FINAL REPORT

For Public Release

Part 1 - Summary Details

CRDC ID: UNE1403

Project Title: Professor of Soil Systems Biology, UNE Armidale

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Research Program: 1 Farmers

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Date submitted: ___26 September 2019__

Part 3 – Final Report

(The points below are to be used as a guideline when completing your final report)

Background

1. Outline the background to the project.

Understanding soil processes is vital to the continued development of sustainable cotton production, both from an intensive farm and natural resource management prospective; all within a changing climate and resource limited framework. Key drivers in developing this understanding are soil biological processes, covering roots and microbial dynamics and how they interact within the overall framework of soil and resilient crop production practices. New technologies have assisted in uncovering the myriad of microbial species living in soil. However, whilst we are becoming reasonably proficient at counting soil microbes, our understanding remains limited in terms of how potentially beneficial microbes aid in the sustainable production of cotton with consideration of wider environmental benefits, and of how specific cotton management practices affect microbial populations .

We have little in the way of proficient indicators with which we can link crop production and ecosystem health, build organic matter in cropping systems and how to measure resilience within cotton cropping. Soil biology, with the exception of some pathogenic species, remains a key unknown in all crop production systems. This jointly funded position, in Soil Systems Biology, reflected the recognition that the activity of soil microbes and roots operate within a complex soil environment, influencing the physics (water use efficiency) and chemistry (nutrition) of the soil. The proposed project recognised that the potential to deliver an improved understanding of the role of the soil biology for agronomic benefits, such as improved water, fertilizer and energy use efficiency (CRDC Strategic R&D Plan 2008-2013. The quest or sustainable competitive advantage), could only be achieved by looking at soil within a systems context and not in isolated disciplines. Hence, the use of the term 'Soil Systems Biology'. The foci of the research was on transformational processes that had the potential to make advances and improvement in cotton production and associated ecosystem services.

To provide a driver for the development of a suite of relevant research initiatives to address these soil biology issues, UNE collaborated with CRDC on developing a full time position in the area of Soil Systems Biology for Cotton Production. The position was funded 50:50 by UNE and CRDC, with an initial 5-year commitment from CRDC. The position was advertised as 80% research, 10% teaching and 10% extension, the latter involving community/industry engagement. In addition to hosting the research position, UNE integrated this position with the developing Cotton Hub at UNE, co-ordinating cotton research within UNE in relation to CRDC requirements.

In 2017 the project, UNE1403, was merged with UNE1604 (UNE Cotton Production Course). The rationale for this was to retain Brendan Griffiths, in his role as the lead contributor to the delivery of the cotton courses, by alleviating some of the academic administrative task associated with the units' delivery. This was justified as Brendan's years of practical field experience as a consultant and grower could not be replicated by a University academic and were critical in providing the connection between the teaching and real world experiences and practices that are required to deliver the best education for the Cotton Production Course students and their future employees. Brendan also brought a skill set that would complement and facilitate some of the UNE1403 milestone delivery.

Objectives

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

1. Appointment - Dr Oliver Knox was appointed to the project April 22nd 2014.
2. Research Focus
2.1. Initial research focus agreed between UNE staff and CRDC in July of 2014.
2.2. Below ground root agronomy.
2.2.1. Rooting depth and Constraints. 2015/16, 2016/17 and 2017/18 season capacitance data was mapped. As part of a joint CRDC CRCSI project the data from the 2015/16 season was compared to satellite imagery. The area of irrigated crop showing signs of restricted rooting depth (not accessing soil nutrient and water below 60 cm) has ranged between approximately 20 and 30% over the three seasons analysed.
2.2.2. Nematodes and plant health. A survey of some of the fields sampled between 2004 and 2006 were revisited and nematode populations assessed. Number were still low, despite the removal of aldicarb (Temik™). The populations also appeared to be more environmentally stable. A paper was written on this work, but has not been accepted for publication. In the support offered to Dr Linda Smith, Linda and Oliver secured a PhD candidate to work on the reinform issues around Theodore, but sadly ill health meant the student had to withdraw only weeks in to their candidature and the project abandoned.
2.2.3 Exudates, pathogens and beneficials. Cotton exudates were produced in a hydroponic system in 2014/15. NMR analysis indicted an absence of carbohydrates, but some amino acids present, but this is thought to be due to the nonsterile nature of the system that was used. Compounds could not be identified from the volumes of exudates collected. The exudates were also used in a *Berkeleyomyces rouxiae,* formerly *Thelaviopsis basicola,* response trial.
2.3. Fertiliser use and management
2.3.1. Review of existing N work. Oliver provided support and advice on N management through grower meetings, the Nitrogen tour in 2016 and involvement with CottonInfo (See 2.3.3).
2.3.2. Drivers of N application rates. This milestone was removed due to the thesis on this topic being undertaken by Geraldine (Gerry) Wunsch. This allowed Oliver to develop more soil health support work as part of this and other projects.
2.3.3. Liaison between UNE staff and industry RDO on N use. Oliver provided trial advice, data analysis and wrote extension material on the REO N trials conducted on farm between 2015 and 2017.
2.3.4. P and K nutrition research. Oliver provided supervisory and paper writing support to Samieh Eskandari, who undertook a thesis looking at mycorrhizal importance to cotton in sodic soils. Oliver assisted Emma Longworth on her current Honours project looking at mycorrhizae in summer crops, which again implies the potential for mycorrhizae to be pathogenic. He has provided mycorrhizal assessment (not commercially available) to Steve Buster with his trials in the south and assisted Tim Weaver with his Sumitomo trial in 2017/18. The work on P and mycorrhizae is the basis of a follow on programme of work. Work investigating K nutrition has largely focused on student support for of Emily Young and Matt Maunder, both CRDC Summer Scholarships. Petiole testing for K was found to be unreliable in both experiments.
2.3.5. Microbiology and deep C. Links with the Carbon group at UNE were established as per the milestone and project UNE1601 was instigated. Most of Oliver's microbiological interests have turned to soil health. Oliver assisted Sally Dickinson in establishing #soilyourundies for the industry and has since been rolling it out to industry, schools and the general public.
3. Establishment of work plans.
3.1. Five year work plan. Work plans were established in consultation with CRDC in June 2014.
3.2. Operational plan for the Cotton Hub
3.2.1. The Cotton Hub at UNE was established and continues to interact with staff from across UNE.
3.2.2. Role and remit of the Cotton Hub. The Cotton Hubs primarily functions as a virtual network, the remit of which is knowledge exchange and dissemination of CRDC and other cotton industry news to approximately 25-30 staff and 6-8 PhD students across UNE. The Cotton Hub has a blog and is actively engaged in the portrayal of the cotton industry across several social media platforms.

3.2.3. The Cotton Hub coordination. Oliver has provided assistance and advice on grant responses to CDRDC EOI and has brokered partnerships within UNE and the wider scientific community in responding to some of the EOI calls.

UNE1403 merged with UNE1604 and activity for the cotton course reported at the November 2018 CRDC People forum.

Methods

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

Milestone 2.2.1

Rooting depth and Constraints

Capacitance data sets and remote analysis

An interrogation of Goanna telemetry's capacitance probe data sets was conducted with the view of locating capacitance locations with minimal or no root development below a depth of 60cm in the soil profile. Initially years, from 2012 – 2014 were investigated, however, these were largely drought years, but allowed some data characteristic to be developed around the amount of movement in a profile recording that was indicative of water extractions and therefore a surrogate for root activity. Data from the 2015/2016 formed the initial analysis with 2016/2017 and 2017/18 subsequently investigated.

A capacitance sensor uses an electronic current between two electrodes to measure the dielectric permittivity of the soil. The amount of moisture in the soil affects the frequency of the emitted current, which can be measured and converted into a measure of the total amount of moisture in the soil. The capacitance probe consists of up to 10 sensors in 10cm increments to 1 meter depth and has a data logger or modem to communicate results back to server. A solar panel and battery provide the power for the unit. The probe remains in the soil for the life of the crop with data normally captured at hourly intervals.

After the cotton growing seasons the complete datasets of the Goanna telemetry capacitance probes was interrogated. The investigation involved the development of an algorithm (John Bowman) to establish probes that were showing limited or no activity on those sensors below 60 cm during the period from the 20th Jan until 1st June each year. This period was chosen to allow sufficient time for the cotton root system to reach full development in all of the cotton growing regions included in the investigation.

Using probe GPS location from the 2015/16 field, Landsat satellite imagery was recovered and the pixel corresponding to the probe site and being closest to the field was selected for further analysis of the NDVI and SVI wavelengths. Probe sites, for which imagery could be recovered during the period of investigation, where then grouped by whether the capacitance probe data indicated rooting constraints or not. The wavelength profiles were then interrogated using ANOVA in Genstat to determine if there was a difference in the crop images as a result of root constraints.

Cone penetrometry and soil pit investigations

As part of the work conducted in this project, and also under UNE1601, the implications of field management, soil chemical condition and the resultant changes in soil physical condition were monitored using a CPII Cone Penetrometer (Rimik, Toowoomba) and a Vane Sheer (Pilcon, Derby, UK) at most field sites visited during the work period.

The cone penetrometer was fitted with a RTK GPS device, a 75 kg load cell and was used with a 75 cm shaft tipped with a 130 mm^2 displacement tip. Data was recovered and analysed using the Rimik Penetrometer File Reader. There is a single reference that cotton roots are impeded at a soil restrictive force of 2500 KPa (Taylor, Roberson & Parker 1966), although this has been standardised by Fritton (1990), the combination of visual and measured soil parameters would imply that this value is significantly higher, probably nearer to 4000 KPa or higher.

The vane sheer was used to map out soil pit faces, normally on something approaching a 30 cm gridded pattern with the 19 mm vane attached. Changes in shear readings, rather than interrogation of absolute values, were used to determine and highlight areas of compaction.

Milestone 2.2.2
Nematodes

Refinement of a method for passive nematode recovery from vertosols

A method was developed for the quick recovery of nematodes from vertosols. The development was compared to Baermann funnel recovery. The standardised method was to recover nematodes from 10 g of soil directly into 20 ml of Ludox, adjusted to a specific gravity of ~1.1 g/cm3, which after shaking and centrifugation, the supernatant could be poured through a 20 µm sieve from which the nematodes could be recovered. This method was comparable to Baermann funnel extractions, but could be undertaken in a fraction of the time and overcame the issues of resuspending pelleted soils of high clay vertisols.

The addition of Na(PO3)6 as a dispersant to alleviate the pelleting of the clay was found to significantly ($P < 0.001$) reduce recovered nematode numbers, which continued to fall as the percentage of Na(PO3)6 increased.

Enumeration of recovered nematodes from sequential Ludox at 1.1 g/cm3 extractions returned an average of 78% of the total recovered nematodes within the first Ludox extraction (s.d. 9.9, n = 6). This fell to 19% in the second recovery with only 2% in the third and final attempt. As such it was possible to theoretically recover 98% of the nematodes within a sample (s.d. 1%) with two Ludox extractions.

This method development was published:

Knox and Griffiths (2017) Communications in soil Science and Plant Analysis. Vol48, no. 3 pp316-325

This method was subsequently used by Elijah Wood when she undertook her GRASS placement and investigated free living population of nematodes with depth, using the long term rotation trial at ACRI.

Population change

Nematodes were also sampled in 2015 from two fields in the Namoi, which were initially interrogated in 2005 for nematode populations in 2005 to 2007 as part of a wider investigation into the interaction between nematodes and vertilcillium wilt (Knox *et al.*, 2006a, Knox *et al.*, 2006b). Although no relationship was established at that time, the low recovery of nematodes, by comparison with European, new Zealand and North American studies, was postulated as being due to the widespread use of aldicarb (Temik®) in the cotton system at that time. The nematode communities were assessed and compared between the sampling years. In all cases, field sampled soil was placed in plastic bags and returned in a chilled ice box to the laboratory. In the laboratory, the samples were sieved through a 2 mm sieve and a 300 g subsample was sent within 48 hours of samples being taken in the field to Biological Crop Protection (Moggill, Queensland, Australia) for nematode community analysis. Roots were collected during the sieving process and the root tissue was cleared using the NaOCl and stained with acid fuchsin method of Byrd *et al.* (1983). Roots were examined under a stereo microscope (20 to 45 x magnification) for the presence of nematodes.

Milestone 2.2.3
Exudates, pathogens and beneficials.

Mycorrhizae and sodic soil constraints

The work on beneficials undertaken on UNE1403 was primarily involved in the supervision of Samieh Eskandari and a few honours and taught Masters students. A lot of this work has had focus on myccorhizae. The methods undertaken for the majority of the work are available in the associated publications (see section 9 of this report).

Mycorrhizal root staining and colonisation

Other mycorrhizal work, including analysis for trials being run by Steve Buster near Griffith and Tim Weaver at Narrabri involved the following protocols.

Soil (~400g) is recovered from the plant line to a depth of about 20 cm using a poly-pipe modified to function as a coring pipe. Canola oil is often used to assist in core recovery from the coring pipe. Cores are returned to the laboratory in plastic bags and the soil is dispersed with the aid of water to which Calgon (sodium hexametaphosphate) has been added at ~2g/l. The soil is soaked for a minimum of an hour and agitated over this period of time. The saturated soil is then transferred to a bucket and agitated further with a jet of water. The soil is allowed to settle for a few seconds and

then the supernatant poured through a 250 um sieve. Recovered roots on the sieve are washed further then the sieve is place in a tray of water and the floating roots recovered to a labelled staining vessel. The vessel and roots are immersed in a 90°C solution of 10% (w/v) KOH and left for an hour. The cleared roots and staining vessel are then rinsed with water, neutralised with 2% HCL for 5 minutes and then transferred to the staining solution at 90°C for 20 minutes. Excess stain is rinsed from the roots with water and the roots recovered from the staining vessels and stored in acidified glycerol until root length colonisation is assessed under a dissecting microscope on a 1 cm gridded Petri dish.

Mycorrhizal DNA recovery and sequence analysis

Identification of fungal composition of the MycoApply product was undertaken by weighing out 1 g of the mycorrhizae in clay suspensions. To this was added 10 ml of Ludox at 1.1.g/cm3, vortexed for 1 minute and then centrifuged at 1000 X g for 5 minutes. The supernatant was recovered to a 10 ml Syringe and passed through a 0.45 um filter. The filter was recovered and the spores washed into 300 ul of sterile deionised water in a 1.5 ml Eppendorf.

200 ul of the recovered spore and hyphal suspension was added to a MoBio Power soil extraction tube to which 100 ul of 0.5 mm glass beads were added along with 250 ul of a solution of 0.2 M NaHPO4 and 0.5 M TRIS (pH 9) that had been filter sterilised with a 0.22 um filter. Three rounds of 60 seconds were then used to disrupt the fungal material prior to completion of the DNA recovery as per the manufacturer's instructions. Recovered DNA was quantified and sent for fungal metagenomic analysis at AGRF.

To recover fungal DNA from field sampled cotton roots, the soil was soaked in a solution of sodium hexametaphosphate (5 g/l) for >16 hours and then roots washed from the soil and collected via sieving and floatation. About 0.5 g of recovered roots was frozen for DNA analysis. DNA was recovered from the frozen root material using the MoBio Power Soil kit. The extraction followed the manufacturer's instructions with the exception that in the initial tissue disruption 100 ul of 0.5 mm glass beads were added to the buffered extraction tube and the tissue was lysed on a Retch bead beater at 30 Hz for 3 minutes, before being placed on ice. Recovered DNA was eluted into 50 ul of 1xTE buffer, quantified for purity (260:280 nm) and amount on a Nanodrop spectrometer and then sent to AGRF for metagenomic analysis using the ITS fungal primer set and illumina sequencing. Results were analysed in PrimerE.

Exudates and pathogens

Exudates of Sicot 74BRF were generated in a hydroponic system. The basic system design was of bottom capped 155 wide by 300 mm deep poly-pipe pots. Germinated seedlings of Sicot74BRF were transferred to 10 ml cups and suspended using a piece of corflute board that acted as a lid to the pots. The hydroponic solution and root system was continually aerated using filtered compressed air, delivered via irrigation bubblers to the bottom of each pot. Five litres of basal hydroponic solution was added to each pot, which contained; 12.5 ml of 1M $Ca(NO_3)_2$, 12.5 ml of 1M KNO_3, 5 ml of 1M $MgSO_4$, 2.5 ml of 1M KH_2PO_4, 2.5 ml of FeEDTA solution and 2.5 ml of Micronutrients. FeEDTA solution is an ion complex of ethylene diamine tetra-acetic acid, each ml of stock solution contains 5 mg of Fe (8.156 g of EDTA Fe Na salt in 200 ml produces 5.6 mg Fe in 1 ml solution). The micronutrients stock solution contains 2.86 g H_3BO_3 (boric acid), 1.81 g of $MnCl_2.4H_2O$ (manganese chloride), 0.11 g of $ZnCl_2$ (zinc chloride), 0.05 g of $CuCl_2.2H_2O$ (copper chloride) and 0.025 g of $Na_2MoO_4.2H_2O$ (sodium molybdite) per litre. Half N, P half N and P, and quarter N and P were also included in the experimental set up with 1M $CaCl_2$ and 1M KCl used to balance the salts from the N and P removal.

Plants were grown in the system for 12 weeks, with samples of the hydroponic solution sampled every four weeks. 100 ml of hydroponic solution was recovered and passed through a 0.22 µm filter before being stored in two 50 ml aliquots at -20°C until analysed.

For exudate analysis, 25 ml aliquots of the exudates were freeze dried to produce a dry powder. This residue was reconstituted in 1 ml of D_2O and transferred to an Nuclear magnetic resonance (NMR) tube for scanning. Peak interrogation and analysis was assisted by Dr Ben Greatrex of UNE's chemistry department.

Sub samples of the hydroponic solutions were also used by Hamid Abd Oun in a chemotactic response trial against the various species of *Berkelyomyces rouxiae* (formerly *Thelaviopsis basicola*). This followed procedures previously used by Lily Pereg and others in her lab and generally involved

adding the exudate to a strip of filter paper on the surface of an agar plate, which had a growing colony of *B. rouxiae* on the other side. The distance grown, area uncultured or zones of inhibition were then assessed to determine if there were differences in the response.

In a separate series of experiments, MSc student Upama Paudel investigated the potential for Silica and diazotroph colonisation of spinach to stimulate plant growth and elevate stress resistance. Her experiment was divided into three parts; (i) silicon trial to optimise Si application to the system, (ii) a bacterial trial to isolate and identify potential beneficials for host plant colonisation from seed applied bacteria, and (iii) a silicon and bacterial trial to evaluate the response of spinach under biotic and abiotic stresses. The final part of this experiment had included in it a Mannitol seed treatment as well as the live biological seed coat. The reasoning for this was that Mannitol is the sugar used in the selection of diazotrophic bacteria, which are often responsible for N fixation (Kumar *et al.*, 2007) and linked to nematode control in cotton (Chawla *et al.*, 2013).

Seed coating was done using CMC (Carboxymethyl cellulose) as an adhesive seed coating material. The seeds were sterilized prior seed coating by immergence in 50% ethanol and 50% beach for 5 minutes, then washed with deionized water for 5 times and soaked for 3 hrs to imbibe the seeds. 6.6 ml of CMC solution (3%) was transferred to sterile sample container (A) and the bacterial solution was added in the container to make a final volume of 20 ml in order to make bacterial suspension at 1% of CMC. In another sterile container (B) 20 ml 1% CMC was added. 5.65 g of surface sterilized seed was soaked overnight in both containers containing CMC and bacterial suspension. The following day, excess bacterial mixture was drained out and the seeds were transferred immediately to 25cm diameter seed coating drum for uniform seed coating and drying. The seed coating drum was tilted at about 45 °C. For treatment containing mannitol, 1.33 g of mannitol was sprinkled in the drum during seed coating and was allowed to rotate until there was a uniform coating on the seeds. Post coating, the seeds were stored at 5 °C until needed.

Five treated spinach seeds were sown in small square pots (6.5×6×5.5 cm) containing 255g of soil. The plants were watered with 15 ml every day, thinned to three plants after 20 days of growing and hand weeded as required. After a month, a leaf of each replicate was removed carefully for recovery of bacteria from leaf tissue. Leaves were grounded with a sterile micro-pestle followed with serial dilution into ¼ Ringer's solution for each replicate. Enumeration of the diazotroph colonisation of the leaf tissue was made by plating these dilutions onto Ashby-Sucrose agar (1.5% agar, 0.5% $CaCO_3$, 0.5% sucrose, 0.02% KH_2PO_4, 0.02% $MgSO_4$, 0.02% NaCl, 0.0005% $FeSO_4$) to which 10 g of Mannitol was added as a 30 ml filtered sterilised solution prior to pouring the agar.

Border cells and Exogenous DNA (exDNA)

Acid delinted cotton seed of Sicot cultivars 730, 43 Bollgard2 Roundup Ready Flex (BRF), 71 BRF, 74 BRF, 746 Bollgard3 Roundup Ready Flex (B3F), 748 B3F and 754 B3F was provided by Cotton Seed Distributors (Wee Waa, NSW) and used under a material user agreement with Monsanto (now Bayer), Australia. Pea seed of the variety Greenfeast and a fodder maize were locally sourced. Batches of 20 seeds of each cotton cultivars, maize and pea seeds were surface sterilised with a 3 minute wash in a solution of ethanol (50% v/v) and sodium hypochlorite (5% m/v available chlorine), followed by three washes in sterile distilled water. At this time cotton seeds were imbibed for 1 hour, whilst pea and maize were given 6 hours in sterile distilled water. The surface sterilised seeds were germinated on a layer of moist filter paper laid over 100 ml of water agar (1.5% w/v) in the bottom of a 20 by 10 cm instrument sterilisation tray, which was incubated in the dark at 28°C for 72h.

Border cells were enumerated germinated root tips having been liberated by immersing the terminal 15 mm of an emerging radicle in 1 ml of sterile deionised water in a 1.5 mL microfuge tube. The root tip was allowed to imbibe for 5 minutes prior to agitation with ten repeated flushes of 100 µL with a pipette. The seedling's radicle was removed and the entire 1 mL suspension transferred to a Sedgewick-Rafter counting chamber (PYSER-SGI, Kent, UK) for enumeration under a compound microscope at 200× magnification. The number of border cells present in 60 arbitrarily chosen, cells on the grid were counted and the border cells per tip estimated for all germinated seedlings. This process was repeated for a minimum of three batches of seed for each cultivar.

The F and V rank data, which is an indication of the level of resistance a cotton cultivar against the wilt fungi *Fusarium oxysporum* and *Verticillium dahlia*, respectively, were obtained from the commercial disease tanking data sets available from Cotton Seed Distributors as of 2018.
Identification of exogenous DNA (exDNA) was initially attempted from the same recovery as that used for the border cell enumeration, however, the exDNA recovery appeared to be close to or below the detection limits of the methods being employed. As such exDNA was recovered by immersion of the last few mm of a root tip into 30 ul of sterile distilled water within a 1.5 mL microfuge tube. After 10 minutes of imbibition, ten 10 ul aspirations were made to liberate border cells and exDNA from the root tip prior to the root being removed.
Quantification of exDNA was undertaken using a Nanodrop 8000 micro-volume UV-Vis spectrophotometer (Thermo Scientific) with measurements taken from 2 µL of the recovered solutions, having blanked the machine against water.

Milestone 2.3.1-2.3.3
Fertiliser use and management

Nitrogen fertiliser use

The majority of the work undertaken under the milestones reporting to investigate N use was focused on the N trials undertaken by CottonInfo during 2014 to 2017. Dr Knox provided statistical analysis of the trials data, which focused predominantly on yield, field residual N, crop uptake, application rates and gin processing data.
In most cases the analysis was routine, although the codes used for cotton classing meant that non-parametric methods had to be used. The analysis of gin data also highlighted that when conducting industry wide experiments the gins themselves offer very different capabilities. Some gins were able to process single round modules and provide information on the four bales that were derived from it, whilst others could only provide information on the six modules loaded into the front of the ginning process. Some gins produced turn out based on an actual seed weight from the module, whilst others produced turnout numbers using data from CSD's variety trials on lint and seed percentages. Classing data was generally considered to be consistent, despite the industry continuing to use a visual scoring over HVI measurements.
Post analysis of the trials data Oliver was involved in assistance with development of trial reports and largely responsible for the development of extension material for various industry publications including the Cotton Production manual, Spotlight and the Australian Cottongrower.
None of this would have been achievable without the work of CottonInfo, the REOs, the growers who facilitated the trials and the gins who returned analysis. A word of thanks has to be given to all involved for their support.

Milestone 2.3.4

P and K

Sampling of cotton (Gossypium hirsutum) for nutrient levels is of importance to the modern Australian industry, due to both the impact of low levels of nutrition on plant health and yield, and the economic and environmental impacts of over-fertilizing. Potassium deficiency has been exacerbated by the increase in boll load on shorter–statured plants typically grown in Australia (Bange *et al.*, 2008), and has led to a shift from relying on soil tests prior to sowing, to sampling throughout the season to track the uptake and availability of the element through the crop life-cycle . Matt Maunder's experiment aimed to determine which sampling method; analysing petiole or blade concentrations of K, was a more accurate and reliable measure of K in cotton, specifically Sicot 74BRF.
The experiment was carried out in a glasshouse at the University of New England, Armidale, NSW, using a low to moderate K grey vertosol soil sourced from Inverell, NSW, to mimic conditions found in the northern Cotton growing regions of NSW.
The experiment was carried out in a complete randomized block, with 5 levels of treatment (0, 40, 80, 120 and 200kg K/ha applied mixed through the soil prior to planting). There were 5 replicates per treatment. The soil had 5 mg/kg of nitrate, 0.4cmol/kg of K and a CEC of 30 cmol/kg. Each pot was 150mm in diameter, constructed of polyethylene pipe and had an individual dripper irrigation system installed. Each contained 18kg of crushed soil (<4 mm), with individual treatments applied

to each pot. Basal elements were applied to all treatments at the rate of 180kg/ha of N, 25kg/ha of P, 40kg/ha of S and 26kg/ha of Mg. Ca application varied from 220kg/ha for treatment 1 to 150kg/ha for treatment 5.
K treatments were selected from the control (0kg K/ha) up to the amount of K taken up by a high-yielding cotton plant (200kg K/ha) (Rochester, 2007). K was applied as KNO3, along with the corresponding basal elements (180kg/ha of N, 25kg/ha of P, 40kg/ha of S and 26kg/ha of Mg) to ensure that K was the only limiting fertilised nutrient. Three cotton seeds of Sicot 74BRF were planted per pot, then thinned to one per pot after successful germination. Irrigation was carried out once the pots reached 20kg in weight, then stopped once a weight of 23kg was achieved (found in the pre-watering to be close to the field capacity of the soil).
The cotton was grown for 112 days in a glasshouse with natural lighting, and daily temperatures of 30°C and a night time temperature of 20°C (December 2016 to April 2017). Plants were watered to weight (23kg of pot, soil and plant) approximately once every 7-12 days. Samples were taken of the separated leaf blade and petiole at set accumulated heat units. The sampling occurred at 9am, and with minimal water stress. Due to plants showing slight yellowing, nitrogen was applied as urea twice, at 50 and 10 units per ha. After the 112 day lifecycle, the plants were cut off at ground level, dried, ground and subjected to the same analysis as the blade samples.
The blade was analysed at two points between veins on a pXRF. The pXRF was set to 15kV, 55 μ A for 56 seconds per sample. The samples where then dried at 80°C for 48 hours, and then analysed again with the pXRF. The samples where then weighed and crushed in a mortar and pestle. The petioles were subjected to a water digestion, where 0.05g of plant material was digested in 7.5mL of deionised water. It was then tumbled at 15RPM at 25°C for an hour, then filtered through Whatman no. 42 filter paper. The samples where then analysed through Inductively Cooled Plasma Optical Emission Spectrometry (ICPOES) with an Agilent Technologies 700 series machine. The blade samples then were made into 0.5000g samples, and combined with 4mL of nitric acid (70%). they were then pre-digested for at least an hour, then subjected to ultrawave digestion. The samples were then subjected to ICPOES. Smaller samples (such as the first harvest, where the leaves where not big enough to make 0.5000g samples where instead analysed through a micro digest. 0.03000g of sample and 0.5mL of Nitric acid (70%) were combined, then following predigesting where subject to an ultrawave digestion, then ICPOES. These results were then compared to a lucerne standard measured using the same process, and adjusted. The plants where then collected by cutting off at ground level. The lint was separated and weighed, and these values (of lint and seed weight) will be referred to as lint yield. The plants were then dried, and digested in the same process as the blades, in order to measure total plant potassium.

Emily Young's project also targeted K uptake by cotton, but was targeted on the potential for co-uptake with other nutrients. The experiment was again conducted in the UNE glasshouse complex. 24 rhizoboxes (1 m^2 front and back surface area with a 2 cm opening at the top). The soil was collected from the 0 - 0.2 m layer at Laureldale Research Station, University of New England, Armidale, NSW, Australia (30.4751200 S, 151.6483920 E). The soil was air dried and crushed (< 5mm) and packed into the boxes to a bulk density of approximately 1.1 gcm-3 with a total of 37 kg of soil in each rhizobox. The rhizoboxes were incubated in the glasshouse at 30/20°C (day/night) for 6 months through a series of wetting and drying cycles to establish soil structure. Boxes were placed in four trolleys of six boxes, spread evenly around the glasshouse with 1.5 m between each trolley. In November 2017, two week old plants of variety Sicot 71 Bollguard Roundup Ready Flex® were transplanted into each box, 0.5 m apart, and watered with 500 ml water every second day and to field capacity (40% gravimetric water content) by weight at the end of each week.
20 weeks after transplanting, rhizoboxes were ranked based on summed plant height and assigned to six treatments with each treatment replicated four time. Plants were pruned to two living nodes to standardise plant growth and 25 kg N/ha was applied to the soil surface as urea and watered in with 500 ml of water.
Twenty four days following the N application, six treatments were prepared, each consisting of 150 kg K/ha with 7.5% Rb in the form of rubidium chloride (RbCl). The treatments were as follows: control (de-ionised water), dipotassium phosphate (K2HPO¬¬4), potassium sulfate (K2SO¬4), potassium chloride (KCl), potassium nitrate (KNO3) and potassium nitrate with ammonium nitrate

(KNO_3 + NH_4NO_3). The final treatment contained double the quantity of N of the other treatments. Each treatment had 4 replicate rhizoboxes, allocated based on combined plant heights during initial mapping. Each treatment was dissolved in 100 ml water per box and syringed into the soil 0.30 m below the surface equally between the 10 openings in the side of the rhizobox. Twenty seven days following treatment applications, petioles of the fourth leaf from the top of each plant were excised and the petiole sap was transferred to a Hanna potassium specific ion electrode field test kit to determine K concentration. All post pruning leaf and stem material were harvested, dried at 60°C for 1 week then weighed and ground to 1 mm.

A 0.50 g subsample of each ground sample was run through an Ultrawave Microwave Digester and Agilent 720/730 Series ICP-OES spectrometer to determine the concentration of K, phosphorus (P), sulphur (S) and Rb. The N concentration of each sample was determined using a Leco TruMac® Series Determinator.

A second sample (0.2000 g) of tissue from each plant was weighed into crucibles and the N concentration of each sample was determined using a Leco TruMac® Series Determinator. Concentration increases in plant tissue K, N, P, S and Rb in each sample were calculated using the blank and standard samples to correct for any error. Total rubidium uptake (mg) was calculated by multiplying Rb concentration and plant dry weight for each plant. The apparent recovery of K from the fertiliser band was calculated for all treatments except the control by subtracting the background Rb found in the control, then multiplying by the amount of Rb and K added to each band.

Using the R statistical package, treatments were tested for normality and variation between treatments was analysed with ANOVA, with statistical significance based on P=0.05. A regression was done on K concentration and petiole K to determine the correlation between the two.

Brendan has also supervised student projects looking at placement of P at the Incitec long term fertiliser trial at Colonsay. The work generated by the students has been the basis of talks given at several workshops and the cotton conference. More recently, Oliver, in addition to his involvement in the GRDC soil constraints and CRDC sub-soil constraints programmes, being run out of USQ by A/Prof John Bennett, has been co-supervising Kirsty Neale in her work on K placement. She is now entering the 3rd of her 4 year study period and the deep ripping and nutrient placement has occurred in anticipation of planting either this summer or next winter, depending on rain.

Results

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

Milestone 2.2.1

Rooting depth and Constraints

Using the Goanna Telelmetry Capacitance probe data set and with the assistance of Brendan Griffiths, Jamie Barwick and John Burrows we have mapped root exploration across the cotton industry for the 2015/16, 2016/17 and 2017/18, seasons. The initial season produced 370 usable probe site, with 750 in 16/17 and 830 in 17/18. The percentage of fields showing no root activity below 60 cm during these seasons was 30, 25 and 20% approximately. Some of the reduction was due to an increase in the total number of site, but we were also able to refine the algorithm to provide improved confidence in the automated analysis. The work implicates that ~25% of the capacitance sites surveyed over those three seasons had rooting constraints below the top 60 cm.

Satellite imagery was mapped to the 2015/16 probe sites for which GPS data was available in conjunction with clear satellite imagery. The analysis showed no significant difference or variation in NDVI measurements of the crops in constrained and unconstrained fields. Other spectra were examined, including SVI, but also found to be of little use in discriminating between constrained and unconstrained fields. The level of missing GPS site data and the loss of coverage from cloudy days may have had some influence on the lack of significance, but it is worth noting that compensatory leaf development in cotton in response to nutrient levels may also have had an effect on this analysis. Cotton leaves under variable water and N conditions alter their growth (Fernáandez *et al.*, 1996), which would result in a canopy appearing a similar shade of green from above due to the indeterminant growth nature of the crop (Constable & Bange, 2015).

Soil sites from around Goondiwindi have also been cored to determine the probable nature of the constraints. In most cases soil test have indicated that sodicity and pH remain the major reasons for the soils becoming restrictive to rooting (Chorom *et al.*, 1994, Gupta *et al.*, 1984). This is also coupled to changes in the industries irrigation practices, where more frequent irrigations working off 50 mm deficits may be resulting in failure of the 'throttle zone' in the soil to open up (Hodgson & MacLeod, 1989) so that we end up with wet soil sitting on top of dispersive wet soil more often resulting in a gradual loss of soil pore space during the season.
Although Na remains an important aspect to this system, in one of the initial sites where this investigation began, assessing the soil against EM showed little difference in any chemical attributes partly due to a lack of variation in the EM. We then turned to the yield map of the field and using cores taken against a yield distribution identified that the most probable constraint to cotton rooting below 60 cm in that particular field is the soils EC, which is driven by chloride potentially present as calcium salts rather than sodic ones. Observations of this type highlight not only the importance of knowing if a soil is constrained, as there is a likely limit on yield potential, but on also identifying the cause as the wrong remediation strategy could exacerbate the issue in some soils.

Milestone 2.2.2

Nematode population change

Analysis of the total recovered nematodes did not indicate any significant difference in nematodes/g assessed either within fields, between years or in combination. The percentage of the nematode population representing plant parasitic nematodes had not changed in the field A and was reflected in the plant parasitic index (PPI) scores for the field, which averaged 2.38, 2.56 and 2.09 for 2005, 2007 and 2015, respectively. However, the PPI had significantly ($P<0.001$) increased in field B from 2.29 in 2005 to 3.18 in 2015.
Community analysis with NINJA indicated that there was significant ($P<0.05$, ANOVA) difference in the maturity, plant parasitic, enrichment and structural indexes and the herbivore, fungivore, bacterivore and omnivore footprints within the assessed field material. This indicated an improved maturity of the analysed ecosystem rather than nutrient enrichment (Figure 1) , due to an increase in the number of higher order coloniser-persisters in the samples.
Although observations from the 2015 sampling indicated that significant changes in the composition of nematode communities were occurring, the total numbers of nematodes supported within the vertosols had not changed. This was taken as indication that Aldicarb had not imposed a limitation on the population size as initially hypothesised, which is in keeping with other work where pesticide changes had not altered nematode population size, but had been associated with a change in species richness.
A paper around this work was written and submitted in 2017 to 'Crop Protection', but rejected. Publication has not been attempted since.

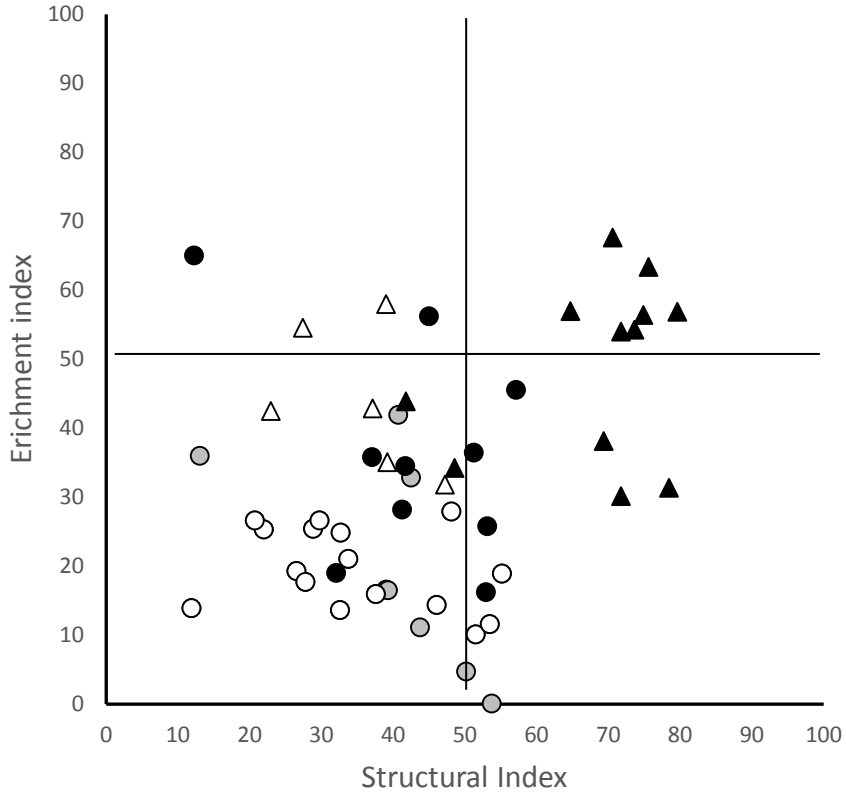

Figure 1 Food web analysis of nematode community assemblages from field A, sampled in 2005 (○), 2007 (●) and 2015 (●) and field B sampled in 2005 (△) and 2015 (▲). The Enrichment index parallels with the nutrient enrichment whilst the structural index correlates with the maturity of the ecosystem

Milestone 2.2.3

Exudates, pathogens and beneficials.

Mycorrhizal DNA recovery and sequence analysis

Microscopic examination of the recovered supernatants from the MycoApply product indicated that the drench was mainly root fragments, some of which were colonised with some fungal spores. The ultra-fine formulation appeared to contain fewer root fragments, but we recovered fewer spores as well, observing about 10-20 spores in 20 ul of the recovered suspension.

The MycoApply packaging claims to contain; Glomus intraradices, G. aggregatum, G. mosseae and Glomus etunicatum. The sequence data we obtained contained the Glomeromycota, Funneliformis mosseae, but no organisms belonging to the genus Glomus or any of the species were identified. Most of the fungal sequences (>99.9%) belonged to Ascomycota.

Root colonisation analysed revealed no significant difference (at the 5% level) between treatments or MAP being added. At the 10% level, sometimes used in biological experiments, there was a difference in treatment, but not MAP, which is contrary to a lot of research where adequate P in the soil can restrict colonisation. Unexpected was that the control had higher levels of colonisation than the MycoApply treatments (Figure 2).

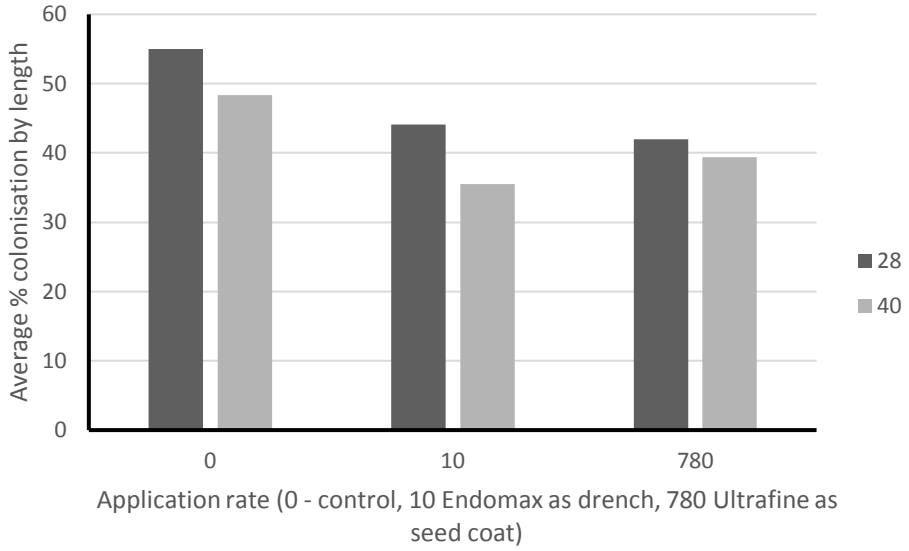

Figure 2 Average root colonised by mycorrhizal fungi for cotton sampled in February of 2018 at ACRI. Treatments were MAP applied at 28 or 40 kg/ha at planting and various applications of MycoApply. MAP application was not significant (P=0.21) and MycoApply treatment differences were significant at the 10% level (P=0.08) with the control higher than both seed treatments.

Analysis of the fungal colonisation of the root tissue produced few mycorrhizal sequence hits and analysis of the sequences failed to show any difference between the treatments. Ordination was also used to see if any pattern of colonisation might be identified from the sequence information, but this was also uninformative (Figure 3).

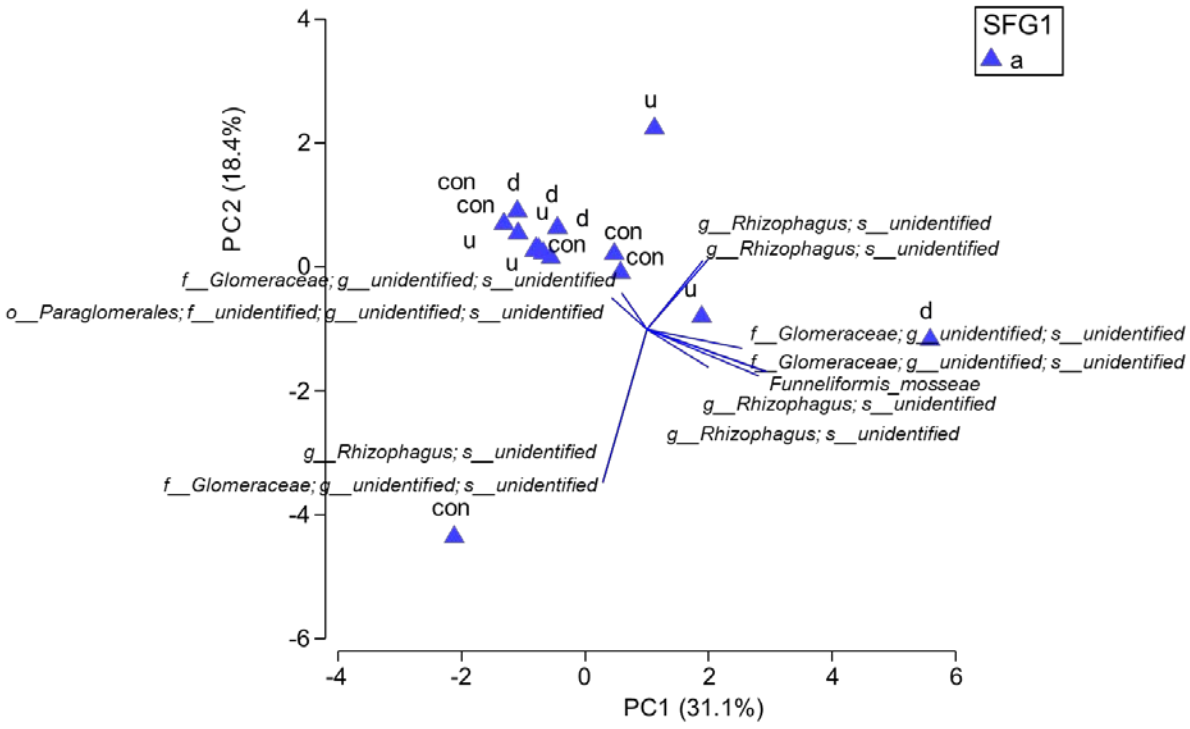

Figure 3 Principal component analysis generated from the number of OTU (specific sequence reads just of Glomeracae data) for the different MycoApply seed treatments. The ordination shows little grouping of the nil control (con), seed drench (d) or seed applied (U) formulations of the product.

The lack of mycorrhizal sequence data from the roots may also be indicative of the late time of sampling of the crop. Earlier season analysis may overcome this if it is indeed the issue. Another problem may be from the way in which the DNA was recovered from the root tissue.

Analysis of Mycorrhizal cotton roots from cut and fill areas in the south

Soils were received, roots recovered, cleared, stained and colonisation assessed from a field site near Griffith where a difference in stand development was noted and thought to possibly be due to cut and fill operations on the field. Colonisation of the cotton roots increased over the sampling period, which was as expected in both poor and good areas of the field, however, there was no significant difference between the two (paired T-test). The mean % root colonisation remained higher in the good sites, but the lack of significance was because one of the three good sites had a lower level of colonisation than the other samples from those areas. This could be a function of what has colonised the plant and this is an area where we still know little. In the ACRI trial reported we had AM inoculated and bigger plants that had lower levels of colonisation. Stephen Allen used to talk about how in some instances VAM could be pathogenic and Samieh Eskandari noted smaller, but heavily colonised plants in her work. Smaller plants with less P uptake and higher colonisation has also been recorded in the honours project work being undertaken by Emma Longworth. Where AM are not supplying P, Zn or water to the plant, but are simply a Carbon drain on the plant the function would not be one of symbiosis, but of pathogenicity. Our current thinking behind this is that generalist mycorrhizae, which tend to be found in commercial inoculums, may cause this reduction in growth and that locally adapted specialists may be needed to provide benefits to cotton. Experiments around these hypothesis form the basis of UNE2001.

Exudates and pathogens

NMR analysis of the cotton exudates from a hydroponic solution produced an apparent signature spectrum for Sicot 74BRF (Figure 4), but the peak intensities were small. There was some Amino Acid detection (Figure 5) within the NMR output, but the compounds were not present in high enough concentrations to permit identification. It transpired that the desired 10 mg of chemical required was for each target compound and not a total. Based on the outcomes, we would have been struggling to achieve this even if we had freeze dried the entire 5 l of hydroponic solution the plants were cultured in. The spectral range for the carbohydrates failed to detect any signature. This was surprising, given previous work in this area (Knox *et al.*, 2014, Li *et al.*, 2009), which had suggested the presence of glucose, fructose and maltose was likely and it may well have been that the failure for the hydroponic system to remain aseptic had resulted in the carbohydrates being removed by foreign organisms (e.g. bacteria and algae) in the system.

Exposure of isolates of *B. rouxiae* to exudates of Sicto 74BRF did not elicit a differential response in fungal development. However, Hamid has recently completed work that indicates that there may be a differential response of *B. rouxiae* toward germinating cotton seeds, with less directional growth toward Sicot 730 than the other current varieties tested.

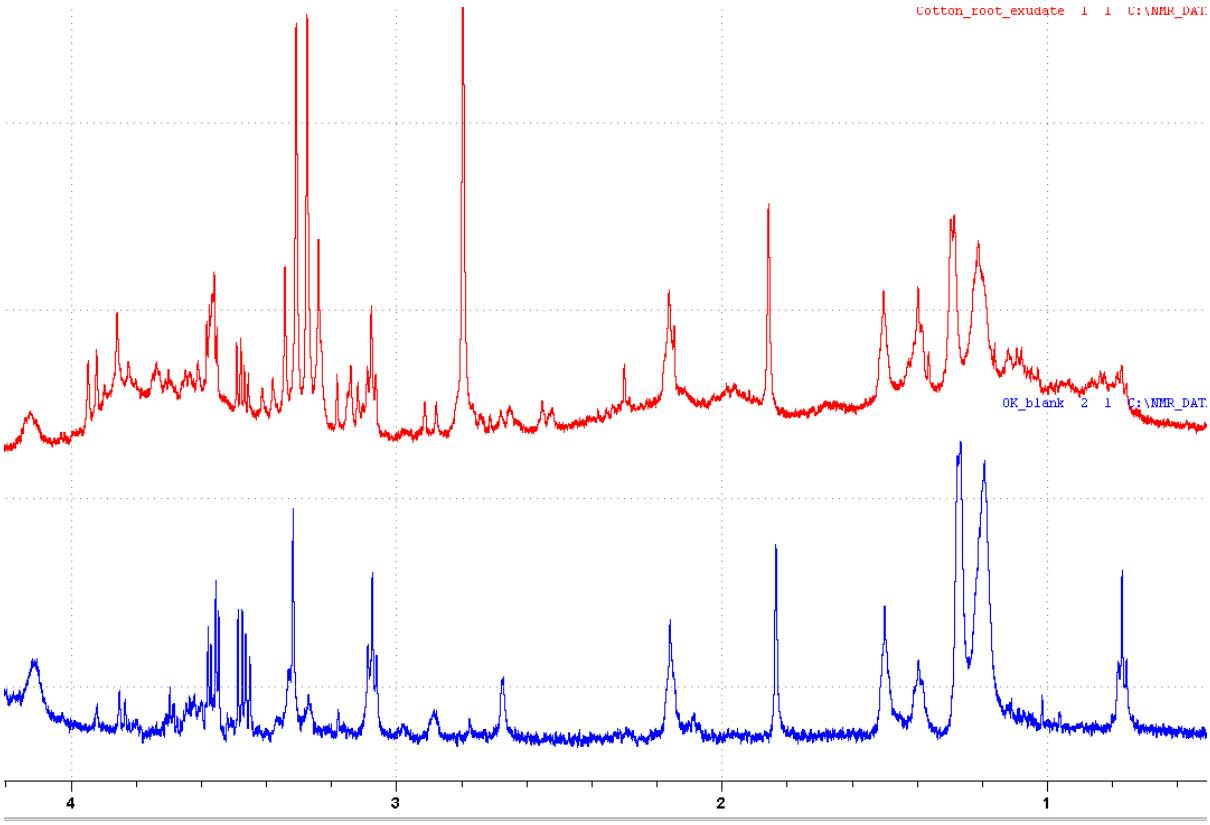

Figure 4 NMR graphical output for cotton exudates from Sicot 74BRF (red) and the control basal hydroponic solution (blue) used to produce the hydroponically grown cotton.

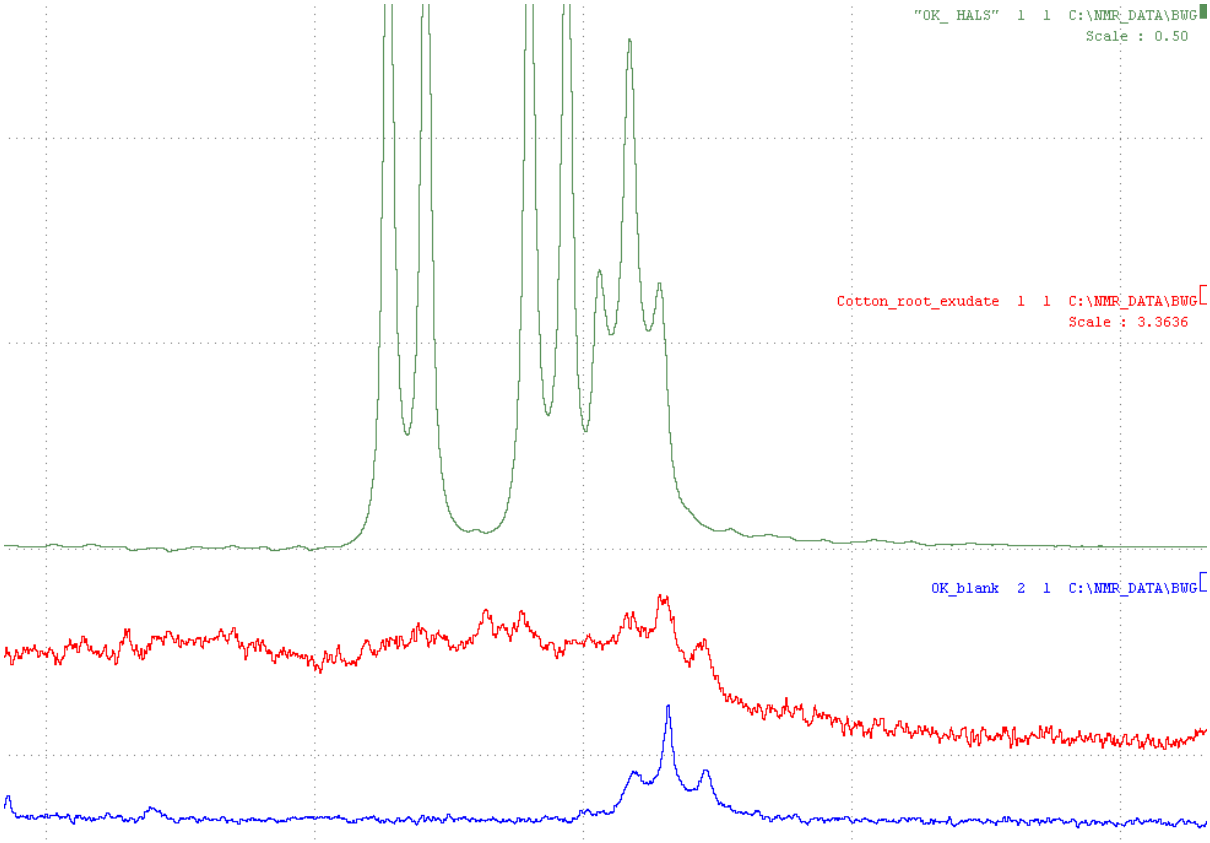

Figure 5 Amino acid scan of the hydroponic solution used to grow Sicot 74BRF. The control media is shown in blue, the plant exudate is in red and the signature of the amino acids solution (containing 10 mg/ml Gly, Glu, Ser, Ile, Asp and Thr) is in green.

Upama's work, on spinach, showed no significant difference in the number of bacteria recovered from the spinach's leaves between any treatments (P > 0.05). However, higher numbers of bacteria were recovered from the two seed coat treatments that contained either mannitol and bacteria or just mannitol.
This work implies that there is potential to modify the localised seed environment with selected substrates that will assist in developing a beneficial rhizosphere and phylosphere, regardless of plant exudation chemistry.

Border cells and exDNA

Border cell counts for the cultivars Sicot 730, 43 BRF, 71 BRF, 74 BRF, 746 B3F, 748 B3F and 754 B3F were estimated at 9839, 6612, 9593, 13126, 10027, 8142 and 6079 border cells per root tip, respectively (Figure 6). Pea border cell counts averaged 3180 per root tip with 2772 border cells/root tip for the maize variety, which was lower than expected for both.
V rank and F rank data, in addition to the varieties previously assessed was only available to add to the original analysis for Sicot 730, 43 BRF, 71 BRF, 74 BRF, 746 B3F, 748 B3F and 754 B3F. No correlation existed for these varieties between border cell number with either F or V rank (r = 0.43 for both). Adding this data to the original data set also did nothing to improve the slight relationship that had been previously observed (Knox *et al.*, 2007).
The Nanodrop methods of exDNA determination produced good standard curves using standards from Invitrogen between 5 and 40 ng/ul, with r>0.99. Analysis indicate that there was difference between cotton varieties, in terms of the exDNA recovered per root tip, but there was no measurable response or change in exDNA levels from border cell suspensions to the presence of fungal spores and conidia of *F. oxysporum*, *V. dahlia* and *B. rouxiae* at 0, 24 or 48 hours post exposure.

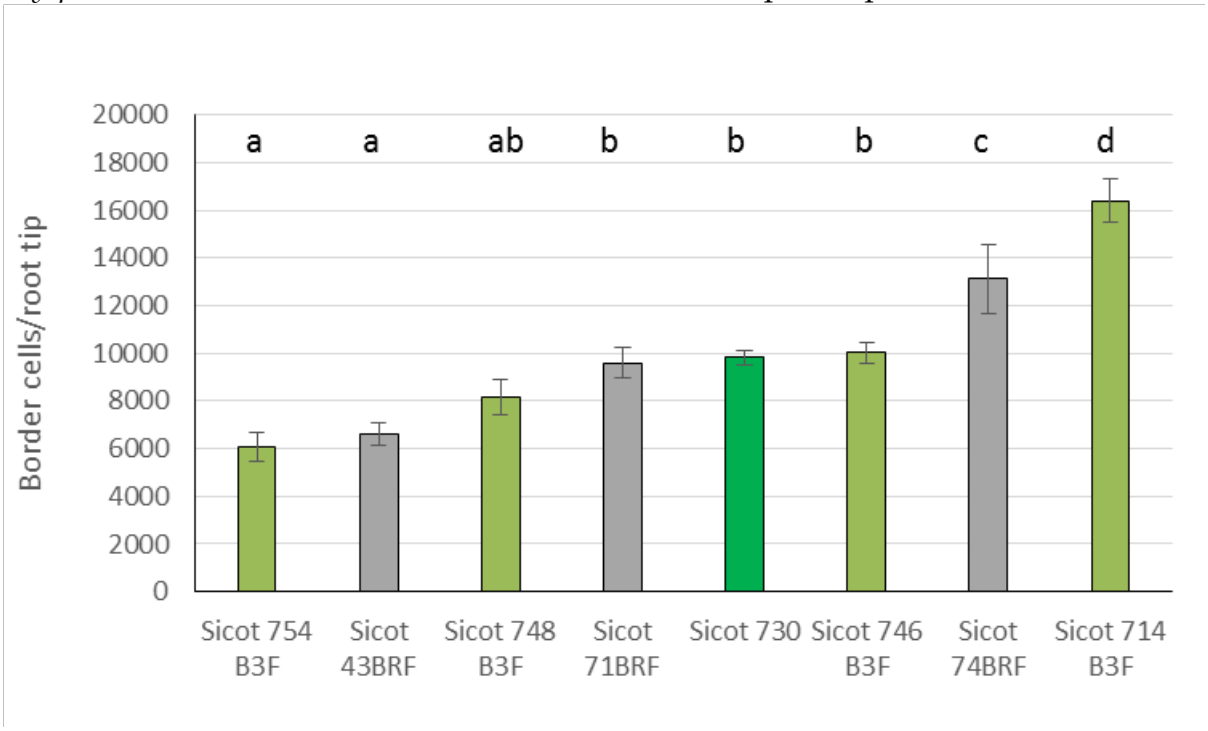

Figure 6 Mean border cell counts per root tip of newer Australian cotton varieties. Error bars indicate the standard error of the mean. Significant (P<0.05) differences are indicated with different lower case letters.

Fertiliser use and management

Nitrogen fertiliser use

During the course of this project Oliver delivered updates to the Cotton Production Manual around N and other nutritional use initially under the substantial re-write undertaken primarily by John Smith in 2014.

His summation work of the CottonInfo N trials also resulted in articles in Spotlight (Regional trials yielding surprising data) and the Cottongrower (Cotton nutritional tour: top tips for growers).

Oliver and Brendan presented work at the CRDC Nitrogen workshop in Moree in 2014 where they used Ian Rochester's existing N levels in CottAssist for the field evaluations. Ian challenged this presentation and subsequently updated the CottAssist N data from a linear to a polynomial response within the weeks, using data he had compiled over the preceding years.

K sampling methods for Australian cotton

The summation of Matt Maunder's thesis was that petiole concentration of K demonstrated a continuous decline throughout the season, in comparison to Blade concentrations which fluctuated, regardless of attempt to limit the effect of water on sampling. The content of the nutrient in the petiole showed a decline during the peak demand period of boll formation and setting, whereas blade content appeared to remain constant. As there was a significant treatment effect, on both yield and final K concentration of the whole plant, it appeared that K availability and uptake had a significant effect on yield. This effect was shown in the petiole concentrations, but not that of the blade. Furthermore, the petiole concentration of K was significantly correlated to blade levels of P, Zn and Na, which are key nutrients in determining yield (Rochester & Constable, 2015). Thus, it appears that petiole concentrations are the more accurate and reliable measure of K availability through the season in cotton.

In addition, it was found that the first blade sample for K, at 400 GDD, was a significant predictor of final yield. As this sample was removed in the petiole data, this suggest that using the blade sample for 400GDD, rather than the petioles for 750 to 1450 GDD samples, may present an optimum sampling method if a limited number of samples is possible. Na levels were found to have a significant negative impact on K levels in the petioles. Na has been found to partially replace the function of K in plants (Zhang *et al.*, 2006). This may be a contributing factor to the low levels of K often observed in industry blade levels due to the availability of Na in many of our soils, however, further study is needed.

The pXRF data showed poor correlation to that of the tissue concentrations. Whilst the linear regression of petiole K concentration to pXRF K results gave a significant p-value (2.31e-15), there was only a R^2 correlation of 0.4. The pXRF was found to be useful in predicting Ca concentration in the blade, with statistical analysis showing an adjusted R^2 value of 0.806 when a linear model was made with the non-dried results. There was also a correlation between P levels from the pXRF and ICP readings, however, there was significant variation in the ICP values and so limited confidence in this technique as a method of prediction.

Co-uptake of K with N

Emily's experiments found that plants fertilised with KNO_3 had the highest average weight of 8.52 g, which was significantly higher than the control, K_2HPO_4 and KCl treatments. This was evidence that addition of nitrogen as a counter-ion in potassium fertilisers increased plant K uptake during vegetative growth, however,

these plants were N deficient. The N deficiency was evident in the average dry weights, with both N treatments having the highest weights.
Plant tissue concentrations for N were significantly greater than the control with 3.75 and 4.07% for KNO_3 and KNO_3 + NH_4NO_3, respectively. N was readily available in both treatments as NO^{3-} and NH^{4+} ions. The application of K fertiliser with N anions increased the K uptake in cotton plants in both N limited and non-limited systems.
P tissue concentrations showed the same nutrient deficiency as the N concentration. The KNO_3 fertilised plants had significantly lower P concentrations than the control and K_2HPO_4. Due to P being immobile in the soil, roots of plants must proliferate to locate P sources. By placing K with the P anion, roots have been reported to increase to find the P, and with it the K in cotton (Chen & Dong, 2016) However, the tissue test in this experiment indicated that the plants were above critical values for P and so there was no increased proliferation due to a requirement for P and therefore no increase in K uptake with P in this instance.
S concentrations in the cotton herbage was highest in the K_2SO_4 treatment, which was expected due to the increased presence of sulphate (SO_4) in the soil for absorption. Again, tissue critical values for S were in the range expected and no change in K uptake in relation to additional S uptake were observed.
Petiole K and tissue K concentrations were not correlated, which was supportive of the work Matt had undertaken in the previous summer. This observation again suggesting that petiole K would not a useful tool for the Australian cotton industry to use as an indicator of plant K status. The high mobility of K around the plant, indicates that assessment of plant K status from a single location gives inaccurate results (Bednarz, 1997). If petiole testing continues to be used, producers risk applying less K fertiliser than the cotton crop needs, which will not address the occurrence of premature senescence due to K deficiency.
Rb uptake in plants that had been fertilised with the N treatments was higher than the other fertiliser treatments and reflects the trend for the K uptake. Both Rb and K are absorbed into the roots through the same ion channels (Britto & Kronzucker, 2008). The apparent recovery and utilisation of the band fertiliser showed that N treatments recovery of Rb was close to 7.5% of the applied treatments (Figure 6). The K and Rb recoveries were similar, therefore demonstrating that Rb could be used as a direct label for K uptake.

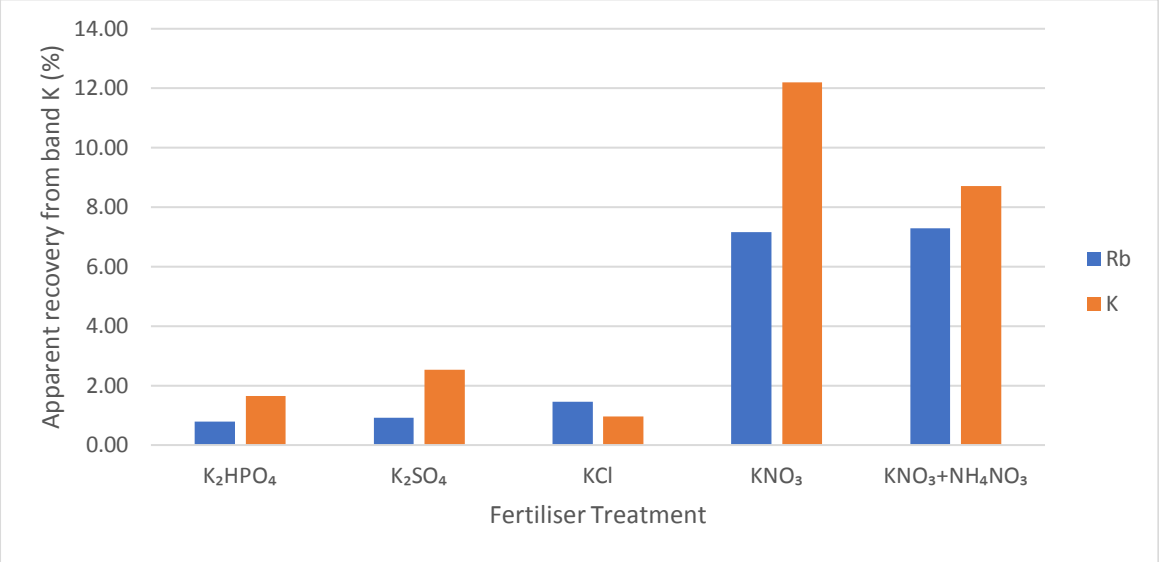

Figure 7 Calculated apparent recovery of K and Rb (%) from Sicot 71 Bollguard Roundup Ready Flex® cotton the banded treatments of no K, K_2HPO_4, K_2SO_4, KCl, KNO_3 and KNO_3+NH_4NO_3 in a Vertosol soil. Plants were harvested 27 days after fertiliser application

Despite the results indicating co-location of K and N increases K uptake, repeating the experiment in a non N constrained soil would allow the results to be confirmed. Due to the N limitation, K was not the most limiting nutrient, which may have meant there was a reduced demand and therefore less uptake of K. Conducting a field experiment across several seasons would also determine the impact when in the Australian environment. Deep placement of the treatments, at say 0.30 m below the surface prior to planting, would allow to roots to move into the band possibly again increasing uptake efficiency.

Outcomes

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

Table 1 The key questions and proposed outputs and outcomes as outlined in the 2014 agreed project delivery for UNE1403 between UNE and CRDC. The final column, Delivered outputs and linkages to outcomes is a summation of what the project has delivered in relation to the originally proposed outputs and outcomes. What is not captured are changes to milestones negotiated over the five years of the project and how outputs were altered to deliver outcomes in relation to those. They are addressed elsewhere (see sections 2 and 4 of this report).

Key question(s) to be researched in project	**Proposed Outputs** *From UNE1403 FRP 2014*	**Proposed Science Outcomes** *From UNE1403 FRP, 2014*	**Delivered outputs and linkages to outcomes** *e.g. Impact and adoption of industry knowledge and practice change*
Extension and scientific response – The Cotton Hub at UNE is proposed to deliver improved response from UNE to cotton production related issues and to better coordinate response to tenders	More coordinated UNE and collaborative tenders, with UNE involvement, to cotton related science and production issues. A source of impartial scientific guidance and assistance on matters that arise within the cotton system.		The UNE Cotton Hub, with CRDC support, has shifted the development of proposals in response to the annual EOI calls. This is evident in that most applications have sought advice or assistance from Oliver prior to submission. In addition, in several cases UNE academics have sought advice on other institutional collaborators and Oliver has had contact from out with UNE seeking academic assistance with projects. This aspect of the Cotton Hub at UNE has worked well, although success has been hindered by the drought.
Is rooting exploration of the soil profile being affected by	Identify if change is occurring on farm and in field through data set analysis. Link the	Identification of trends in cotton rooting profiles over a 5 year period and the soil	In collaboration with Brendan Griffiths and Goanna Telemetry we now have three years of field rooting depth data to work with, which covers a far wider

management and varietal development?	changes observed to soil parameters and/or changes in farm management.	biotic and abiotic factors that restrict this in Australian cotton production systems.	proportion of the industry than previously possible. The rate of change on soil conditions that may be limiting rooting are not evident in the data set to date. There are several reasons for this; reduced production, too few years and continued delivery of new varieties. In terms of proposing mitigation strategies for continued production, the data gathered to date does imply that fields managed for constraints are generally more profitable than those that are similarly constrained and forced toward higher production targets, which are rarely achieved.
What exudates does cotton produce and what impact do these have on disease and nutrition?	Identification of sugars and amino acids suspected of having a role in altering rhizosphere communities.	The exudates of cotton variety Sicot 74BRF and the implications of these signature chemicals on the pathogenic and beneficial soil microbiota.	Root exudates were produced and screened with NMR, but specific chemical signatures were not possible from the amount of exudate material generated. Alternative methods of analysis are being investigated. In the meantime, a MSc student (Upama Paudel) was able to change plant colonisation by diazotrophs by adding mannitol to the seed coat, proving one of the earlier proposed hypothesis that manipulation of the existing soil biology may be as effective as applying live biologicals.

			The work moved on to border cells and exDNA, which was not found to respond to the causal pathogens of wilt or black root rot. Cotton border cells appear to produce a fraction of the exDNA that is associated with pea and maize.
What factors contribute to the impact of the reinform nematode in Australian cotton?	Nematode population diversity measures, existence of nematode predators, and management and environmental factors that alter the destructiveness of reinform nematodes.	What losses in the trophic groups of nematodes can result in reinform proliferation in the presence of a suitable host plant? Presence of nematode predatory invertebrates and fungi are present in Australian cotton regions.	Linda Smith has been working primarily in this area and our joint venture into this was curtailed. This was due to the short term tenure of PhD candidate Bernard Walker and the continued desire not to bring the reinform nematode into NSW to conduct experiments. Work on other free living population has been conducted at UNE, which challenges work from the USA and Netherlands on the potential for nematodes to move vertically due to the high proportion of clay in our soils.
What is the best nitrogen management plan for Australian cotton production and why does it appear to not being adhered to by growers?	A review of the current recommendations for N fertiliser use across the industry. The generation of a proposal to look at the social and economic drivers of grower fertiliser decision basis.	Submission of a review of the Nitrogenous fertiliser work associated with Australian cotton production systems and future expectations and trends.	Oliver has contributed to several workshops on nitrogen and one CRDC supported industry tour. He has written several articles for the associated industry grey literature and supported the REO's N trials, which served as an excellent demonstration of the lack of benefit from too much N.

			Changes in the reporting against this milestone were made when it became apparent that there were more qualified social scientist (Geraldine Wunsch) delivering on similar objectives.
What role does the soil microbiology have in the sequestration of carbon at depth under cotton rotations where maize is being utilised?	Is the carbon that is being seen to be increased at depth coming directly from the plants in the rotation or the movement of dissolved organic carbon or does it require to pass through the microbial community to become stabilised? Is this carbon recalcitrant or is it mobilised back into the food web during the rotation or with changes in the soil water table?	The presence and distribution of C4 derived carbon in the soil bacterial and fungal community over the course of a cotton rotation involving maize. The potential for the soil biology to contribute to or remove carbon sequestered at depth under cotton rotations involving maize.	This aspect of the project was largely moved to assistance and delivery of UNE1603 with Drs Yui Osanai, A/Prof Brian Wilson and Katherine Polain. The work in UNE1603 is reported elsewhere in detail, but has confirmed that the maize rotation has potential to sequester C under cotton, however, the nature of that C and the availability to microbes lower in the profile is now also recognised. Oliver has also worked with others on aspects of chickpea root morphology that also appears to relate to C (Rabbi *et al.*, 2018).

6. **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 adaptation of the Ludox based centrifugation technique for passive nematode recovery represents the potential for more rapid and higher throughput of nematode recovery from smaller volumes of the soil types more often encountered under irrigated cotton systems (Knox & Griffiths, 2017).
      Root recovery of mycorrhizal DNA methodology has not currently been published. The method reported here was a first attempt as far as we know to use a system based on this sequencing approach. Post the work we report here, Dr Knox has been working with Jonathon McLaughlin on a refinement of the methodology to attempt improvement in his work looking at P acquisition in clover. The major additional modification is the freeze drying of the root material to facilitate even greater tissue, hyphal and arbuscule disruption prior to DNA recovery. This additional step appears to be yield greater mycorrhizal DNA recovery, however, his tissues have been fresher and finer than the cotton roots reported here.
   c) **required changes to the Intellectual Property register.**
      None

Conclusion

7. **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?**

The observation of rooting constraints in 25% of our industries production area may question how and if we can alleviate these constraints to increase productivity. However, our observations to date would suggest that a better recourse is actually to manage the affected fields or areas for the constraint rather than trying to push the system beyond its current limits. There is work being conducted elsewhere that may eventuate in strategies to alleviate these constraints, but for now our advice would simply be appropriate management for realistic yields.

The improvements in methodological recovery of nematodes from heavy clay soils has the potential to increase and improve nematology related work. However, whilst reinform remains the only major nematode threat to Australian cotton the implementation of such a technique will remain limited. Observations of changes to the nematode community structure, in fields that were reassessed during this project in NSW also implies the industries continually improving environmental credentials, but the causes of these changes and the occurrence of *Axonchium* sp. or spp. in our system remain unanswered.

The work on crop fertility has confirmed some suspicions that already existed around blade and petiole testing for K. Whether this will reduce testing of K from these tissues or investigation and development of other techniques to facilitate predictions around the potential for premature senescence, due to low K availability, will need to be seen. Meanwhile the work on the CottonInfo N trials was a great extension tool to grow grower confidence in reducing the amount of N applied. As well as changes in application, the trials provided greater confidence in the use of split application. There still remain issues around corporate driven N decisions and unrealistic yield predictions that are resulting in over use of N fertilisers, but hopefully a continued drive in this area will continue to see improvements in the industries N use and associated environmental credentials, whilst also paying homage to the incredible work that Ian Rochester did.

The exudate work has remained disappointing, but the recent observations, all be it in Spinach as to the potential to manipulate the indigenous soil biota with simple sugars to alter plant colonisation and potential stress tolerances is exciting. It may well be possible to provide some level of disease protection through such systems and we propose to continue to use students to investigate this.

The Cotton Production Course continues to deliver up to date and field relevant training for our graduates and those in the industry seeking upskilling or learning more about this incredible crop. UNE remains committed to its delivery and looks forward to a continued partnership with CRDC in its continued growth and development.
The Cotton Hub at UNE remains active and has continued to create and share positive stories around cotton production and the industry, whilst fostering greater inter- and intra-institutional collaboration.

Extension Opportunities

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

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

Oliver will continue to develop and seek students willing to assist or undertake projects involving the study and use of simple carbon and amino acid substrates to stimulate plant responses in cotton. This will build on observations made during this project. He has also been liaising with colleagues who are developing GCMS and LCMS techniques for amino acid and sugar identification from other plant tissues. Oliver remains positive that we will be able to isolate and screen cotton root tip exudates.

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

The nematode work (vertical movement and change in the decade between sampling is to be submitted to a special review on insects in agricultural systems to be considered for 'Arogonmy'. The border cell work and the production of exDNA is still being written up. It is envisaged that there is at least one and potentially two papers in that body of work, which currently needs to be recovered from lab books due to a electronic loss of data, which has delayed this process.

Oliver's role as tech lead in soil health for CottonInfo has had him requested to continue to promote the involvement of soil biology in our cropping systems. Recent workshops on soil health have been summarised for Spotlight and a Cottogrower article offering an alternative reason for long fallow disorder is proposed.

(c) for future research.

UNE2001 has been funded, which will continue to support Oliver in his role with CottonInfo and in exploring the relationship between mycorrhizae and cotton further. Oliver's wider interest in soil biology and soil health will also be fostered under this new project.

The Cotton Production Course has returned to a standalone project and UNE has committed to continue to support its existence during this drought. A review of the impact and future of the units is currently underway and will align with the wider Agricultural Course Reviews being undertaken at UNE in 2019.

9. 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)

Copies of publications to date have been loaded into the Clarity portal in format that do not compromise the publishers copyright of the peer reviewed and published material.

Other material that has been published due to either direct or associated activity from this project is as follows:

Spotlight and Cottongrower

Articles on soil biology and CRDC research were prepared for Spotlight. The first appeared in the Spring 2016 edition and resulted in three articles in the 'Looking below the Surface' series. Oliver oversaw the UNE contribution to the 'Shedding the light on deeper issues' autumn 2018 Spotlight article.

The N tour and the REO trials were also written up for Spotlight and the Cottongrower.

Work with Bryan Griffith on nematodes resulted in the Cottongrower April-May 2015 article, 'If what goes up must come down then does what goes down come up?' We are still trying to publish this as a peer reviewed article.

Work conducted by Gupta Vadakattu was reformatted and submitted to the Cotton grower. It was published in the October – November 2016 edition under the title 'How Does a Cotton system change the soil biology'.

Oliver has written Cotton Course articles for both Spotlight and the Australian Cottongrower since the merger of UNE1403 and UNE1604.

Videos and CottonInfo YouTube

Oliver shot footage with Paul Grundy (QDAF) for the Come clean – go clean series, which he helped author with Tonia Grundy, featuring the Lone Stranger, Pronto and Farm Cleanse. Oliver also contributed guidance and advice on the methods as well as footage to the #soilyourundies campaign, which he continues to support.

Peer reviewed publications

The following papers have been published;

Charles GW, Sindel BM, Cowie AL, Knox OG (2019a) Determining the critical period for weed control in high-yielding cotton using common sunflower as a mimic weed. Weed Technology, 1-8.

Charles GW, Sindel BM, Cowie AL, Knox OG (2019b) The value of using mimic weeds in competition experiments in irrigated cotton. Weed Technology, 1-9.

Knox OGG, Osanai Y, Polain K, Pereg L, Nachimuthu G, Wilson B, Waters W (2019) Lessons from extension activity related to cotton rotation impacts on soil—A scientist's perspective. Soil Use and Management, 35, 141-149.

Rabbi SMF, Tighe MK, Knox O, Young IM (2018). The impact of carbon addition on the organisation of rhizosheath of chickpea. Scientific Reports, 8, 18028.

Eskandari, S.; Guppy, C. N.; Knox, O. G. G.; Backhouse, D.; Haling, R. E., (2018) Understanding the impact of soil sodicity on mycorrhizal symbiosis: Some facts and gaps identified from cotton systems. Applied Soil Ecology 126, 199-201.

Eskandari, S.; Guppy, C. N.; Knox, O. G. G.; Flavel, R. J.; Backhouse, D.; Haling, R. E., (2017) Mycorrhizal contribution to phosphorus nutrition of cotton in low and highly sodic soils using dual isotope labelling (32P and 33P). Soil Biology and Biochemistry 105, 37-44.

Eskandari, S.; Guppy, C. N.; Knox, O. G. G.; Backhouse, D.; Haling, R. E., (2017) Mycorrhizal colonisation of cotton in soils differing in sodicity. Pedobiologia 61, 25-32.

Knox, O. and B. Griffiths (2017). "Refinement of Passive Nematode Recovery from Cotton Growing High Clay Content Australian Vertisols." Communications in Soil Science and Plant Analysis 48(3): 316-325.

Knox OGG, Anderson CMT, Ross JL, Tann CCR, Gupta VVSR (2016) Organisms with potential to assist in the control of *Helicoverpa armigera* in Australian cotton production systems. Crop and Pasture Science, 67, 1288-1296.

Eskandari, S., C. N. Guppy, et al. (2016). "Improving mycorrhizal colonisation of cotton in sodic soils." Rhizosphere 2: 48-50

Oliver G G Knox, Vadakattu V S R Gupta and Richard Lardner (2014) Field evaluation of the effects of cotton variety and GM status on rhizosphere microbial diversity and function in Australian soils. Soil Research (52)213-225

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

Updates and progress to various aspects of this project have been publicised on the Cotton Hub at UNE via the blog (https://blog.une.edu.au/cottonhub/) and also via the twitter account @CottonHubUNE.

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.

The project UNE1403, Prodessor of Soil Systems Biology, was established between UNE and CRDC in 2014. Dr Oliver Knox was recruited from Scotland's Rural College (SRUC) to the post marking his return to Australia and the cotton industry. In the first few months of the project the Cotton Hub at UNE was established and the remit of this aggregation of academics established. Over the past five years the Cotton Hub, under Oliver's coordination, has been privileged to host the CRDC board twice at UNE, to develop a network of no fewer than 25 academics across five of UNE's nine schools, support several PhD candidates and foster more collaborative responses to funding calls, both within UNE and in collaboration with other research partners. The Cotton Hub has also generated several Spotlight and Cottongrower articles to increase the industry awareness of research activities associated with the hub as well as developing a growing social media presence via both an on-line blog and the Twitter handle @CottonHubUNE.

The research conducted under this project has also resulted in a modified and improved method for the passive recovery of free living soil nematodes from heavy clay soils, such as the grey and brown vertosols that much of the industry relies on. The method represents a saving in sample processing time as well as a reduction in potential sample size, in theory making more rapid nematode recovery and analysis possible.

In addition to this, the work has used capacitance probe data sets to establish the extent of the industry where cotton is being grown in just the top 60 cm of the profile. The analysis conducted over three seasons from 2015 to 2018, showed that approximately 25% of fields suffer from sub soil constraints that prevent root exploration below 60 cm during peak vegetative growth. These numbers are similar to physical studies conducted through the McIntyre in the 1980's, but have allowed areas from all of the cotton growing valleys to be studied across most years. The work has gone on to look at the nature of the constraints likely to be reducing root exploration and also observed that where an awareness of the constraint is known and appropriate management is put in place, profit margins remain good. Potential remedial management to alleviate some of these constraints is now being undertaken in other projects.

Border cells were again investigated under this project. Whilst newer cotton varieties have more than those that were commercially available 10 years ago there remains no link with the resistance to wilts or black root rot. Exogenous DNA (exDNA) from these cells was also quantified and appeared to have little effect on these pathogens, but it was also noted that cotton appears to produce less exDNA than peas and maize.

Finally, the project took on the delivery of the Cotton Production Course. The units continue to deliver a scientific approach to aspects of cotton production, protection, system development and position in the wider environment and to attract the majority of their students from industry.

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