FINAL REPORT

For Public Release

Part 1 - Summary Details

CRDC ID: DAQ1803

Project Title: Understanding the ecology of reniform nematodes in cotton

Project Start Date: 11/11/17 **Project Completion Date:** 31/12/2019

Research Program: 1 Farmers

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Part 3 – Final Report

Background

1. Outline the background to the project.

The reniform nematode (*Rotylenchulus reniformis*) is a new and growing threat to the cotton industry in central and south east Queensland and possibly further afield should its current distribution increase. This nematode has the potential to be extremely damaging to the current affected cotton industry areas. Approximately 205,000 bales of cotton are lost to reniform nematode annually in the United States, which translates to roughly 11% of cotton losses attributed to all cotton diseases (Khanal et al., 2018).

The reniform nematode has only recently been identified as causing cotton yield loss within the Theodore region. Since this pest was identified in 2012, QDAF has been undertaking studies to identify the distribution of this nematode in the Theodore and wider central Queensland area and undertaking rotational crop studies. To date this work has been largely successful at the field scale at controlling reniform population levels, but not in its eradication. The distribution of the reniform nematode in the soil profile has also been investigated. Results suggest that the nematode can survive deep in the soil profile (at least 100 cm). This is concerning as the deep-lying populations may assist in repopulating the upper soil profile when cotton is grown following a season with a non-host.

Control methods for reniform in Australian cotton are almost non-existent (Allen et al., 2012). Aldicarb was the only nematicide registered for use in Australia, but its registration was recently not renewed and global production is being stopped. In the absence of chemical control strategies, crop rotations remain as a potential damage limitation option, but there is currently insufficient information on the reniform host-pathogen-soil interactions and general ecology in Australian cotton systems to design suppressive rotations or implement additional cultural control methods.

Spread of the reniform nematode across various cotton-producing states in the USA was rapid with yield reduction and economic impacts experienced after this pest arrived and quickly developed within their systems. During this period chemical control was available but was never able to eradicate this pest meaning that cultural control methods were also needed.

This project seeks to develop knowledge of this pathogen epidemiology, which is crucial for developing targeted management strategies. Developing a basic understanding of the reniform nematode's ecology and behaviour under Australian cotton cropping conditions has to be a key industry research objective to improve control and reduce the threat of the potential spread of this pathogen. To address this we will investigate the limitations to reniform growth and development, look at crop and weed host ranges, soil distribution patterns and movement in relation to cotton plants and system management. Through the development of improved epidemiology understanding of this pest, we will be able to develop spread and control mitigation strategies suitable for deployment in the Australian cotton industry.

Objectives

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

Milestone 1. Research question: Where in the soil profile are reniform nematodes?

1.1 What is the epidemiology between cotton roots and rising reniform numbers in the soil profile?

1.1.1 Examine the vertical movement of the reniform nematode in vertisol. A glasshouse trial was conducted to study the vertical movement (both upward and downward) of the reniform nematode in vertisol. This objective has been achieved.

1.1.2 Assess the reniform population at depth. The field survey was conducted to examine the nematode population at various soil depths in various growing seasons. This objective has partly been achieved. This was delayed due to reduced staffing. The remaining samples are scheduled to be processed in January 2020.

Milestone 2. Research question: What is the potential risk to the rest of the industry?

2.1 What are the constraints to reproduction in Australian cotton soils for reniform and root-knot nematodes?

2.1.1 Conduct seasonal soil sampling to assess the presence, absence, and severity of the reniform nematode. This objective has been achieved.

2.1.2 Conduct a population trial to estimate the threshold level of reniform on cotton. Glasshouse trial was conducted using three different population levels of nematode on three different cotton varieties. This objective has been achieved.

2.1.3 Conduct pot trials with different soil types from different regions to test max and minimal temperature: Characterised reniform activity and reproduction in combination with root visualisation techniques. This objective has not been achieved due to the delay in project commencement and technical difficulties in establishing a reniform culture. It was planned to use reniform from a single egg-mass population for this trial but due to the technical difficulties, the reniform population could not be established and thus the trial did not progress.

Milestone 3. Research question: What management tactics can be used to manage reniform?

3.1 What crops are non-hosts and can they be incorporated into farming systems?

3.1.1 Conduct a host/non-host trial to check if a potential rotation crop is a host or non-host of the reniform nematode. A glasshouse trial was conducted to test the host/non-host status of wheat, corn, forage sorghum, and grain sorghum. This objective has been achieved.

3.1.2 Investigate the nematicidal seed treatment to manage the reniform nematode. A field trial was performed in Theodore to test the efficacy of Syngenta seed treatment products against reniform nematode. This objective has been achieved.

3.1.3 Investigate the biological product against the reniform nematode. A field trial was conducted to test the effect of a biological product containing Bacillus species called FertiLink on reniform nematode. This objective has been achieved.

3.2 What's the impact of flooding of soils on mixed nematode populations including reniform?

3.2.1 Conduct pot trials with different degrees of wetting and flooding. This objective has not been achieved due to the delay in project commencement.

Milestone 4. Research question: Can we adopt information from the US into the Australian system?

4.1 Are nematodes in Australian cotton systems the same as in the US?

4.1.1 Study the genetic diversity of the reniform nematode. Reniform nematode populations collected from different host crops were tested for their diversity in collaboration with Dr. Dean Brooks of the University of Queensland. This objective has been achieved.

Methods

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

Milestone 1. Research question: Where in the soil profile are reniform nematodes?

1.1 What is the epidemiology between cotton roots and rising reniform numbers in the soil profile?

1.1.1 Examine the vertical movement of the reniform nematode in vertisol.

A pot trial was conducted in a climate-controlled cabinet to study the vertical movement of the reniform nematode in vertisol soil. Both upward and downward movement of the reniform nematode in the presence of host (cotton) and non-host (sorghum) plant was assessed. For this trial, field soil was collected from Theodore (Central Queensland) that has consistently low or no reniform or other plant-parasitic nematodes. The soil was frozen for 24hrs to further reduce the survival of any plant parasites present in the soil. Once thawed, the soil was homogenised to a fine tilth using a custom-designed soil mixer developed by CSIRO. Nematode extractions and microscopy were then performed to confirm the absence of plant-parasitic nematodes. Tall, thin plastic pots (16Hx6.5Wx6.5L cm) were filled with soil and inoculated with reniform nematodes either at the top or bottom of the soil profile, except the pots prepared for control plants. For the top inoculation, two small holes (≤1cm) were made at an equal distance from each other and 1 ml of water suspension containing approximately 240 nematodes per ml were added to the holes using a pipette (500 microlitres in each hole). For the bottom inoculation, a thin layer of soil (about 1-1.5 cm) was added at the bottom of the pot which was then rinsed with water and inoculated with nematode as described above and filled with rest of the soil.

One-third of the top and bottom inoculated pots were sowed with cotton seeds, another one third with sorghum, and the rest of the one-third pots kept without any plants. The cabinet temperature was maintained at 26/22°C (+/- 3°C) day/night with the photoperiod of 16/18 hours day/night (maximum Photosynthetic Photon Flux Density of 800 micromoles m^{-2} s^{-1}). The relative humidity was maintained at about 80%. Pots were watered every second day. As the water requirement of cotton and sorghum is different, they received different volumes of water but all the plants of each crop received the same amount. Pots with no plants received enough water to remain just moist. All the plants were completely randomised once every week.

Plants were harvested at two different time points, half of the sorghum plants (from all the treatments) harvested at 28 days after sowing (DAS) and the second half at 60 DAS. Pots with no plants were also harvested along with sorghum pots at 28 and 60 DAS. Similarly, half of the cotton plants (from all the treatments) harvested at 29 days after sowing (DAS) and the second half at 62 DAS. The pots were cut into three (top, middle, bottom) equal sections (Fig. 1) and nematodes were extracted separately from each section using the Whitehead tray method. Additionally, the roots of the plants from different sections were collected, cleaned, and stored separately to assess the extent of nematode infestation using root staining methods as described below.

Figure 1. Soil core cut into three equal sections (top, middle, and bottom) to assess the vertical movement of the reniform nematode. Soil and root subsample in each section was analysed separately to quantify the reniform nematodes.

Nematode extraction from soil sample using the Whitehead tray method.

The volume of the soil from each of three sections was measured and placed into a whitehead tray (metal sieve with tissue paper layer placed inside a tray) and water was added to moisten the soil. Trays were placed inside a cabinet for three days of incubation. After the incubation period, the metal sieve containing the soil was removed and the remaining solution was poured through two fine sieves, one to remove soil debris (150-micron sieve) and one to collect the nematodes (38-micron sieve). The nematodes were rinsed from the sieve surface into a vial which is then analysed under the microscope for identification and quantification of plant-parasitic nematodes. All plant-parasitic nematodes were reported as numbers per 200 ml soil.

Quantification of nematode root infestation using staining method.

Root staining was done by modifying the method developed by Byrd et al. (1983). In summary, the following steps were taken to stain the roots containing female reniform nematode and egg masses:

i. Cut infected roots into pieces and put 1 gram of well-mixed fresh root subsample in a 15 ml centrifuge tube and add household bleach (NaOCl) diluted to a concentration of 1% available chlorine. Keep the root immersed in bleach solution for about four minutes to clear roots.
ii. Drain the bleach solution from the tube and rinse roots with tap water twice and then fill the tube with tap water to soak the roots for 15 minutes to remove residual NaOCl.
iii. Drain the water and fill the tube with 12 ml of tap water.
iv. Add 300 to 400 microliter of a stock solution of acid fuchsin, prepared by dissolving 3.5g acid fuchsin in 250 ml acetic acid and 750 ml distilled water, to each tube containing root sample.
v. Boil about 300 ml water in a 1000 ml beaker on a hot plate and arrange tubes containing root sample in the beaker in an upright position for about 10 minutes until the solution inside the tube start to produce bubbles due to heat. Then remove the beaker from the hot plate and allow it to cool to room temperature.
vi. Upon cooling, stain the staining solution and add 12 ml of 50% glycerol (acidified with a few drops of 5N HCl) into each tube.
vii. Spread roots along with glycerol into a Petri dish and examine/quantify adult reniform nematodes and egg masses (if present) under a stereomicroscope.

1.1.2 Assess the reniform population at depth.

The field survey was conducted in Theodore to examine the nematode population at various depths in early and late in the growing season. The cropping and sampling history of fields has been examined and a total of seven fields were assessed for sampling at pre-plant and post-harvest of 2018/19 season. Fields were sampled using a deep-corer to collect populations to a depth of 1m with the aim to investigate the movement of reniform at depth through the season. Fields selected have a long history of sampling, low or high populations of reniform, and one field has both reniform and root-knot nematode. Cores were divided into three sections: 0-30 cm, 30-60 cm, and 60-100 cm and analysed separately. Soils samples are being processed and counted.

Milestone 2. Research question: What is the potential risk to the rest of the industry?

2.1 What are the constraints to reproduction in Australian cotton soils for reniform and root-knot nematodes?

2.1.1 Conduct seasonal soil sampling to assess the presence, absence, and severity of the reniform nematode.

During the yearly early-season disease surveys, the soil is collected from every field surveyed in QLD and NSW and assessed for plant-parasitic nematodes. This sampling strategy is being tested for its usefulness as an early detection method of the reniform nematode. The soil samples were collected from the 10 sampling stops along the disease survey transects (as shown in Fig. 2) and mixed well. Plant-parasitic nematodes are extracted using the Whitehead tray extraction method as described in section 1.1.1 and reported as the number of

nematodes per 200ml of soil. This sampling was not as extensive as to cover the whole field, therefore, it provides only an idea of whether reniform is present or absent in a particular field.

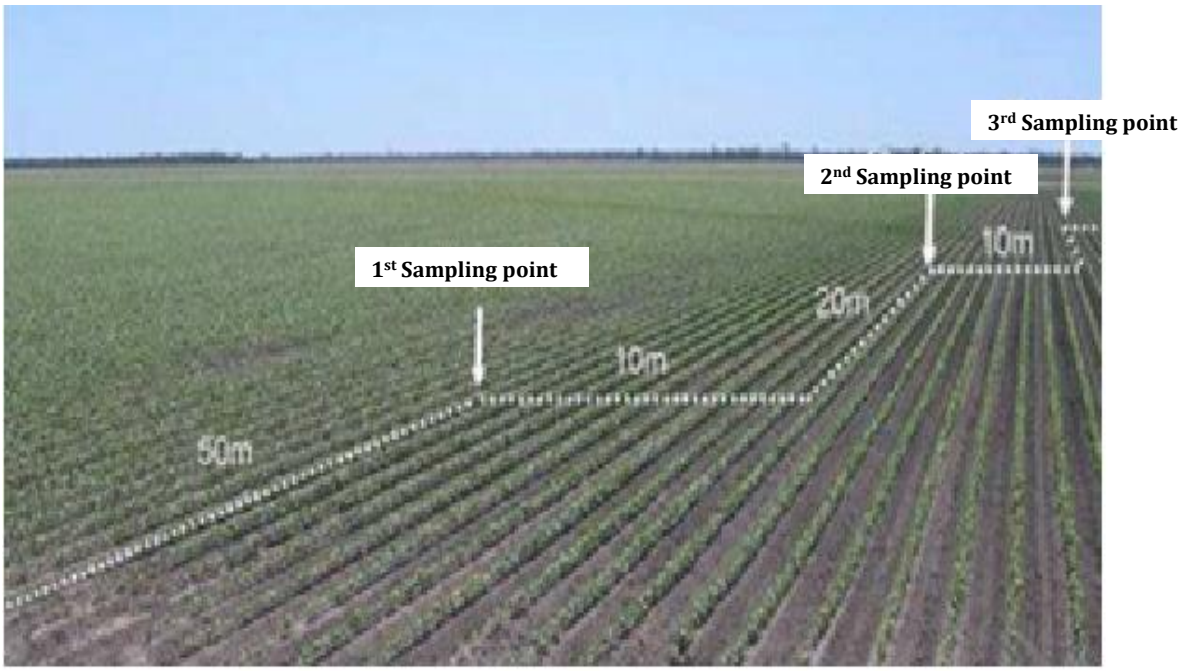

Figure 2. Disease survey transects. The first sampling point/stop is 50 m from the starting point and each successive sampling point is 10 m across the rows then 20 m ahead. Samples were taken from a total of 10 sampling points in each transect. Photo: Chris Anderson

A follow-up assessment was conducted in the late-season using an extensive soil sampling protocol of fields to determine the soil population. Fields were chosen and divided into 10-hectare blocks, and 100 soil cores were collected using a soil corer from 100 different sampling points in the field in a zig-zag manner as shown in Fig. 3. Cores from each of the blocks were well-mixed separately and a subsample was stored for the nematode extraction and analysis. A total of fifty late season samples (2017/18) were collected from various fields in Central Queensland, including Emerald, Moura, and Theodore. Extractions are complete and counts have been finalised.

Extensive soil sampling protocol

i. Take samples in a zigzag pattern across a field (as shown in Fig. 3)
ii. Scrape off the dry topsoil and sample between 10 – 15 cm deep
iii. Use a small trowel or soil corer
iv. Take 100 samples for every 10 ha and place directly into a bucket
v. Mix thoroughly and subsample
vi. Place approximately 400 g in a clearly labelled plastic bag
vii. Label bag on the outside with a permanent marker
viii. Label towards the bottom of the bag
ix. Extraction depends on live nematodes, therefore keep samples cool in an Esky container without an ice block
x. Do not store samples in a fridge

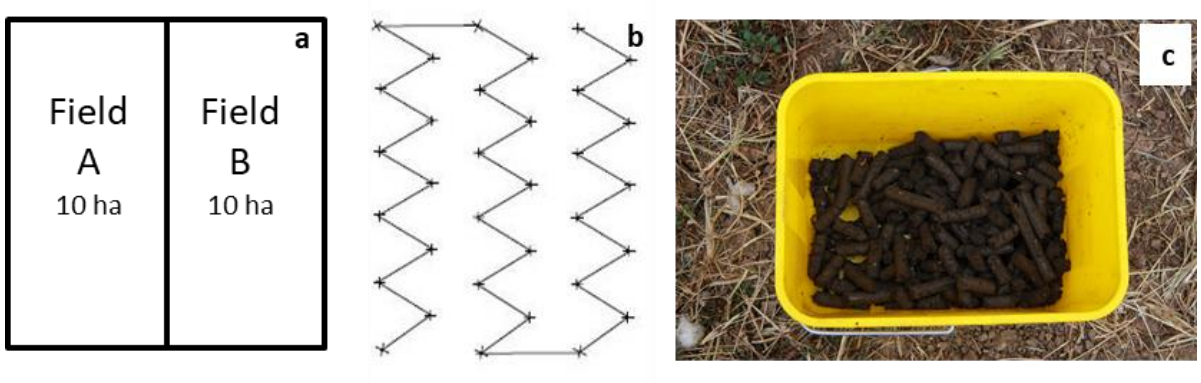

Figure 3. Extensive nematode sampling procedure in the field. a) Fields were divided into 10 ha blocks, b) Zigzag sampling pathway and sampling points, c) soil cores collected from a single 10 ha blocks were collected in a single bucket.

2.1.2 Conduct a population trial to estimate the threshold level of reniform on cotton.

A glasshouse trial was conducted to assess the effect of different populations of reniform nematode on plant performance of three different cotton varieties. Bulk field soil was collected from a cotton field at Theodore known to have no reniform nematode. The collected soil sub-sample was tested in the laboratory to confirm the absence of nematode. The soil was homogenised to a fine tilth and mixed with sand in 4:1 (soil: sand) ratio to increase workability using a custom-designed soil mixer developed by CSIRO. Three popular 'Bollgard 3' varieties namely 'Sicot 714', 'Sicot 746', and 'Sicot 748' were selected for this trial. Seeds were sown to the seedling trays filled with sterilised sand. Then, 11-days-old seedlings were transplanted to experimental pots containing 1.8 l of soil. Sand in the root system was cleared off completely before transplantation by dipping the seeding roots in clean water.

Treatments: Plants were inoculated with three different populations of nematodes while control plants got no nematode inoculation. Inoculation level was based on the pre-plant threshold of the reniform nematode as found in studies conducted in the USA which is 500 nematode per 200 ml soil sample. The level of inoculation was determined for this trial as follows:

Treatment 1: Control: 0 Nem/1.8 l soil
Treatment 2: Low nematode: 100 Nem/200 ml= 900 Nem/1.8 l soil
Treatment 3: Threshold nematode: 500 Nem/200 ml= 4500 Nem/1.8 l soil
Treatment 4: High nematode: 1000 Nem/200 ml= 9000 Nem/1.8 l soil

Fresh nematodes were extracted from the fresh soil collected from Theodore. Multiple extractions were carried out using the Whitehead tray method to get the required number of nematodes for the inoculation. All the plants were inoculated with the required number of nematodes over a period of one week. Due to the technical difficulty in calculating the exact number of nematodes in a limited timeframe, the actual number of nematodes added was slightly different than planned. The actual numbers of inoculated nematodes and treatments were as described in Table 1.

Table 1. Reniform nematode inoculation levels in different treatments.

Variety	Treatments	Number of nematodes added (approx.)	Number of replicates
B3 714 B3F	Control	0	8
	Low nematode	850	8
	Threshold nematode	4450	8
	High nematode	9350	8
B3 746 B3F	Control	0	8
	Low nematode	850	8
	Threshold nematode	4450	8
	High nematode	9350	8
B3 748 B3F	Control	0	8
	Low nematode	850	8
	Threshold nematode	4450	8
	High nematode	9350	8

All the plants were completely randomised once every week. Plants were watered gently and slowly to prevent over-watering and leaking.

Figure 4. Experimental setup of the reniform nematode population trial.

Harvest: Plants were harvested 26 weeks after sowing. Aboveground plant parts were kept in a paper bag and dried in an oven at 60°C for three days to get the dry shoot biomass. The root was cleaned and separated from the soil. Clean roots were weighed and kept in the refrigerator until the sub-sample was taken for nematode quantification. The remaining root tissue was dried in an oven at 60°C for three days to measure the dry root biomass. Soil from each of the replicates was kept in individual plastic bags in storage at room temperature until they will be used for the nematode extraction.

Nematode Quantification: Nematodes infestation in the root tissue was quantified using the staining method as described in section 1.1.1 while nematode abundance in the soil will be determined using the Whitehead extraction method as described above in section 1.1.1.

Parameters: From this trial, the following data were collected at different time points to assess the effect of different nematode population on plant growth and performance:

i. Plant height: Plant height was measured at three different time points during the plant growth: First at 7 weeks-49 DAS days, second at 12 weeks- 84 DAS, and third at harvest- 26 weeks- 185 DAS
ii. Number of balls
iii. Yield
iv. Shoot and root biomass
v. Nematode infestation on the root
vi. Nematode population in soil

Milestone 3. Research question: What management tactics can be used to manage reniform?

3.1 What crops are non-hosts and can they be incorporated into the farming systems?

3.1.1 Conduct a host/non-host trial to check if a potential rotation crop is a host or non-host of the reniform nematode.

A pot trial was conducted to test whether grain crops namely wheat, corn, forage sorghum, and grain sorghum is a host or non-host of the reniform nematode. All these crops are potential rotation crop in cotton cropping system in the cotton-growing regions of Australia. The trial was conducted in the climate-controlled walk-in cabinets at the Ecosciences Precinct in Brisbane. The cabinet temperature was kept at 28/20°C (+/- 3°C) day/night with the photoperiod of 14/10 hours day/night (maximum Photosynthetic Photon Flux Density of 800 micromoles m^{-2} s^{-1}). The relative humidity was maintained at about 65% (+/-15%).

Soil collected from two different cotton fields in Theodore was used for this trial. One of the fields has been known to have a reniform population while another field had no reniform nematodes. The absence of nematode in the collected soil was also confirmed in the laboratory. Field soil was mixed with sand in 4:1 (soil: sand) ratio to ease the handling of soil. The plastic pots of 1.3-litre capacity were used to grow the directly sown seeds. Three to four seeds of each crop were sown in each pot which was thinned out to only one plant per pot after germination.

Cotton seeds were sourced from the Cotton Seed Distributors (CSD) Australia, while the seeds of other crops were provided by the Pacific Seeds Toowoomba. Only one variety of each crop was selected and tested for this trial. All the seeds were tested for their germination rate before sowing. The list of crops and varieties tested on this trial are listed in Table 2.

Table 2. Different crops and their varieties tested as host and non-host of reniform nematode

S.N.	Crop	Variety	Source	Tested as
1	Cotton	Bollgard 3 Sicot 746	CSD	Host
2	Corn	PAC 606 IT	Pacific Seeds	Non-host
3	Grain Sorghum	MR Buster	Pacific Seeds	Non-host
4	Forage Sorghum	Jumbo	Pacific Seeds	Non-host
5	Wheat	Kittyhawk	Pacific Seeds	Non-host

A total of 16 plants were grown of each crop, totalling 80 pots. Half of the plants were grown on infested soil while the other half were grown in clean soil, making two treatments for each crop with 8 replicates in each treatment. Plants were watered as needed in the interval of 2-3 days and all the pots containing certain crops received an equal volume of water. All the plants were completely randomised once every week.

Figure 5. Experimental setup of the host/non-host trial.

Plants were harvested nine weeks after sowing. The aboveground plant part was weighed for the fresh biomass and dried in an oven at 60 °C for three days to obtain the dry shoot biomass. Roots were cleaned and washed gently to remove any soil particles. Clean roots were weighed and kept in the refrigerator until the sub-sample was taken for nematode quantification. The remaining root tissue was dried in an oven at 60 °C for three days to get the dry root biomass. Soil from each of the replicates was stored in an individual plastic bag at room temperature until they were used for the nematode extraction.

Nematodes infestation in the root tissue was quantified using the staining method as described in section 1.1.1. Similarly, the nematode abundance in the soil was determined using the Whitehead tray extraction method as described above in section 1.1.1. The only difference in this trial was the number of nematodes was calculated per 100 gm of dry soil instead of 200 ml of soil.

3.1.2 Investigate the nematicidal seed treatment to manage the reniform nematode.

Two field trials were conducted on two farms in Theodore, Queensland to test the efficacy of Syngenta seed treatment products (listed in Table 3) against reniform nematode. This trail was conducted in collaboration with Syngenta, CSD and two local growers. The product contains a new nematicide (SYNSTN1) which has the SDHI mode of action (SDHI – Succinate dehydrogenase inhibitor). A second nematicide tested has the active ingredient Fluopyram, also a succinate dehydrogenase inhibitor (SDHI) fungicide, which has been shown to have nematicidal properties (Faske and Hurd, 2015). Two trials were conducted. Soil samples were collected pre-plant, mid-season and late-season to determine the effect of nematicides applied as a seed treatment on the field populations of the reniform nematode.

Table 3. Syngenta product, formulation code, the active ingredient and formulation concentration of products used in two field trials investigating the effect of two products on reniform nematode population in the soil when planted to cotton

PRODUCTS	Formulation Code No.	Active Ingredient	Formulation Concentration
Vibrance CST	A-21606-B	75 g/L azoxystrobin + 75 g/L metalaxyl-M + 12.5 g/L fludioxonil + 35 g/L sedaxane (197.5 FS)	197.5 g/L
A-21616-A 600FS	A-21616-A	600 g/L fluopyram	600 g/L
EXF10670V 500FS	EXF10670V	500 g/L SYNSTN1	500 g/L
Cruiser 600FS	A-9765-N	600 g/L thiamethoxam	600 g/L

Treatments

Following 6 treatments were applied in this field trial:

T1 = Vibrance CST 200 ml (Fungicide: Pythium, Rhizoctonia)
T2 = Vibrance CST 200 ml, Cruiser 460 ml (Insecticide)
T3 = EXF10670V 500 ml, Vibrance CST 200 ml, Cruiser 460 ml
T4 = EXF10670V 700 ml, Vibrance CST 200 ml, Cruiser 460 ml
T5 = EXF10670V 900 ml, Vibrance CST 200 ml, Cruiser 460 ml
T6 = A-21616-A 500 ml, Vibrance CST 200 ml, Cruiser 460 ml

3.1.3 Investigate the biological product against reniform nematode.

A field trial was conducted to test the effect of a biological product containing *Bacillus* species called FertiLink on reniform nematode on a cotton farm at Theodore. Its effect on reniform nematode was tested when applied pre-plant at three rates (100 ml, 200 ml and 300 ml/ha). The population of the reniform nematode at the end of the season was measured in the soil from the top 15 cm of the soil profile.

Milestone 4. Research question: Can we adopt information from the US into the Australian system?
4.1 Are nematodes in Australian cotton systems the same as in the US?

4.1.1 Study the genetic diversity of the reniform nematode.

Rotylenchulus reniformis is a major pathogen of cotton throughout the US. In Australian cotton, reniform has only been detected in Central Queensland and is common within horticultural cropping in Queensland north of Bundaberg. Reniform nematode populations collected from different host crops were tested for their genetic diversity in collaboration with researchers at the University of Queensland. Soil from various locations and crops were collected in Queensland such as Bowen tomatoes, Mareeba coffee, Gumlu melons, North Queensland bananas, Bundaberg sweet potatoes, and Emerald/Theodore cotton. Nematodes were extracted from soil and *R. reniformis* juveniles were individually picked, placed into 95% ethanol and frozen. The molecular analysis of these reniform populations was carried out by Dr. Dean Brooks at UQ.

Available molecular markers

Ribosomal RNA genes (rRNA - 18S and 28S) and their intergenic spacers (ITS1 and ITS2) are the primary molecular data that have been used to compare the genetic diversity of reniform nematode (Agudelo et al., 2005, Nyaku et al., 2013, Palomares-Ruis et al., 2018). The 18S and 28S genes are the most appropriate to use for comparisons internationally because they will be impacted less by population-specific factors.

The most comprehensive rRNA data available come from China but from an unpublished data set (Deng and Zheng unpublished). Some microsatellite loci have been used in the US (Arias et al., 2009, Leach et al., 2012) but using a population genetics approach (such as microsatellites) to compare individuals between continents is inappropriate. Some mitochondrial sequences (of the COI gene) are also available from the US and Japan.

A simple comparison of the genetic diversity of Australian reniform nematode and those found internationally is difficult because the rRNA genes used in most studies have two highly divergent gene duplicates that are present within each individual nematode (Nyaku et al., 2013) and these duplicates may each have further duplications. The two rRNA duplicates are amplified simultaneously by the primers most commonly used for reniform rRNA genes and may have led to the overestimation of the genetic diversity found in the reniform nematode, while also missing any genetic diversity that is actually present.

Samples and DNA extraction

DNA was extracted from 96 individual reniform nematodes using a REDExtract-N-Amp Tissue PCR Kit (Sigma-Aldrich Co.) as per the methods of Leach at al. (2012). Sixteen nematodes were used from the following host plants; cotton, banana, coffee, sweet potato, quinoa, and tomato. We also tested a Chelex extraction method and a DIY spin column extraction method, but neither of these approaches resulted in enough DNA for PCR analysis. The REDExtract-N-Amp kit yielded about 15ng of DNA from each individual nematode, which is a low amount but enough for PCR analysis.

Primer design and testing

We designed a number of new primer pairs to a) avoid the issues that are common among past studies that looked at the genetic diversity of reniform nematode, and b) still make comparisons with existing international data possible. For this purpose the new primers were designed to independently target each rRNA duplicate (A and B copies) at the following regions; the 28S gene and the intergenic spacers ITS1 and ITS2. New primers targeting the 28SA gene and the ITS2A intergenic spacer gave the best results and were used for further analyses. We also obtained new COI primers from a researcher in Europe, and these primers have successfully amplified the COI gene region for other *Rotylenchulus* species.

Although the new primers did successfully amplify the gene regions of interest for reniform nematode they still require some optimization before they can be published. The primers and protocols outlined by Van Den Berg et al. (2016) are the best-published primers available presently.

PCR amplification

A total of 27 individuals amplified with strong PCR bands. Individuals that successfully amplified underwent Sanger sequencing (Macrogen, Korea) at the 28SA and ITS2A gene regions. Of the samples sent for sequencing, high-quality sequence data were obtained for 18 individuals at the 28SA gene region and 13 individuals at ITS2 (Table 4). Partial sequences were obtained for others but these were not used for comparisons with international samples. Four sequences associated with COI were also obtained during initial testing of these new primers from reniform associated with sweet potato.

Table 4. Final sequences by the host plant

Host Plant	28SA	ITS2A	COI
Cotton	4	1	-
Banana	4	3	-
Sweet potato	4	7	-
Coffee	6	2	4

The low PCR success was probably because of PCR inhibitors present in the reaction that originate from the soil nematodes were sampled from. Individuals that failed PCR amplification did so for every gene they were tested with and this indicates that the problem is with the sample itself and not with the primers. We tried purifying nematode samples using magnetic PCR Clean DX magnetic beads after extraction but this did not increase the PCR success rate. This is a methodological hurdle that needs to be resolved before any population-level genetic studies can be undertaken for reniform nematode although it does not impact the higher-level comparisons being made with international reniform sequences here.

Results

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

Milestone 1. Research question: Where in the soil profile are reniform nematodes?

1.1 What is the epidemiology between cotton roots and rising reniform numbers in the soil profile?

1.1.1 Examine the vertical movement of the reniform nematode in vertisol.

A glasshouse trial was conducted to study the vertical movement (both upward and downward) of the reniform nematode in vertisol soil. Analysis of the counts of nematode in the root tissue and soil indicated that there is limited movement of reniform in the control pots (without any plant) and sorghum pots, while substantial movement within the cotton pots upward from bottom inoculation points have been found. Reniform has been found to infect only cotton roots (Fig. 6) but not the sorghum roots.

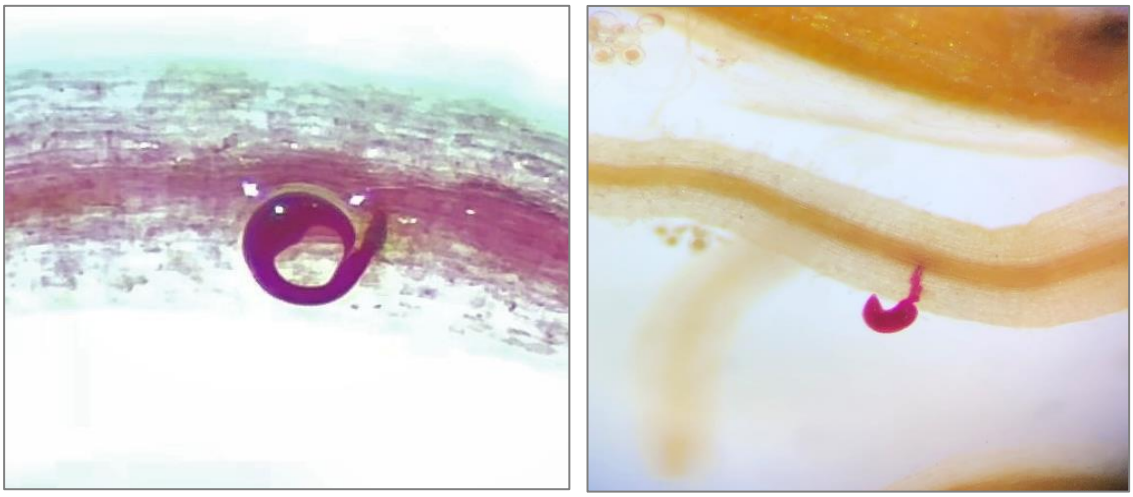

Figure 6. Female reniform nematode (stained purple) feeding on a cotton root

***Control (No plants)*:** In the treatment without any plants, most of the reniform were found in the inoculated section (Fig. 7) which indicates there was no or very little movement of nematodes in the absence of host plant.

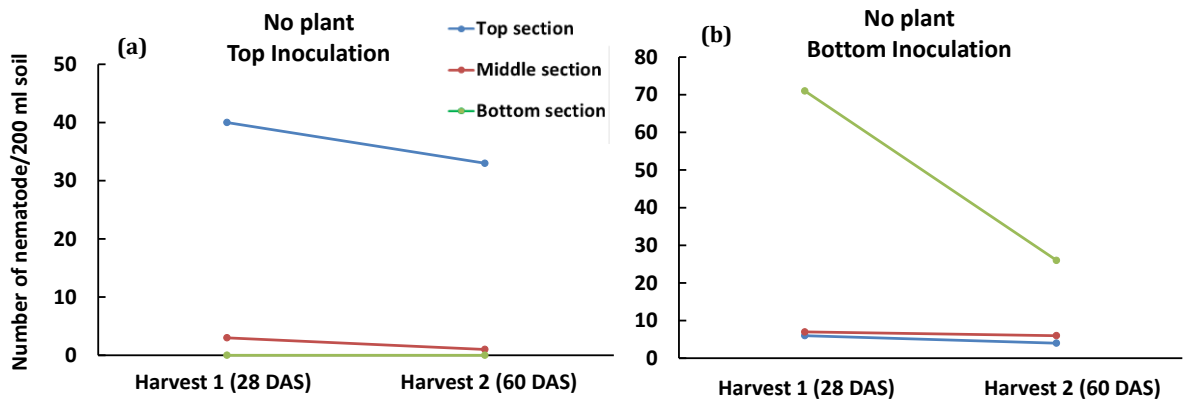

Figure 7. The number of reniform nematodes found in the top, middle and bottom sections of soil in the pots without any plants a) when inoculated with reniform nematode at the top of the pot, b) when inoculated with reniform nematode at the bottom of the pot.

***Sorghum*:**

Nematode on sorghum root tissue: Reniform nematode was not found in the roots of sorghum plants.

Reniform nematodes on sorghum soil: In the soil of the sorghum plant, reniform was found mostly in the inoculated sections and there was no increase in the population of reniform. This confirms that the sorghum is a non-host (Fig 8).

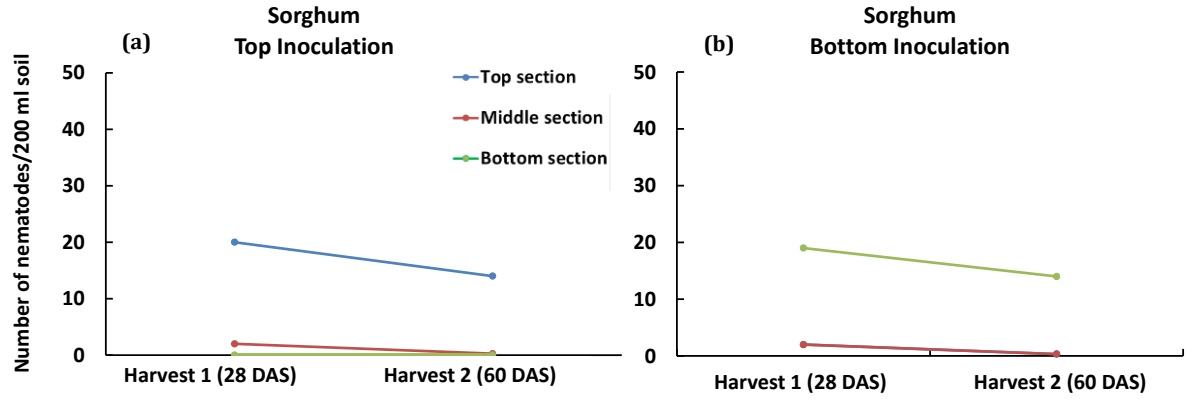

Figure 8. The number of reniform nematodes found in the top, middle and bottom sections of soil in the pots with sorghum plants a) when inoculated with reniform nematode at the top of the pot, b) when inoculated with reniform nematode at the bottom of the pot.

Cotton:

Nematode on cotton root tissue: The top inoculated nematode stayed on the top section while the bottom inoculated nematode moved upward toward the top section. It is likely that the reniform juveniles can sense the food source (root tissue) and therefore headed towards it at the earlier stage of plant growth (Fig. 9).

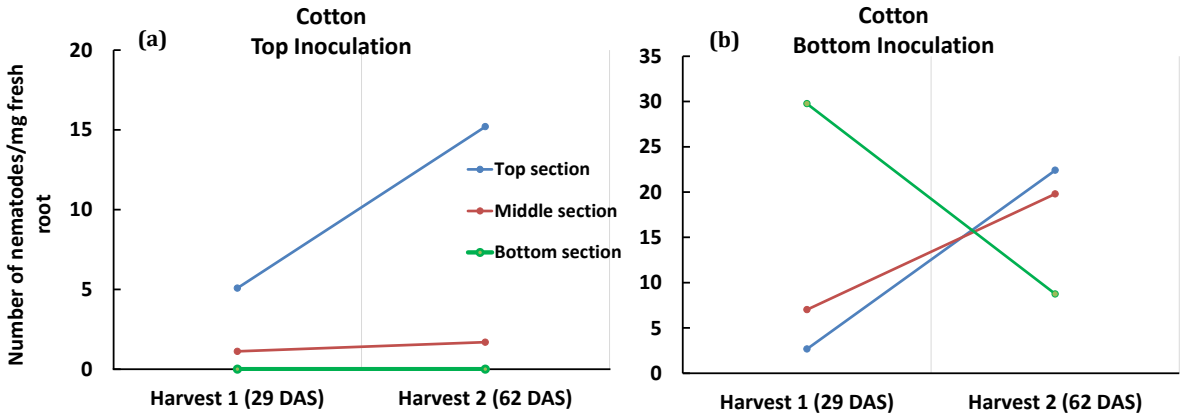

Figure 9. The number of reniform nematodes found in the root tissue of cotton plants located in the top, middle and bottom sections of soil a) when inoculated with reniform nematode at the top of the pot, b) when inoculated with reniform nematode at the bottom of the pot.

Nematode on cotton soil: When inoculated on the top section, most of the nematodes did not move while few moved to the middle section (Figure 10). On the other hand, bottom inoculated nematodes moved towards the top section. Also, there was a large increase in the nematode population indicating successful and significant reproduction during the period of two months.

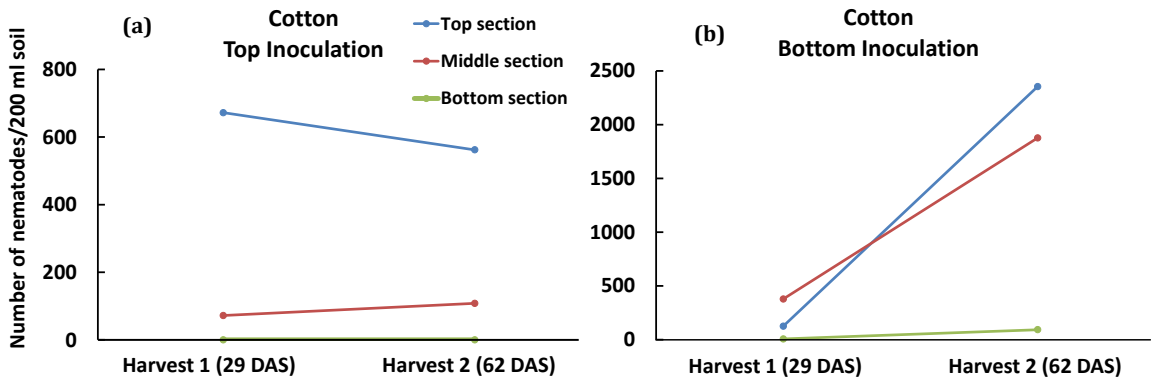

Figure 10. The number of reniform nematodes found in the top, middle and bottom sections of soil in the pots with cotton plants a) when inoculated with reniform nematode at the top of the pot, b) when inoculated with reniform nematode at the bottom of the pot.

1.1.2 Assess the reniform population at depth.

The field survey was conducted to examine the nematode population at various depths in the various growing seasons. A large number of nematodes were found in all the depth of soil which varied between fields in early season (2018/2019) samples.

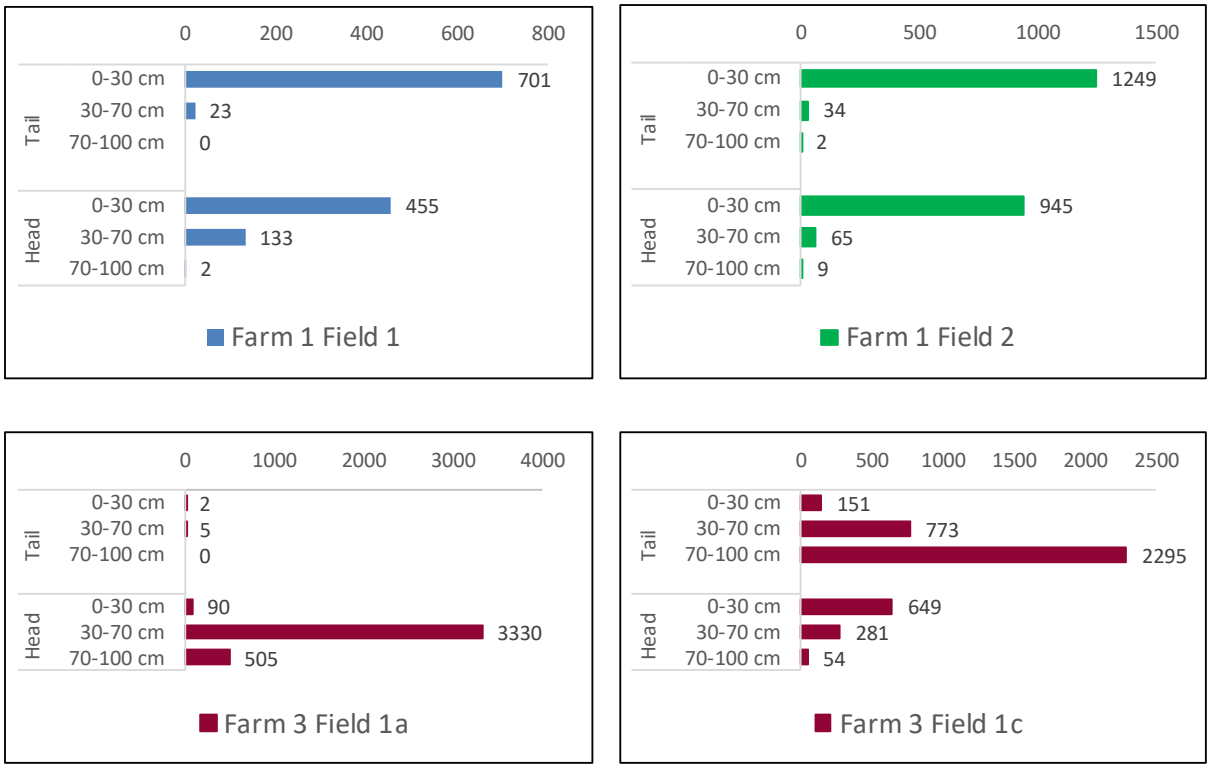

Figure 11. The number of nematodes found in three different depths (0-30 cm, 30-70 cm, and 70-100 cm) of the soil of four different fields of two farms. The 'Head' represents the samples taken from the head ditch side while the 'Tail' represents the samples taken from the tail drain side.

Milestone 2. Research question: What is the potential risk to the rest of the industry?

2.1 What are the constraints to reproduction in Australian cotton soils for reniform and root-knot nematodes?

2.1.1 Conduct seasonal soil sampling to assess the presence, absence, and severity of the reniform nematode.

In the 2017/18 season, four out of five farms in Emerald and one farm in Moura, not previously assessed, were determined to have reniform nematode when sampled early season during disease surveys (Table 5). A follow-up assessment late-season using an extensive sampling protocol of fields to determine soil population detected reniform in five out of seven fields assessed (Table 5). The early season sampling has proven to be a useful tool for the detection of reniform nematode without undertaking an extensive sampling protocol.

No reniform nematode has been detected outside CQ.

Table 5. Soil sampling early and the late season for detection of reniform nematode

		Early season survey sample 2017/18		Follow-up field population sample late season			
Farm/Field	Location	Sample date	Rr/200 ml	Sample date	Field section: Rr/200 ml		
Field 1	Emerald	31/10/2017	27	24/07/2018	A: 0	C: 205	E: 380
Field 2	Emerald	31/10/2017	2	24/07/2018	B: 38	C: 168	D: 85
Field 3	Emerald	1/11/2017	2	24/07/2018	C: 2	D: 0	E: 0
Field 4	Emerald	1/11/2017	5	24/07/2018	D: 0	E: 0	F: 0
Field 5	Emerald	31/10/2017	0	Not sampled			
Field 6	Emerald	31/10/2017	20	24/07/2018	B: 7	D: 0	F: 61
Field 7	Emerald	31/10/2017	18	Not sampled			
Field 9	Emerald	31/10/2017	413	24/07/2018	A: 2	B: 16	C: 14
Field 10	Moura	1/11/2017	25	25/07/2018	A: 0	B: 0	
Field 11	Moura	1/11/2017	0	Not sampled			

2.1.2 Conduct a population trial to estimate the threshold level of reniform on cotton.

A glasshouse trial was conducted using three different population levels of nematode on three different cotton varieties.

Yield: Higher nematode inoculation significantly reduced the cotton yield in variety ‘Sicot 714’ ($F = 3.872$, $p = 0.02$) (Fig 12). Although not significant, a similar reduction was found in ‘Sicot 746’. Nematode treatment has no effect on the total number of open bolls (data are not shown).

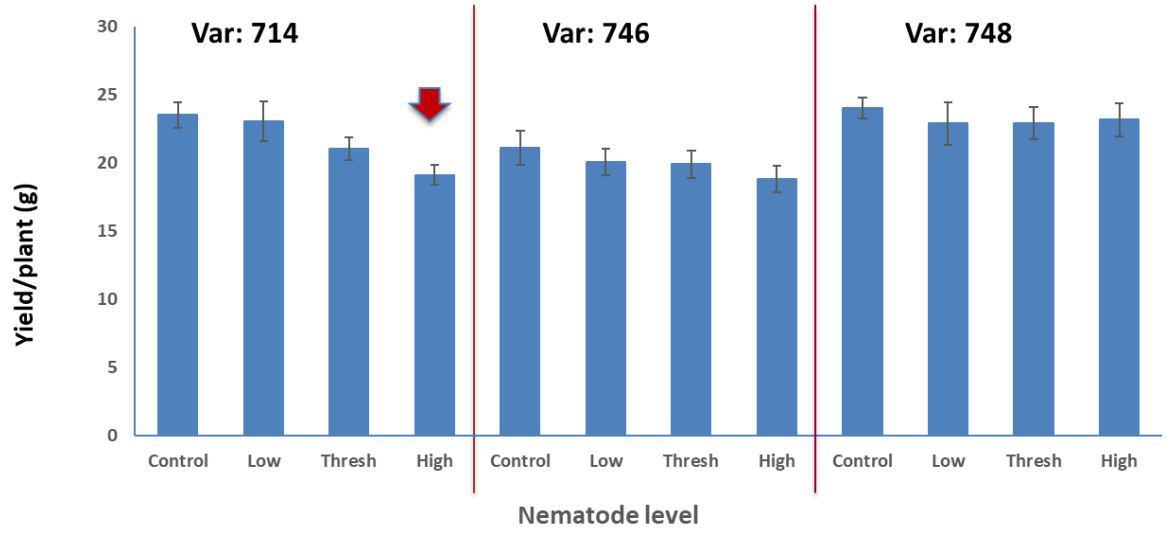

Figure 12. Cotton lint yield (with seed) per plant of three different varieties of cotton under different nematode inoculation levels (Control: no nematode, Low: 850 nematodes/pot, Threshold: 4450 nematodes/pot, High: 9350 nematodes/pot). Each pot contained 1.8 litre of soil. The red arrow indicates a significant reduction in the yield ($P<0.05$).

Shoot and root biomass: Higher nematode significantly reduced the shoot biomass of 714 ($F = 4.5$, $p = 0.01$) (Fig. 13) while nematode treatment has no effect on root biomass of any variety (data are not shown).

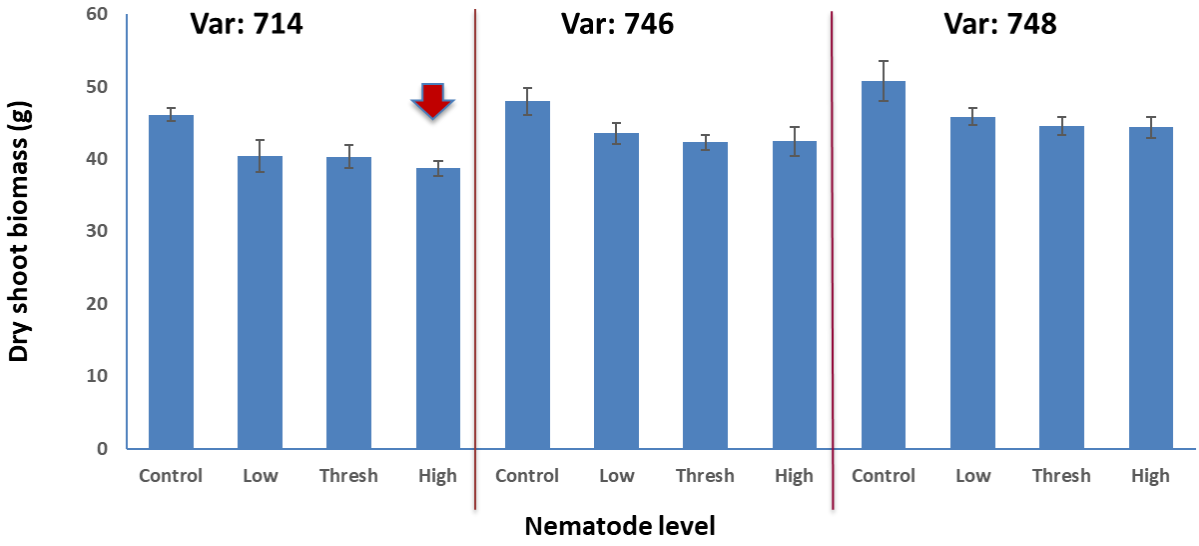

Figure 13. Dry shoot biomass (per plant) of three different varieties of cotton under different nematode inoculation levels (Control: no nematode, Low: 850 nematodes/pot, Threshold: 4450 nematodes/pot, High: 9350 nematodes/pot). Each pot contained 1.8 litre of soil. The red arrow indicates a significant reduction in the shoot biomass ($P<0.05$).

The number of open bolls: Nematode treatment has no effect on the total number of open bolls (Fig. 14). Irrespective of the treatment, variety 748 produced significantly more open bolls compared to two other varieties (Kruskal-Wallis chi-squared = 14.496, $p < 0.001$).

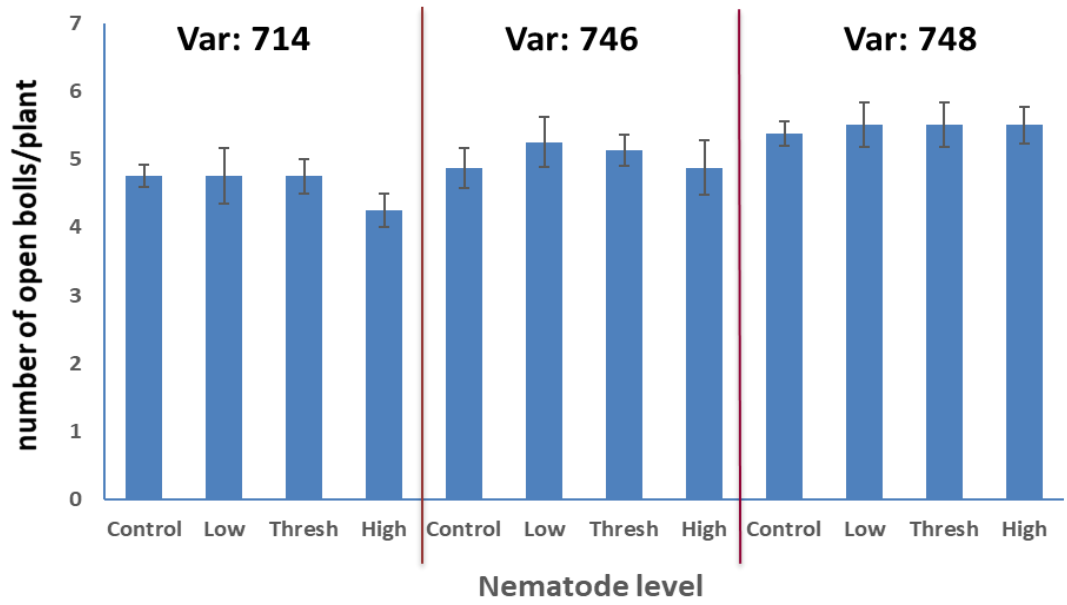

Figure 14. The number of open bolls per plant of three different varieties of cotton under different nematode inoculation levels (Control: no nematode, Low: 850 nematodes/pot, Threshold: 4450 nematodes/pot, High: 9350 nematodes/pot). Each pot contained 1.8 litre of soil.

Plant height: Plant height in different growth stages did not differ within the variety. Irrespective of the treatments, variety 748 was significantly taller compared to two other varieties at each point of the measurement (50, 85 and 185 DAS) (Fig. 15).

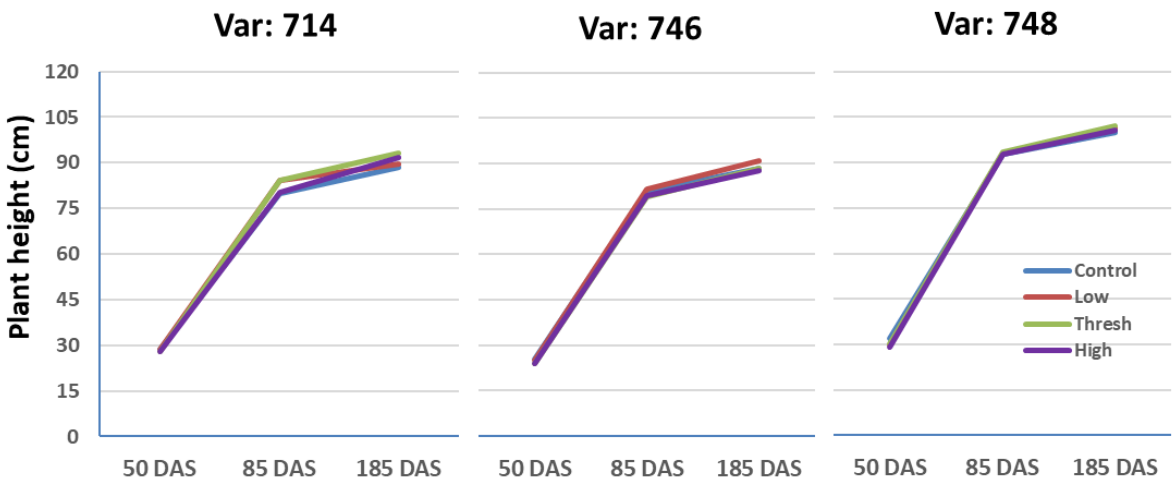

Figure 15. Average plant height of three different varieties of cotton under different nematode inoculation levels (Control: no nematode, Low: 850 nematodes/pot, Threshold: 4450 nematodes/pot, High: 9350 nematodes/pot) at three different growth stages (50, 85, and 185 days after sowing). Each pot contained 1.8 litre of soil.

Milestone 3. Research question: What management tactics can be used to manage reniform?

3.1 What crops are non-hosts and can they be incorporated into the farming systems?

3.1.1 Conduct a host/non-host trial to check if a potential rotation crop is a host or non-host of the reniform nematode.

A pot trial was conducted to test the host/non-host status of wheat, corn, forage sorghum, and grain sorghum. No infestation on any other crops except cotton indicating all the rest of the

crops are non-host of the reniform nematode. While analysing the soil sample, we found a great number of reniform nematodes in the soil containing cotton plants while the soil from other crops contained nil or only a couple of reniform nematodes Table 6).

Table 6: Average number of reniform nematodes found in 100 grams of dry soil. The average number of reniform smaller than one was rounded to one.

Crop	The average number of reniform/100 gram dry soil
Cotton	2150
Corn	1
Forage Sorghum	1
Grain Sorghum	1
Wheat	1

3.1.2 Investigate the nematicidal seed treatment to manage the reniform nematode.
A field trial was performed in Theodore to test the efficacy of Syngenta seed treatment products against reniform nematode.

Trial 1 Field 1

There was no significant difference between field populations pre-plant between treatments (Table 7). Mid-season, seed treatment T1 (Vibrance only) resulted in a significantly lower population of reniform nematodes per 200 ml of soil compared to T2 (Vibrance plus Cruiser). The absence of Cruiser meant that plants had less protection against soil pests such as soil-dwelling beetle larvae and pests that feed on the hypocotyl and cotyledons such as thrips and aphids, than seed treated with insecticide. It is possible that T1 plants may have been smaller due to the impact of insect pests resulting in lower root mass for reniform nematode to feed and reproduce, hence a lower population of reniform in the soil. Future trials should include assessment of plant growth. At harvest, there was no significant effect of seed treatments compared to seed treated with Vibrance or Vibrance plus Cruiser (Table 7).

Table 7. Field 1: The effect of seed treatments on average population of reniform nematode pre-plant, mid-season and harvest data per 200 ml soil

Treatment	**Pre-plant 13/9/2017**	**Pre-plant** **Log10 (No. nematodes/200ml + 1)**	**Mid-season 19/12/17**	**Mid-season** **Log10 (No. nematodes/200ml + 1)**	**Late-season 27/3/2018**	**Late-season**
T1 Vibrance CST 200 ml	31.3	1.382	366.5	2.134 a	5820	3.743
T2 Vibrance CST 200 ml, Cruiser 460 ml	90.0	1.756	645.7	2.787 b	5843	3.750
T3 EXF10670V 500 ml, Vibrance CST 200 ml, Cruiser 460 ml	80.3	1.862	846.5	2.894 b	6686	3.785
T4 EXF10670V 700 ml, Vibrance CST 200 ml,	90.0	1.598	1137.3	2.951 b	6023	3.759

Cruiser 460 ml						
T5 EXF10670V 900 ml, Vibrance CST 200 ml, Cruiser 460 ml	49.3	1.481	587.0	2.618 ab	5966	3.730
T6 A-21616-A 500 ml, Vibrance CST 200 ml, Cruiser 460 m	58.5	1.662	526.8	2.569 ab	5966	3.728
L.S.D (P=0.05)		NS		0.4883		NS

Trial 2 Field 2

There was no effect of seed treatments on soil populations of reniform nematode in trial 2 mid or late-season (Table 8). Decrease in population late-season compared to mid-season was consistent across all treatments and was likely due to the soil drying down in the top 15cm and possible movement of reniform deeper into the soil profile where the soil was moist.

Table 8. Field 2: The effect of seed treatments on the average population of reniform nematode pre-plant, mid-season and harvest data per 200 ml soil

Treatment	**Pre-plant 13/9/2017**	**Mid-season 20/12/17**	**Late-season 27/3/2018**	**Late-season Log10 (No. nematodes/200ml + 1)**
T1 Vibrance CST 200 ml	54.2	885.5	782	2.884
T2 Vibrance CST 200 ml, Cruiser 460 ml	78.3	795.0	701	2.8
T3 EXF10670V 500 ml, Vibrance CST 200 ml, Cruiser 460 ml	116.3	1152.2	868	2.885
T4 EXF10670V 700 ml, Vibrance CST 200 ml, Cruiser 460 ml	82.5	1282.5	699	2.83
T5 EXF10670V 900 ml, Vibrance CST 200 ml, Cruiser 460 ml	79.7	1082.5	762	2.842
T6 A-21616-A 500 ml, Vibrance CST 200 ml, Cruiser 460 m	83.3	840.7	841	2.834
L.S.D (P=0.05)	NS	NS		NS

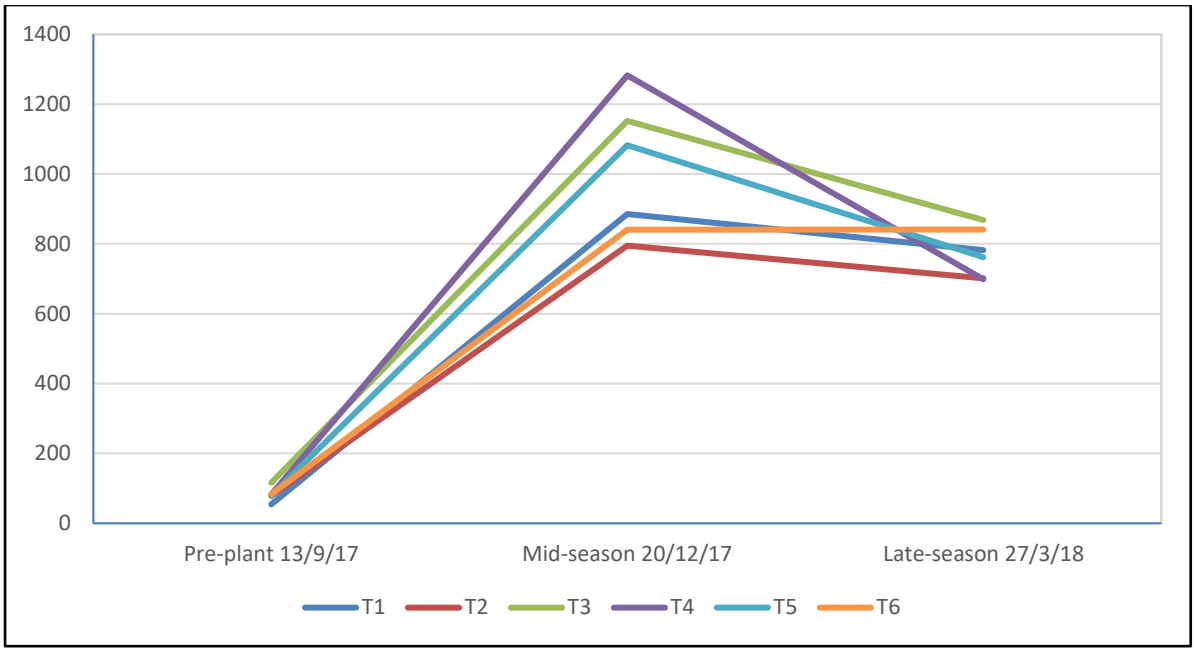

Figure 16. Treatment effect on reniform population/200 ml soil over three time periods

3.1.3 Investigate the biological product against the reniform nematode.

A field trial was conducted to test the effect of a biological product containing *Bacillus* species called FertiLink on reniform nematode. There was no significant effect on the population of reniform nematode at the end of the season when measured as *Rotylenchulus reniformis*/200 ml soil in the top 15 cm (Table 9).

Table 9. The effect of FertiLink treatments on the average population of reniform nematode (*Rotylenchulus reniformis*) pre-plant and end of season data per 200 ml soil.

Treatments	Pre-plant Avg. number of Reniform/200 ml soil	End of Season Avg. number of Reniform/200 ml soil
0 ml/ha	109	708
100 ml/ha	122	596
200 ml/ha	144	575
300 ml/ha	132	900
L.S.D.	NS	NS

Milestone 4. Research question: Can we adopt information from the US into the Australian system?

4.1 Are nematodes in Australian cotton systems the same as in the US?

4.1.1 Study the genetic diversity of the reniform nematode. Reniform nematode populations collected from different host crops were tested for their diversity in collaboration with researchers at the University of Queensland.

Our results indicate that the reniform nematodes in Australia are typical of those associated with cotton systems in the US and elsewhere in the world. Although we can broadly conclude that Australian reniform nematodes do not differ genetically from those found in the US, there are suggestions in the literature of biological differences between reniform nematode populations that should not be ignored and the parthenogenetic Japanese strain at least is highly divergent and separate from other reniform (McGawley et al., 2010, Adam et al., 2019).

The conclusions that can be drawn from the genetic data we have are limited by the low genetic resolution of the methodologies that have been employed internationally and duplication of the genes that are used to compare reniform. The application of more comprehensive and modern genomic approaches could reveal additional genetic diversity in reniform nematode that does relate to differing degrees of pathogenicity. We have made some headway toward providing better molecular tools to investigate the genetic diversity of reniform nematode but the full development of these tools was outside the scope of this project.

Phylogenetic comparisons

We compared our results with all international sequences for the 28S (A copy), ITS2 (A copy) and COI genes that were available from online databases. No genetic variants were found for any gene region in Australian samples that were not also found in reniform nematode internationally. No genetic variation within Australia was uniquely associated with one host plant. Australian sequences fit within the global genetic variation of reniform nematode (Fig. 17 and 18) and look to be typical of a single species gene pool.

We found two divergent genetic variants within the A copy of the ribosomal RNA genes (28S and ITS2) of Australian reniform nematode that warrant further investigation (Figure 18). These variants are found together within some individuals but not in others. The first genetic variant is typical of reniform nematode found globally, and the second genetic variant is only represented in sequences from China (Deng and Zheng unpublished). Further investigation is required to determine whether these rRNA variants are biologically meaningful as it is possible that these are further copies of rRNA genes within the 28SA variant which is a common pattern in other nematodes (Qing, 2019).

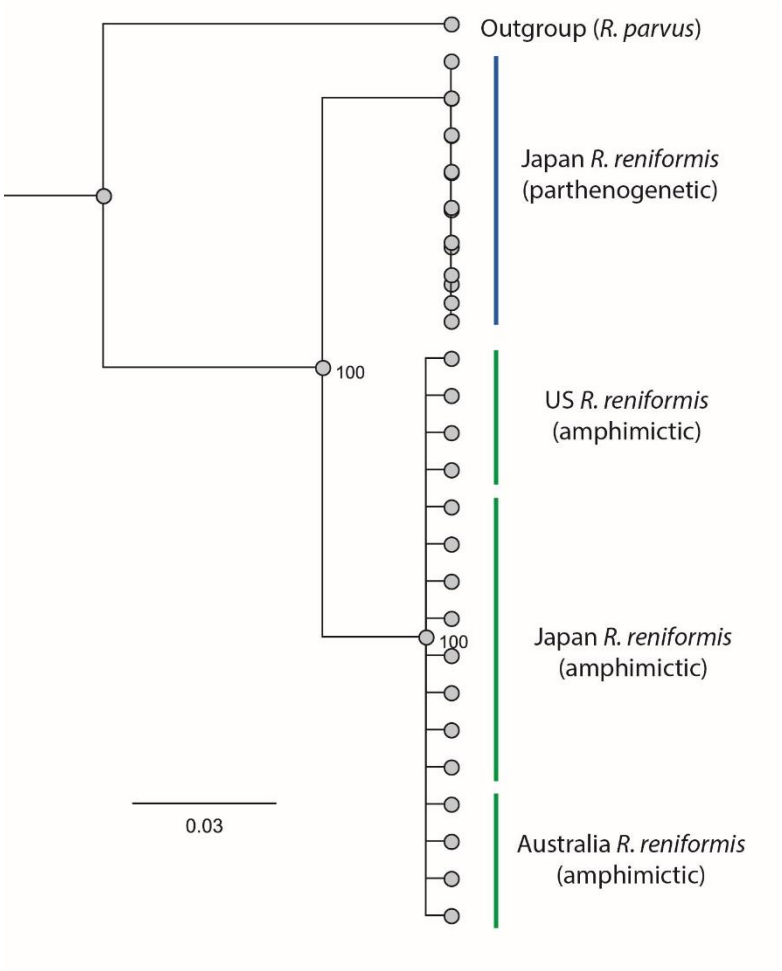

Figure 17. Maximum likelihood phylogenetic analysis showing the relationship between Australia's reniform nematode and those from the US and Japan at the COI mitochondrial gene region. Although few samples are available, this gene region has no problems with the gene duplications found in the rRNA genes.

Figure 18. Maximum likelihood phylogenetic analysis showing the separation of the two Australian reniform 28SA variants. Sequences obtained from Genbank have their tip labelled by their Genbank accession number. Sequences from other countries are mostly from China.

Outcomes

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

Outcome 1: Early-season deep core samples from the field shows the variable abundance of reniform nematode in the soil profile. In some fields, they are concentrated in the top 30 cm while some have a large population in 30-60 cm below the surface. Interestingly, in some fields, the largest population of the reniform was found at the depth of 60-100 cm below the soil surface. These results clearly show that the reniform can live and survive deep in the soil profile. As the results of the vertical movement pot trial suggest, they move towards the food source (cotton roots) once the cotton seedling starts to grow. Root exudates or plant root volatile emitted by cotton root may be the cues that reniform nematode can sense to locate the root.

Outcome 2: The regular field survey has provided information about the prevalence and spread of the reniform nematodes in Australian cotton fields. So far, reniform nematodes have been found in the cotton field in Central Queensland only. The population trial has provided some interesting results on the varietal response of cotton plants. Higher nematode inoculation had a significant negative impact on the plant's growth and the yield on variety 'Sicot 714'. This indicates the reniform nematode may significantly reduce the crop yield if the number of nematodes in the soil reaches a certain threshold. It also indicates that different cotton varieties may have different thresholds for reniform. As these results were obtained from the greenhouse pot trial, they may not represent the field condition. Detail field trials are needed to test these results in the actual field condition.

Outcome 3: Host/non-host trial has clearly shown that the rotation crops such as corn, forage sorghum, grain sorghum, and wheat are non-host of the reniform nematode. There was not any root infestation by reniform nematode on these crops. Interestingly, the number of reniform nematodes found in the soil of these crops was close to zero compared to cotton soil. As the reniform nematodes are known to survive in soil without host plants for an extended period of time, such a reduction in their numbers in non-host soil is an interesting finding. Field trials conducted to test the commercial product with nematicidal properties or biological control agents has yielded no effect on the soil nematode population. Thus, crop rotation seems to be the only option to manage the reniform population in the field.

Outcome 4: The genetic diversity study has shown that the reniform nematodes found in different crops across Queensland are not different from each other. This is concerning as the reniform nematode has positively been identified in other crops in Gatton (South East Queensland), which may pose a great threat to the cotton field outside central Queensland. As they are genetically identical population, cross-contamination of the reniform nematode to the cotton field from other crop is possible although it has not been confirmed if the reniform in other crops are virulent to cotton. This study also confirmed that the Australian reniform population is not different than the international population which means we are not dealing with any different reniform, therefore, the management recommendations from abroad can also be practiced in Australia.

4 Please describe any:-

a) Technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);

- None

b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and

The root staining procedure developed by Byrd *et al.* (1983) was modified to a simpler procedure which enabled us to successfully stain multiple root samples at once for quantification of adult reniform nematode and their egg masses.

c) required changes to the Intellectual Property register.

- None

Conclusion

5 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?

Key outcomes that will have a significant impact on cotton production are:

- The reniform nematode has the potential to cause significant yield losses to cotton production. In a greenhouse trial, it has significantly reduced the yield of cotton variety Sicot 714. It is important to remember that this was a pot trial conducted in the glasshouse condition, thus this result may not represent the field condition.

- The reniform nematode is abundant in the soil profile up to a metre of depth. In some fields, the deeper soil profile (60-100 cm below soil surface) had higher numbers of nematodes compared to the upper soil profile.

- The glasshouse trial showed they move towards the cotton root from the deeper soil profile.

- Planting a non-host crop such as corn, sorghum (forage or grain), and wheat is a good strategy to reduce populations of nematodes in the soil. A greenhouse trial showed that these are non-host of reniform nematodes and help reduce the reniform number in the soil. This trial was conducted in the greenhouse settings, thus a field trial with similar treatment needs to be conducted to confirm these results in the field.

- The reniform nematodes found in different crops across Queensland are genetically similar. They are also not different from the international population.

- The overall trend is of increasing reniform populations, which is commonly associated with back-to-back cotton.

- Varieties with resistance are needed.
- Farm hygiene is extremely important to reduce the risk of introducing reniform into other regions.

Extension Opportunities

6 Detail a plan for the activities or other steps that may be taken:

(a) to further develop or to exploit the project technology.

(b) for the future presentation and dissemination of the project outcomes.

(c) for future research.

This project has provided an overview of the epidemiology of the reniform nematode in Australian cotton. Although there is no separate funding to conduct any scientific study on reniform this year, regular field surveys to monitor the reniform population, their presence or absence in the Australian cotton field will continue. As few glasshouse trials have been conducted and some interesting results have been found, it would be worthwhile to test these findings in the field. Some new research ideas for the future could be:

1. Testing different rotation crops in the field to see if they really help to lower the reniform population by killing them or by pushing them towards deeper soil profile.
2. Testing if the cotton plants can be primed using biotic or abiotic products to make them more resistance to reniform.
3. Testing different cotton varieties to see if they differ in their response to the reniform nematodes in the field condition.

7. 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)

1. A talk titled "Reniform Nematode – An Update" was jointly presented by Tim Shuey and Dinesh Kafle at FUSCOM, June 2018 in Griffith, NSW.

2. A Three Minute Talk (3MT) titled "The genetic diversity of reniform nematode" was presented by Tim Shuey at the Australian Cotton Conference, August 2018 in Gold Coast, QLD.

3. A talk titled "The effect of cropping regime on field populations of reniform nematode (*Rotylenchulus reniformis*) in cotton soils in Theodore, Queensland" was presented by Linda Smith at the Australasian Soil-borne Diseases Symposium, September 2018 in Adelaide, SA.

4. A talk titled "The effect of cropping regime on field populations of reniform nematode (*Rotylenchulus reniformis*) in cotton soils in Theodore, Queensland" was presented by Linda Smith at the Australasian Soil-borne Diseases Symposium, September 2018 in Adelaide, SA.

5. A talk titled "Reniform Nematode Update" was jointly presented by Linda Smith and Dinesh Kafle at the Grower's meetings at Theodore (QLD) and Emerald (QLD) in July 2019.

6. A talk titled "Understanding the ecology of reniform nematodes in Australian cotton" was presented by Dinesh Kafle at the Australian Cotton Research Conference, October 2019 in Armidale, NSW.

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

- None

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 internet. 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.

Since the first detection in the Dawson/Callide region of Central Queensland in 2012, reniform nematode has become a serious concern for cotton growers and researchers as well. Although reniform is considered one of the major diseases of cotton in the US, it is still considered a minor problem in the Australian cotton industry due to the limited scientific studies of the epidemiology of reniform in the Australian cotton cropping system. This project had a number of objectives to address knowledge gaps and obtain data to understand how the reniform nematode is interacting with cotton plants in Australian soil so that we can improve the management practice.

In this project, large numbers of cotton fields from both NSW and QLD have been monitored every season to confirm the presence or absence of plant-parasitic nematodes, and to provide an indication of possible nematode problems in the field. To understand the ecology of reniform nematode in Australian cotton, three glasshouse pot trials were conducted specifically to assess the vertical movement reniform nematodes in the vertisol soil, host/non-host suitability to different crops for reniform nematode, and effect of different reniform nematode population on growth and yield of different cotton varieties. The genetic diversity of the reniform populations found in different crops including cotton was compared to each other while it was also compared with international isolates. Additionally, three different field trials were conducted during this project period to investigate the effectiveness of seed treatment products (with nematicidal property) and a biological control agent to control the reniform nematode.

The seasonal field survey has provided information about the prevalence and spread of the reniform nematodes in Australian cotton fields. So far, reniform nematodes have been found in the cotton field in Central Queensland only. Early-season deep core samples (2018/2019 season) from different fields in Theodore shows the variable abundance of reniform nematode in the soil profile. In some fields, they were most abundant in the top 30 cm while

some fields had a large population in the 30-70 cm below the surface. Interestingly, in some of the fields, the highest population was found at the depth of 70-100 cm below the soil surface. These results clearly show that the reniform can live and survive deep in the soil profile thereby providing a reservoir of nematodes that may reinfest the planting zone when cotton is sown. The vertical movement pot trial confirmed that the reniform nematode can move upward from deeper soil profile in the presence of a suitable host (cotton) once the seedling starts to grow.

The reniform population trial has provided some interesting results on the varietal response of cotton plants. Although all the varieties were treated similarly, reniform had a direct negative effect on growth (shoot biomass) and yield of variety 'Sicot 714' while 'Sicot 746' and 'Sicot 748' were not affected. This indicates the reniform nematode may significantly reduce the crop yield if the number of nematodes in the soil reaches a certain threshold and different cotton varieties may have different thresholds for reniform. The host/non-host trial has provided evidence that the rotation crops such as corn, forage sorghum, grain sorghum, and wheat are non-host of the reniform nematode. These crops were not infested by a reniform nematode, and interestingly, the reniform population in the soil of these crops was dropped to almost zero. Thus, crop rotation using any of these crops would be a good option to manage the reniform population in the field. It would be worthwhile to conduct a detailed field trial to evaluate whether similar results can be achieved in the field.

In the field trials, commercial seed treatment products with nematicidal properties had no effect on the reniform population in the soil. Similarly, another field trial with a biological product containing *Bacillus* species named FertiLink showed that this product has no effect on the nematode population in the soil.

The genetic diversity study has shown that the reniform nematodes found in different crops across Queensland are not different from each other. Although it is not clear if the nematodes on other crops are virulent to cotton, they may pose a great threat to the cotton field because of the cross-contamination of reniform nematodes from other crops to the cotton field. This study also confirmed that the Australian reniform population is not different than the international population, therefore the management practices from abroad can also be recommended in Australia.

The research results obtained during this project have been widely disseminated throughout the industry through presentations at different conferences and grower's meetings.

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