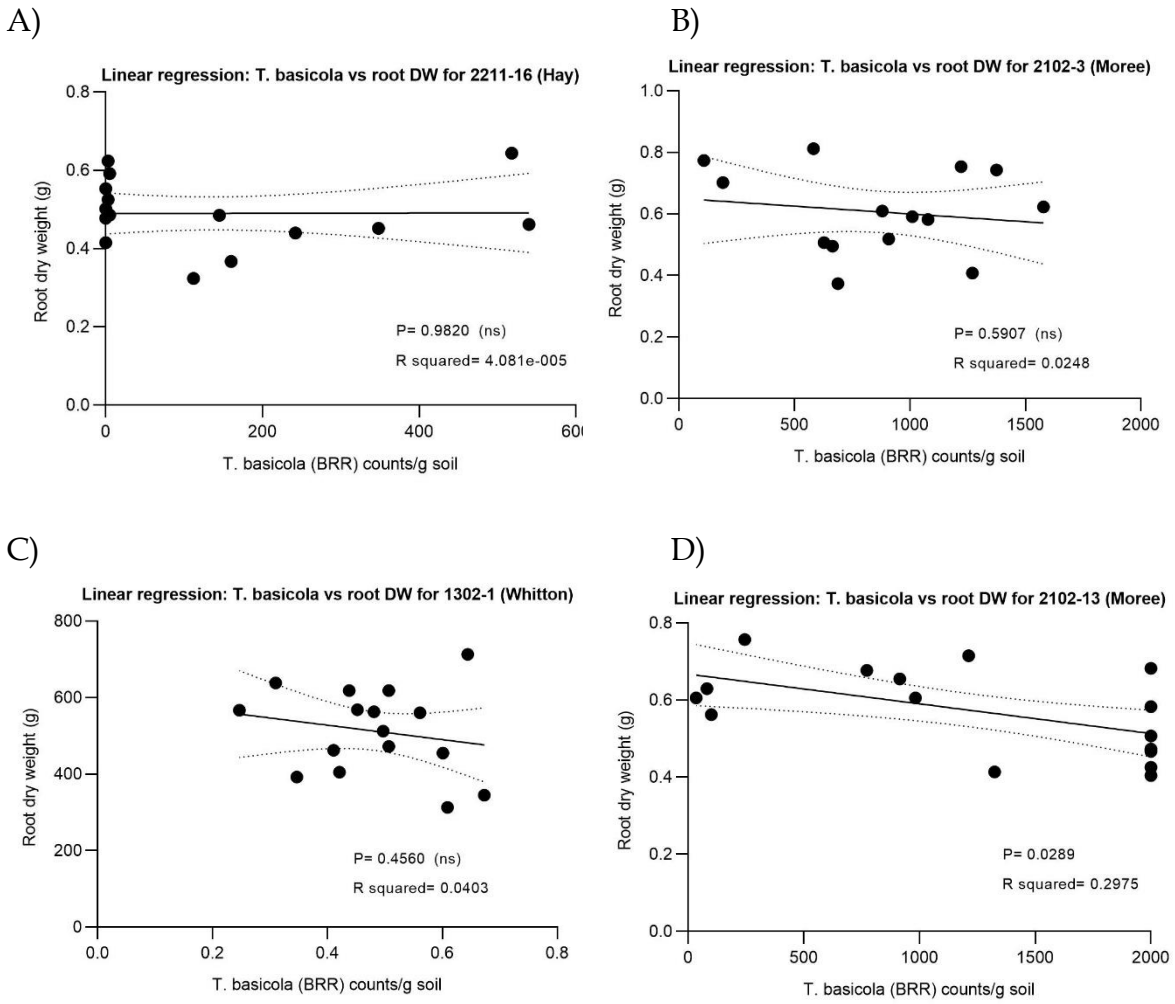


Figure 17 Linear regression showing the Interaction between T. basicola (BRR) and root dry weights for different farms or sites: (A) the correlation for 2211-16 from Hay was not significant as it was for (B) 2102-3 for Moree and (C) 1302-1 from Whitton. The correlation was statistically significant (P= 0.0289, n=16) for sample 2102-13 from Moree.



5.2.3.5. Quantification of other pathogenic fungi and its correlation with T. basicola, Part b

*The number of other pathogenic fungi infecting the field samples varied depending on the site/farm and on the soil dilutions. The counts ranged from around 6 colonies/g soil for sample 2102-13 (Moree) and up to 255 colonies/g soil in sample 1469-1 (Whitton) (*Figure 18 *and* Figure 20*). In general, there was a trend where whenever BRR was elevated, other pathogenic fungi were mostly low and vice versa, as already described in Par a.*

The correlation between these two variables was highly significant (P= 0.0001; Figure 19*a), confirming the observed trend.*

5.2.3.6. Effect of T. basicola (BRR) and other pathogenic fungi levels on the reduction of cotton seedlings biomass, Part b

*No significant correlation (P= 0.1183) was found between other pathogenic fungi and shoot dry weights of the cotton seedlings (*Figure 19*b). Except for sample 1469-1 from Whitton (*Figure 20*), in which BRR was hardly detected, but other pathogenic fungi were in medium to high levels. In this sample root dry weights of the seedlings were lower in soils at 50% dilution compared to the root dry weights at 20% (with a reduction up to 18.6%). However, the correlation between root dry weights and other pathogenic fungi was not significant (P= 0.3921, See* Figure 20*), which may indicate that other factors were also involved in the biomass reduction.*

Figure 18 T. basicola (BRR) counts (green bars) compared to other pathogenic fungi (yellow bars) from soils sampled from different farms/sites, which were diluted to different percentages to achieve different levels of Inoculum. n=16 per sample, with 4 replicates per soil dilution. Green bars= T. basicola (BRR) levels. Yellow bars= Other pathogenic fungi levels.

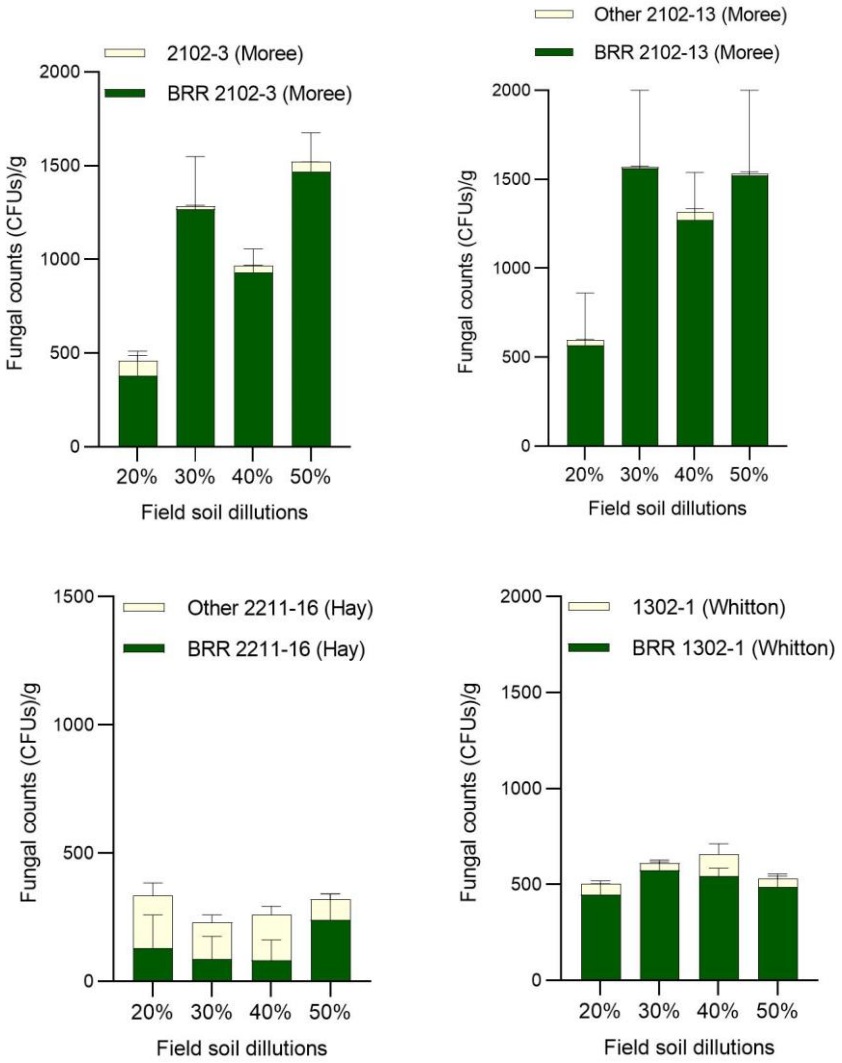


Figure 19 Linear regression showing (A) the Interaction between T. basicola (BRR) and other pathogenic fungi levels. The correlation was statistically significant (P= 0.00010, n=80). And (B) the Interaction between other pathogenic fungi levels and shoot dry weights from different sites/farms. The correlation was not significant with P= 0.1183 (n=80).

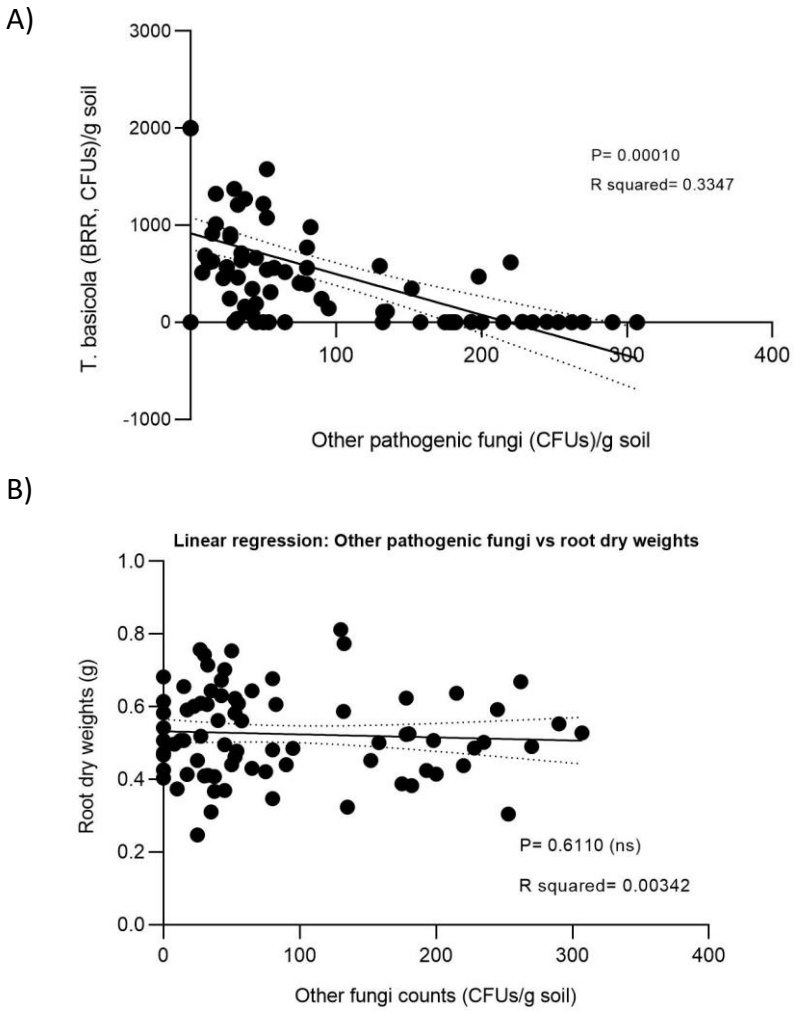
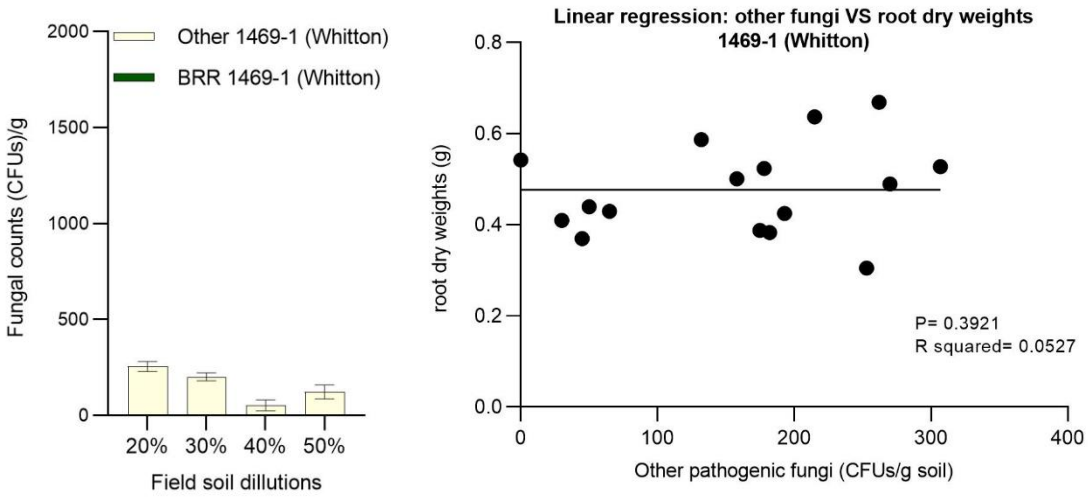


Figure 20 T. basicola (BRR) and other pathogenic fungi levels in soils from Whitton (1469-1) after planting cotton seedlings for 12 weeks in a growth room. On the left, BRR was not detected In Whitton samples, the figure shows other fungi only (yellow bars). On the right, the correlation between root dry weights and other pathogenic fungi was not significant (P= 0.2755, n=16)



VI. Discussion

Soils from the different sites/farms included in this study varied in their levels of T. basicola (BRR) as expected. From the total number of samples, 8 samples had below detectable level or zero colonies, 6 sites had less than 10 BRR/g soil, 8 had 10-30 BRR/g soil, 11 samples had 30-100 colonies and 9 samples had more than 100 colonies (up to 2000)/g soil after the soils were planted with cotton for 6-10 weeks under controlled conditions. T. basicola also varied depending on the dilution of the original soil (from 20% to 50%) for each site. The sometimes very high variability between the soil dilutions during the testing made correlations with some of the response variables difficult. High variability of T. basicola recovered from different cotton soils has been previously reported by Holtz and Weinhold (1994), Wang and Davies (1997) and O'keeffe (2017), which has helped to push for better methods for sampling and testing.

The ready to use test provided a repeatable, quantitative method at very low, intermediate and very high levels for T. basicola (BRR), and other pathogenic fungi such as Fusarium and Verticillium. Up to 8 of the initial farm soils had BRR counts below detectable levels. In two different experiments, sample 1469-1 from Whitton recorded the lowest counts of T. basicola after growing cotton for 6 and 10 weeks under controlled conditions. In contrast, sample 1302-1 from Whitton recorded high counts of BRR in both experiments when the soil was sown with cotton. In addition, two samples from Moree, 2102-3 and 2102-13 recorded more than 1500 BRR counts/g of soil when the sample had 30-50% infected soil. These results gave some clarity of the usefulness of the test.

The correlation between shoot dry weights of cotton seedlings with T. basicola counts was not significant in Part 2a of the experiment after 6 weeks under controlled conditions. However, the correlation was highly significant when the seedlings were left for 10 weeks in Part 2b of this project (P= 0.0003). This correlation showed that with just 30 BRR counts/g soil, dry weights could negatively be impacted. The effect was more pronounced after 100 BRR colonies/g soil. Positive correlations between these two variables have been found before for T. basicola in tobacco (Specht, 1985), cotton (Mauk, 1988; Chapman, 1990 and Rothrock, 1992, Hulugalle et al., 2012) and other crops such as beans (Reddy & Patrick, 1989) under controlled and field conditions. We see an opportunity to use the statistical model found in this project and paired it with cutting-edge data analytics. Current machine learning models may be able to predict more efficiently the reduction of cotton biomass and thus reduce the risks associated with BRR and possibly other pathogenic fungi. In addition, machine learning models can be useful when adding new variables related to management practices such as soil Carbon and or antagonistic bacteria and fungi, helping cotton farmers more comprehensively.

*The effect of the disease measured on roots as browning or severity in Part a of the experiment showed that severity was always higher when the soil dilution contained 50% infected soil compared to 20% (*Table 1*). However, severity measured as browning of the roots did not correlate with the levels of BRR (P=0.1545, R squared= 0.0324), which has been previously found in Australian cotton by Nehl, Jhorar and Mondal (2004). These authors concluded that lateral roots needed to be measured as well as the tap roots to really correlate BRR with root damage. In our experiment tap roots were the ones analysed for disease severity. On Part b of the experiment, as roots were more lignified after 10 weeks, browning of the root was not a reliable way to measure the effect of BRR and root dry weights were measured instead. Data analysis showed a decrease in root dry weights with the increase of infected soil. However, the differences were not always significant and varied from site to site, but root dry weights were always higher at 20%. The correlation of the two variables was not significant (P= 0.3174; R squared=0.01333), which may be an indication of the effect of prolonged, warmer temperatures on T. basicola as the experiment run for 10 weeks. Similar results were previously found by Rothrock (1992) and Holtz et al. (1996) in cotton under controlled conditions.*

The existence of mixed pathogenic infections in cotton seedling makes the quantification of the damage even more difficult and complex. In this study, other pathogenic fungi were also detected by the ready to use test, these fungi mainly included Fusarium and Verticillium. However, the effect of

these other fungi on cotton biomass was not positively correlated in any of the experiments. The correlation in both cases was not significant either for shoot dry weights in Part a (P=0.8842) or for shoot and root dry weights in Part b (P=0.1183 and P=0.6110 respectively). However, these statistical correlations cannot solely explain the changes in biomass observed, for example, sample 1469-1 from Whitton, where BRR was below detectable level in two different experiments, recorded up to 300 colonies of other pathogenic fungi/g of soil. The reduction in biomass is there but cannot be simply explained by any of the fungi quantified. Thus, a cautious analysis of other factors, for example of some soil characteristics, could help elucidate this reduction in biomass. Previous reports had already highlighted the importance of high clay levels to reduce the effect of soilborne pathogens such as Pythium, Rhizoctonia and T. basicola in cotton (Johnson and Doyle, 1985), where heavy soils with more than 20% clay tolerated better the disease. It may also be worthwhile to correlate some more of these soil features in the near future to get a better understanding of these results.

*Finally, the relation between BRR and other pathogenic fungi was inversely correlated in both experiments (P=0.0021 and P=0.0001). High levels of BRR were always recorded with low levels of other pathogenic fungi and vice versa (*Figure 19*a). A general overview of the interaction between pathogens is that the relation is mostly synergistic due to the fact that once the first pathogen invades the plant, it opens the pathway for others to infect, but that is contrary to what was found here. A study by Chapman (1990) reported a similar interaction between T. basicola and Rhizoctonia solani where high T. basicola resulted in reduced disease by R. solani. On the contrary, in a final report to CRDC in 2016, Kirby and associates concluded that there was no correlation between incidence of T. basicola and Verticillium when historical data were analysed. In addition, when a controlled pot experiment was setup for 4 weeks, the incidence of BRR was higher when plants were infected with both T. basicola and Verticillium compared to single pathogen infection. In our case, the cotton seedlings were grown for 6-10 weeks, which may also help to explain the differences in response.*

VII. Conclusions

From the results found in this project, which includes significant correlations between T. basicola counts and decrease in cotton seedling biomass, it was possible to quantify the levels of the disease in different soils with reasonable accuracy. To really use these correlations to the advantage of cotton producers, we suggest that the statistical models should be paired with cutting-edge data analytics to offer the best solution to reduce the risks associated with BRR. In addition, other variables such as soil properties and the presence of beneficial and pathogenic fungi need to be taking into account when using these models.

VIII. References

Chapman, M. A. 1990. The interaction of Thielaviopsis basicola (Berk. and Br.) Ferr. And Rhizoctonia solani Kuhn as pathogens of seedling cotton. Ph D Thesis. The University of Arizona. 94 p.

Holtz, B.A., Roberts, B.A, Hancock, J. and A.R. Weinhold. 1996. The influence of soil flooding and temperature on the survival of Thielaviopsis basicola. Phytopathology 86:110-111.

Hulugalle, N. R., T. B. Weaver, L. A. Finlay, and P. Lonergan. 2012. Soil properties, black root-rot incidence, yield, and greenhouse gas emissions in irrigated cotton cropping systems sown in a Vertosol with subsoil sodicit. Soil Research, 50: 278–292. http://dx.doi.org/10.1071/SR12088.

Johnson. L. F., and J. H. Doyle. 1986. Relations hips of seedling disease of cotton to characteristics of loessial soils in Tennessee. Phytopathology 76: 286-290

Nehl, D. B., Jhorar, OM P, and A. H. Mondal. 2004. Managing black root rot of cotton, Final report (2001-2004). Cotton Research and Development Corporation. 47 p

O'Keeffe, K. and A. Thomas. 2017. Assessing green manure vetch to reduce the impact of black root rot on cotton production. Final report to CRDC. 6 p

Reddy, M.S. and Z.A. Patrick. 1989. Effect of Host, Nonhost, and Fallow Soil on Populations of Thielaviopsis Basicola and Severity of Black Root Rot, Canadian Journal of Plant Pathology, 11:1, 68-74, DOI: 10.1080/07060668909501150

Rothrock, C. S. 1992. Influence of soil temperature, water, and texture on Thielaviopsis basicola and black root rot of cotton. Phytopathology 82:1202-1206.

Youn, S. P. and S. J. Allen. 1991. Thielaviopsis basicola: A potentially important pathogen of cotton in Australia. A paper presented at the 'Second Graduate Seminar in Plant Pathology and Mycology University of New England. NSW Agriculture. Agricultural Research Station, Narrabri.

Wang, H., and Davis, R. M. 1997. Susceptibility of selected cotton cultivars to seedling disease pathogens and benefits of chemical seed treatments. Plant Dis. 81:1085-1088.

IX. Key word index

- *Cotton seedlings*
- *Black root rot levels*
- *Thielaviopsis basicola*
- *Soil test*
- *Inoculum density*
- *Disease severity*
- *Other pathogenic fungi*
- *Soilborne pathogen*
- *seedlings*

X. A full list of industry and scientific publications, presentations, extension activities and other outputs.

1. *Participation at Australian Soil Science conference (suspended for 2020) but will be attended in 2021*
2. *FUSCOM 2020- Farmers and Scientific participation*
3. *Twitter*
4. *Honours Project: "Can Trichoderma and Bacillus be used as biocontrol agents against T. Basicola (BRR) in cotton?" And in vitro study. Fabiel Hernandez-Espinosa. International Student 2020. He will be presenting at FUSCOM 2020, scientific program.*

Please contact your R&D manager if you would like to adopt a different approach.

Part 4 – Summary for public release

This summary will be published on Inside Cotton, CRDC's digital repository, along with the full final report (if suitable for public release). It is designed to provide a short overview of the project for all interested parties. Please complete all fields, ensuring that this exceeds no more than two pages.

Project title:	**Ready to use soil** test to manage black root rot risks	
Project details:	CRDC project ID:	MLAB1901
	CRDC goal:	*Click here to select a Goal*
	CRDC key focus area:	*Click here to select a KFA*
	Principal researcher:	Dr Maria Manjarrez, Research and Development
	Organisation:	Microbiology Laboratories Australia
	Start date:	*July 2018*
	End date:	*June 2020*
Objectives	• *To verify if the ready to use Test is robust enough to recover infective T. basicola propagules in different soils or farms* • *To correlate the levels of T. basicola from field soils or "naturally" infected soils against plant symptoms or disease in cotton* • *To determine what other fungi are predominantly involved in causing BRR, if any*	
Background	*From the CRDC project 1624 it was concluded that a "ready to use test" selected in that project for quantification of BRR needed to be fine-tuned using "artificially" infected soils. The initial development of the test used one soil type with or without black root rot. The initial quantification using the ready to use test was merely based on the contrasting levels without taking into account what was happening with the plant (disease incidence).* *To verify those results, soils with different levels of BRR and with different properties needed to be tested. If the test was robust enough, the end result will be a tool to help reduce the risk associated with the disease.* *The test needed also to be adjusted to test for the interaction with other plant pathogens causing root rot as previous results showed that black root rot may be the result of different fungal species acting together to produce the disease. The results may be used to design a multi-species detection test.*	
Research activities	*Our team worked with ±50 soil samples with different BRR histories, from different cotton producing regions (Whitton, Hay, Hillston, Griffith, Macquarie, Lachlan, Canargo, etc). Soil samples were used to establish 4 experiments under controlled conditions. In Experiment 1 and 2, trap plants such as green beans, soybean and pansy were used to facilitate and speed up quantification of T. basicola. However, the trap plants used gave variable, unrepeatable results, which made detection of the pathogen unreliable. After adjusting the methodology and by following advice from the CRDC research team, experiments 3 and 4 were setup using Sicot 620 cotton seeds supplied by Cotton Seed Distributors (CSD). Both experiments gave repeatable results when using the ready to use test with some statistically*	

	significant data. Statistical correlations were achieved between BRR levels and reduction of cotton seedlings biomass under controlled conditions. The test was also detecting other pathogenic fungi such as Fusarium and Verticillium.
Outputs	*From the total number of soil samples, results showed that 8 samples had below detectable level or zero colonies, 6 sites had less than 10 BRR/g soil, 8 had 10-30 BRR/g soil, 11 samples had 30-100 colonies and 9 samples had more than 100 colonies (up to 2000)/g soil after the soils were planted with cotton for 6-10 weeks under controlled conditions. T. basicola also varied depending on the "dilution" of the original soil (from 20% to 50%) for each site. The sometimes very high variability between the soil dilutions during the testing made correlations not always significant. However, some important results were obtained.* *Our project concluded that the ready to use test can achieve with reasonable accuracy, quantification of T. basicola at very low, intermediate and very high levels in soils from different farms or sites, which have different chemical and physical properties. This conclusion came after results showed statistical correlations (and or linear regressions) between shoot dry weights and BRR levels (P= 0.0003). These mathematical models may be used to help reduce the risks associated with the disease.* *In addition, results showed that plant biomass started decreasing at just 30 BRR colonies/g of soil, which is new information that could be used to correlate better with what is happening in the plant. There was not big difference in biomass reduction after 100 BRR colonies/g soil, however some soils showed up to 1500 BRR colonies/g of soil after planting cotton under controlled conditions.* *Another important result was the detection of other pathogenic fungi such as Fusarium and Verticillium using the ready to use test. Although there were no significant correlations between the levels of these fungi and the reduction in cotton seedling biomass, results can be used to better manage seedling diseases in such a cases as fungicide management.*
Impacts	*As results showed a mathematical correlation between BRR levels with biomass reduction (and root "quality"), the ready to use test could be used to reduce the risks associated with the disease (at least partially as other factors may be involved in this complex production system). In discussing the results, we see an opportunity for new cutting-edge data analytics to be paired with the mathematical model so it can "learn" to predict the disease incidence better in the near future so farmers can fully take advantage of this tool.*
Key publications	*1.Participation at Australian Soil Science conference (suspended for 2020) but will be attending in 2021* *2.FUSCOM 2020 presentations- Farmers (October) and Scientific (November, 2020)* *3.Twitter* *4.Honours Project: "Can Trichoderma and Bacillus be used as biocontrol agents against T. Basicola (BRR) in cotton?" An in-vitro study. Fabiel Hernandez-Espinosa. International Student. 2020. He will be presenting at FUSCOM 2020, scientific program.*

Appendix 1
Example of Report generated after soil samples were tested using the Ready to use test. One report per soil sample.

microbiology laboratories australia
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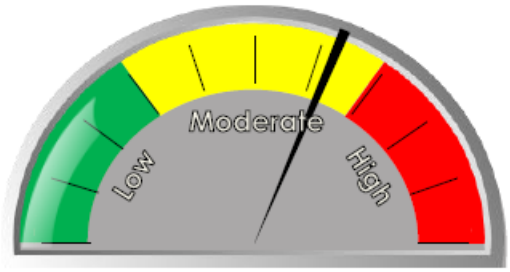
Microbiology Test

Name: **Cotton** Sample: **Cotton** Analysis no.: **1469-1** Date: **5/05/2017**

Customer name	Cotton	**Date received**	5/05/2017
Client name	1st May	**Agent**	Microbiology Laboratories A
Sample name	Cotton	**Advisor**	
Crop	Rice (after cotton)	**Authorised by**	Dr Maria Manjarrez
Date sampled	1/05/2017	**Analysis no.**	1469-1

Black Root Rot-Cotton

Disease Pressure Indicator



Data

		Yours	Guide
Black Root Rot infective biomass	Colony Units Per g/soil	65.0	100.0

	Yours	Guide
Disease Pressure Indicator	6.3	10.0

Risk key: Low | Moderate | High

* Assumes a sampling depth of 20 cm and a bulk density (BD) of 1.1 g/mL. For other depths and densities use mg/kg × (depth (cm)/10) × BD (g/mL).

Comments

The levels of infective Black root rot complex were moderate in this sample. Black root rot colonies were slower to develop than in previous samples. The potential for disease in this soil is unlikely to be very high. Use the appropriate agronomy note to help minimise and manage crop risk throughout the growth season.