Sugar Research Australia

FINAL REPORT 2018/009

DEVELOPMENT OF COMMERCIAL MOLECULAR BIOLOGICAL ASSAYS FOR IMPROVED SUGARCANE SOIL HEALTH AND PRODUCTIVITY

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ABSTRACT

Project research has shown that the DNA-based molecular assays for *Pachymetra chaunorhiza, Pratylenchus zeae* and *Meloidogyne* species quantify soil populations in field samples, confirming research undertaken in project 2016047, and has also confirmed the importance of sampling strategy and storage for obtaining representative data. Some further research is needed with *P. chaunorhiza* to ensure the accuracy of the molecular assay; the focus should be on sample storage conditions, amongst other things. Soil samples were processed from soil health projects, plant breeding selection trials and industry samples sent to the Tully soil assay laboratory.

The effects of storage temperature and time on results obtained from manual and molecular assays for *P. chaunorhiza, P. zeae* and *Meloidogyne* species were examined. Storage at 40^0C for even a short period led to failure of molecular assays to detect the parasitic nematode species. *P. chaunorhiza* manual counts were also affected, but only after 14 days.

For molecular assay of nematode samples, storing soils at 4^0C or 25^0C was the most effective way to ensure accurate results, but this was not so for the manual assay. Molecular assays for mycorrhizae and free-living nematodes (used to assess soil biological health) developed for other industries were found to be potentially useful for the sugarcane industry

EXECUTIVE SUMMARY

Soil-borne disease is a major limitation to productivity and profitability in the Australian sugarcane industry costing growers >$100 million annually. Soil and root health are a recognised major constraint to sugarcane productivity and profitability in Australia. Three root conditions lead to a substantive portion of the losses associated with poor root health; these are Pachymetra root rot (*Pachymetra chaunorhiza*), root lesion nematode (*Pratylenchus zeae*), and root knot nematode (*Meloidogyne* species). Previous research based on manual assays has developed yield risk thresholds for each condition. This has provided the basis for recommendations made by the SRA Soil Biology Assay lab to industry, which assists growers to implement appropriate management strategies.

Quantitative molecular (qPCR) assays were developed/evaluated for the three main organisms in a previous project 2016047. A key advantage of this technology is that one soil sample can be assayed for several organisms; this potentially may lead to significant cost efficiencies. Molecular assays also provide for the rapid processing of large sample batches, something not able to be achieved with manual assays. In addition, extracted DNA can be archived and later assayed for other organisms, if and when assays for these organisms have been developed.

To determine the accuracy of the molecular assay for the two parasitic nematodes species, manual and molecular assays for the two nematode species were compared utilising the manual count skills of five experienced nematologists in four separate nematode laboratories, based on the assay of a single set of soil samples from an experimental field site. The correlations between molecular and manual count methods for root lesion and root knot nematodes were very good in these soils. However, relatively poor correlations in manual vs molecular assays in soils from a couple of other sites raised the potential issue of nematode species variation; detailed sequencing of representative individuals led to the discovery of *Pratylenchus parazeae* in the Burdekin region. This species is known from overseas; the finding highlights the need to investigate the distribution and effects of *P. parazeae* on sugarcane growth and yield (as compared to *P. zeae*).

The correlation of manual vs molecular assay results for *P. chaunorhiza* at times gave poor results. Detailed examination of isolates from the Mackay region failed to show this was the result of pathogen variation. Data suggests that soil storage conditions may influence survival of *P. chaunorhiza* DNA; further investigation of the influence of this factor on the correlation is necessary.

Soil storage experiments found that molecular and manual data were affected differently when samples were stored at either 4ºC, 25ºC or 40ºC. *P. chaunorhiza* molecular assay results were stable when soils were stored at either 4ºC or 25ºC for any period up to 84 days; both manual and molecular assay populations declined with storage at 40ºC for >42 days. Degradation of DNA within *P. chaunorhiza* spores appears to occur before their physical degradation. High temperature storage led to very low manual and molecular assay results for *P. zeae*. Though long-term storage at either 4 or 25ºC reduced *P. zeae* manual assay results, molecular assay outcomes were not affected by these storage conditions, even up to 84 days. Soil storage conditions are important for obtaining meaningful assay outcomes.

A molecular assay protocol for groups of cereal free-living nematodes was provided by SARDI; these were developed to assess soil biological health in cereal soils based on the soil health index developed by Ferris et al 2001. Soils from two sugarcane soil health field trials were assayed for these free-living nematode groups. Whilst further studies are required to validate the method, the current results appear very promising and suggest that these same assays may be used to assess the health of sugarcane soils. Provision of a soil health assay service for Australian cane growers to benchmark management effects on soil biological health would be a significant development for the Australian sugarcane industry.

In a collaborative arrangement, Anders Claassen (USQ PhD student) used existing tests for arbuscular mycorrhizal fungi (AMF) to study the ecology of AMF in sugarcane. A key finding was that sugarcane is very selective; two very different biomes may exist - one in the sugarcane soil and a different biome in sugarcane roots growing in that same soil. Further work is required to study the effect of specific AMF groups on crop growth and implications for better fertiliser management.

Project staff also collaborated with Johann Pierre (CSIRO), with funding provided by 2018009 for molecular assays to map the spatial distribution of sugarcane roots and *P. zeae* across a commercial field.

The research undertaken in this project has demonstrated the value of molecular assays as a research tool and provided a basis for assessing the value of the service to cane growers. Further work is needed to investigate the reason for poor correlations between manual and molecular-based assays with *P. chaunorhiza*. There is also a need to develop a delivery model which outlines turnaround times, provision of grower technical support and data management, and development of a fee structure that works for all parties.

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1. BACKGROUND

1.1 Industry situation

- Poor root and soil health is widespread in the Australian sugarcane industry, with moderate-severe yield limitation a result. Such a situation is not a surprise, given the monoculture farming system widely adopted by the sugarcane industry.
- Yield decline research conducted from the late 1970s showed that major root pathogens contribute to this yield limitation (*Pachymetra chaunorhiza*, and several nematode species (*Pratylenchus zeae* and *Meloidogyne javanica*, *M. incognita* and *M. arenaria*)).
- Other root pathogens / parasites are also present, including, an undefined group of dematiaceous fungi, a range of endoparasitic and ectoparasitic nematodes and *Pythium arrhenomanes*, *P. graminicola* and *P. myriotylum*.
- Beneficial microbes are also present in cane soils, including free-living nematodes (which enhance nutrient cycling / prey on other nematodes), arbuscular mycorrhizal fungi (AMF; assist plant nutrient uptake (P and Zn)); nematode-trapping fungi (capture nematodes) and bacterial / protozoan species (attack insect and nematode pests).
- Labour-intensive assays used routinely to detect *P. chaunorhiza*, *P. zeae*, *Meloidogyne* spp and free-living nematodes (FLN) are time-consuming and currently limit assay capacity.
- A previous SRA project developed molecular assays for *P. chaunorhiza*, *P. zeae*, and *Meloidogyne* spp and showed that under experimental conditions, the molecular assays provided consistent and accurate results.
- Molecular assay of soil for a broad range of pests, pathogens and beneficial microorganisms will be important for providing better soil health information, with enhanced commercial assay value.

1.1.1. Project context

The research reported here was undertaken to test the predictive capability of the molecular tests under industry circumstances, to determine the applicability of several other assays developed in other industries to the sugarcane industry and to develop systems for assay delivery.

A successful outcome would provide industry with the ability to assay bulk samples quickly (rapid turnaround of results) and to diagnose several organisms using the same sampled soils. The ability to assay for other groups of organisms (FLN/AMF/roots) also offers the possibility of developing a soil health assay, a needed tool for assessing the impact of farming systems on soil and root health.

The project built on the outcomes from project 2016047 (developing molecular assays for major root pathogens / parasites) and also led to collaboration with projects investigating soil health, mycorrhizae and molecular assays for living sugarcane roots.

2. PROJECT OBJECTIVES

- **Objective 1**: Apply current PCR-based assay technology for *P. chaunorhiza*, *Pratylenchus zeae*, *Meloidogyne* species and other SARDI DNA-based assays to sugarcane soil health projects to introduce routine delivery of this technology to the sugarcane industry, providing base-line biological data for these projects.
- **Objective 2**: Develop a specific molecular assay(s) for at least 1 new target organism, by correlating results with a traditional assay. A target organism(s) will be selected during the course of the project.
- **Objective 3:** Calibrate the new test(s) against traditional assay methods to confirm potential industry assay recommendations.
- **Objective 4:** Fully implement the delivery of DNA molecular assays for sugarcane soil health assessment in a cost-effective manner and assess the need for further assay development.

3. OUTPUTS, OUTCOMES AND IMPLICATIONS

3.1 Outputs

The molecular assays for *P. zeae* and *Meloidogyne* spp should now be considered for further industry adoption. However, there remains a need to explore some variability between manual and molecular assays with *P. chaunorhiza*. If molecular assays are adopted, then some consideration needs to be given to the commercial pricing structure for the assays and a suitable delivery system (where assay data will be stored / how samples will flow to the SARDI laboratory, what will happen with samples when low industry volumes prevail in association with urgent timelines). There will be an additional opportunity to consider soil health indices alongside other industry researchers.

The target audiences for project outcomes are industry researchers, Productivity Board staff seeking to understand district or regional-based soil constraints and sugarcane farmers across the industry who wish to access specific information to manage individual crop-level soil issues.

Adoption of the technology has so far been limited due to the need for SRA Executive decisions on assay costs, database storage of information and a projected launch of the technology more broadly to industry.

3.2 Outcomes and Implications

The project has developed a number of assays that could be applied to both commercial sugarcane cropping situations and also to other industry research projects. Assays for *P. chaunorhiza, P. zeae*, Meloidogyne spp, free-living nematodes (FLN) have been shown to be of value. A new test was developed for *Pratylenchus parazeae*. Some refinement of a couple of these will be needed (*P. parazeae* / FLN). Research has also shown the value of living root DNA assays and those developed for AMF – both of these resulted from close collaboration with other research projects.

Further refinement of these assays, plus the application of nematode community analyses to FLN data, will provide a sound basis for further refinement of a soil health assay to benchmark farming systems research / farmer application. This is an important stepping-stone for the future of the sugarcane industry.

Industry value will come from the application of farming systems that improve soil and root health (and hence productivity improvement). Changing the soil environment is never rapid, so industry value will take some time to eventuate. What is important is that the research offers the opportunity to quantify positive changes in the soil biology – leading to adoption of the best practices for longer term sustainability.

Environmental outcomes for the sugarcane industry are very important in the context of Great Barrier Reef management and survival. The soil health assays could be used with other environmental monitoring (for example, DIN) to identify the best environmental approaches for farmers. Environmental outcomes will tend to dictate social outcomes in these circumstances (for instance, the social licence to farm).

4. INDUSTRY COMMUNICATION AND ENGAGEMENT

4.1 Industry engagement during course of project

Key messages delivered to industry have been the possibility of:

- Results for multiple organisms per soil sample,
- Soil biological health assessment based on nematode community analysis (Ferris et al, 2001).
- Greater capacity to process samples for industry-wide surveys,
- Expanding the array of molecular assays relevant to the sugarcane industry.

Communication activities largely centre on the following: i. shed meetings, ii. SRA information meetings, iii. researcher forums (soil health meetings). Limited information on the adoption of project outputs is available as research project staff have been the main users of the technology.

4.2 Industry communication messages

A concise summary of the communication message is as below: -

- **Assays**: molecular assays have been developed for the three main root pathogens / parasites (*Pachymetra chaunorhiza, Pratylenchus zeae, Meloidogyne* species). More refinement may be needed with *P. chaunorhiza*.
- **Reliability**: research has shown the assays to largely be reliable, with some exceptions, and how soil samples should be stored/ dispatched.
- **Other molecular tests**: have either been developed, or adapted, for both deleterious and beneficial organisms.
- **Soil health assay**: with a little more research, a soil health assay based on nematode community analysis could be developed and adopted by researchers and industry staff.

5. METHODOLOGY

Introduction

The main objective of research conducted in this project was to apply the technology developed in 2016047 (molecular assay development for *Pachymetra chaunorhiza*, *Pratylenchus zeae* and *Meloidogyne* species) to industry situations, to ensure that the molecular assays provide reliable assay results for the three pathogen / parasites. With successful and reliable application, the technology could be made available for industry adoption via the Tully soil assay laboratory. It is imperative that accurate results are returned to industry to ensure that the best management decisions are made and to minimise the yield constraints imposed by these organisms.

A key feature of the work was to compare results from traditional manual assays to those obtained via the SARDI molecular assay laboratory. The original project proposal embraced two types of research trials to make these comparisons: these were: i. soil health projects (trials from 3 projects), and ii. plant breeding trials (2 trials located in two different environments). As the project progressed, comparisons were expanded with the inclusion of soils from trials conducted by other researchers and from commercial industry samples.

Additional significant aims of the project were to: i. develop one more assay for an organism likely to be an issue in the sugarcane industry, and to ii. assess other molecular assays for other types of organisms, as developed by researchers in other industries. Key assays included those for FLN, AMF and living sugarcane roots.

5.1 Comparing molecular and manual assay data

5.1.1 Soil health projects

Three soil health projects were included in the scope of 2018009; these were: i. 2017005 (led by Danielle Skocaj) – a comparison of paired sites with traditional vs new farming systems, ii. 2018008 (led by Barry Salter) – sugarcane farming systems for soil health, and iii. HCPSL project (led by Lawrence DiBella) – rotation crops as affecting soil health. The project provided resources to test up to 400 soil samples per year from these projects.

Soil sampling was undertaken according to standard industry practice. Paired samples were sent to the Tully soil assay lab and the Adelaide SARDI laboratory or sent directly to SRA Tully with paired samples then concurrently dispatched to each laboratory.

Target organisms initially were *Pachymetra chaunorhiza, Pratylenchus zeae* and *Meloidogyne* species. Later on, free-living nematodes (FLN) were also assayed; these were grouped according to functional groups (manual counts) with DNA assays based on FLN found in southern Australian cereal crops. DNA assays for AMF (mycorrhizae) were also tested.

As only limited samples from soil health projects catered for a comparison of molecular and manual assays for *P. chaunorhiza, P. zeae* and *Meloidogyne* species, the focus of soil health project assays was on assessing molecular assays for AMF and FLN community analyses, to detect treatment effects on soil health.

5.1.2 Paired industry commercial samples

Introduction
Soil health project assay comparisons relied on project leaders submitting samples. Fewer than 400 soil samples were submitted for analysis, despite soil health project meetings (Friday 19th October 2018 / Monday 25th November 2019) being conducted through the course of 2018009 research.

Given the limited number of these samples, it was decided to increase the number of comparisons by assaying paired industry samples submitted to the Tully soil assay laboratory. Soil for molecular assay was sent to the SARDI DNA laboratory and a subset of the same soil submitted for manual assay at the Tully soil assay lab. Samples were assayed for *Pachymetra chaunorhiza*, *Pratylenchus zeae* and *Meloidogyne* species.

Method
Additional soil (approximately 1.0kg) was requested for routine soil samples sent to the Tully assay laboratory from across the Australian sugarcane industry. On receival, the soil was mixed, and then sub-sampled for manual assay at the Tully assay laboratory and for molecular assay at SARDI.

Pachymetra root rot
Over 200 paired samples were assayed. The results were analysed using 'box and whisker' plot by region (molecular vs manual counts).

Pratylenchus zeae (root lesion nematode)
Results arising from both manual and molecular assays were subjected to linear regression.

Meloidogyne spp (root knot nematode)
Assay results arising from both manual and molecular assays were compared using similar linear regression analysis (as per *Pratylenchus zeae*).

5.1.3 Plant breeding trials

Introduction
The SRA plant breeding program uses large field trials planted in each district to select high-yielding clones for progression to commercial release. In each region, (northern, Herbert, Burdekin, Central and Southern), at least four stage 3 trials (FATs) are planted. The clones under evaluation are known to differ in resistance to *Pachymetra chaunorhiza* and can lead to varying soil inoculum levels. Two such trials were targeted for assessment using the molecular assay to measure changes in *Pachymetra* levels over time and contribute to the comparison of count and molecular results, and preliminary resistance ratings for the clones. Samples also provided an opportunity to assess for resistance to *P. zeae* using the molecular assays.

Methods
Soil sampling in each of these trials followed the same general method, which is outlined below.

- ***Sampling depth***: 25cms, with 8 cores taken at random from the centre two rows.
- ***Sampling position***: from the shoulder of the planting mound.
- ***Sampling tool***: 4cm diam soil auger

Soils were placed in a 9-L bucket during collection, and then transferred to labelled plastic bags; samples were taken to the Tully Experiment Station. Over the next 7-21 days, the soils were sieved (0.5cm aperture), mixed thoroughly by hand and bagged ready for manual counting or molecular assay. Soils from all plots were dispatched to SARDI for molecular assay shortly afterwards.

Tully trial (TUL17-35)

Location: Tully Sugar Experiment Station. ***Planted***: 9 August 2017.

P harvest: 25 August 2018. ***1R harvest***: 26 August 2019, **2R harvest:** October 2020.

Clones / varieties: 149. ***Replicates***: 1-2. ***Plot size***: 4 rows x 10m ***Total plots***: 192

First soil sampling (end of plant crop)**:** 16-18 October 2018**,** all plots were sampled and assessed using qPCR tests for *P. chaunorhiza, P. zeae* and *Meloidogyne* spp. Ten soil samples were selected to compare count and qPCR data.

Second soil sampling (end of second ratoon): 2-6 November 2020

All plots were again sampled and assessed using molecular assays for *P. chaunorhiza, P. zeae* and *Meloidogyne* spp. 2020 and 2018 data were analysed correlated with soil populations present at the end of the plant crop. This included changes in populations and any interactions between the soil pathogen / parasites.

Thirty plot soils were selected for *P. chaunorhiza* manual assay to compare with molecular assay results.

Soil *Pachymetra* molecular results for each entry were compared to resistance ratings developed by glasshouse trials (not reported). All entries had been assessed at least once for resistance to *Pachymetra*. Resistance classifications for reference varieties used to benchmark yielding ability, had been developed using multiple experiments.

Mackay FAT (Central trial RAC16-31)

Location: Farm of Steve Orr, Rosella (near Mackay). ***Planted***: 19 October 2016.

P harvest: 24 July 2017; ***1R harvest***: 10 July 2018**; 2R harvest**: 1 Nov 2019; **3R harvest**: Sept 2020.

Clones / varieties included: 115 **Replicates:** 1-2 **Plot size**: 4 rows x 10m **Total plots**: 192

First soil sampling (First ratoon): 23-25 November 2018

Second soil sampling (Third ratoon): 1 October 2020.

Pathogen assessments
For sampling times 1 and 2, all soil samples were tested using molecular assays for *P. chaunorhiza, P. zeae* and *Meloidogyne* spp and data compared with to track changes over time for each clone.

At sampling times 1 and 2, 10 and 50 soil samples respectively were assessed visually to determine Pachymetra spore counts to compare with the molecular results.

***P. chaunorhiza* isolates**
Initial results from the Mackay site indicated that the *P. chaunorhiza* molecular assay was underestimating levels compared to count data. Several isolates of *P. chaunorhiza* were obtained from the Mackay site. Soil DNA was also tested using next generation sequencing to identify variants not detectable by the current qPCR.

Effect of clone resistance on *P. chaunorhiza* levels
Not all entries in the Mackay FAT had been assessed for Pachymetra resistance; resistance data were available for 61 individual plots. For some commercial varieties (standard canes used to benchmark yielding ability), a number of resistance assessments had been made; these provided more accurate data.

5.1.4 Nematode laboratory comparisons

Introduction
A critical aspect of this project was to compare the reliability of the molecular assays compared to manual counts. An opportunity arose to investigate this further using soil samples submitted to the Tully soil assay laboratory by QDAFI Bundaberg. The Bundaberg farming system researcher sent samples to both the Tully assay laboratory and the QDAFI nematode laboratory, Brisbane. Initial results suggested significant variation in nematode species populations. This led to questioning of the reliability of nematode laboratory assays and the need for further comparisons. As the Tully laboratory was involved, the opportunity was taken to compare the SARDI molecular assay results with manual counts made on the same soils by five nematologists in four different laboratories.

Method
The field site was an experiment established by Neil Halpin, QDAFI, Bundaberg. Several sugarcane rotation

treatments were incorporated into the replicated trial (randomized complete block design). Soil was collected from five different treatments, with each of the three field replicates represented. The soil was then sent to five different nematode laboratories and the SARDI Molecular Diagnostic Centre. Close communication between all nematologists and the field technician meant that soils were sampled and dispatched to all laboratories with similar transport and storage times (two days maximum between sampling and receival). Laboratories involved were: i. QDAFI Brisbane (two separate nematologists), ii. QDAFI, South Johnstone, iii. SRA Woodford, and iv. SRA Tully. Soils were carefully mixed and subsampled before dispatch.

In pre-assay meetings, details related to the proposed nematode assay method were discussed and each nematology laboratory adopted identical assay protocols to ensure assay uniformity. Counts were performed using the 'whitehead tray' technique, with a single paper tissue extraction 'membrane' beneath the soil and 48 hours nematode extraction time.

Nematode species were identified using high magnification and a 5 ml open counting chamber. The focus was particularly on the following species: *Pratylenchus zeae*, *Meloidogyne spp* and several minor plant parasitic species. Results were analysed using Pearson correlation statistics, with a comparison between all labs (five nematologists) and SARDI molecular assay of the same species.

5.1.5 Sample integrity

Introduction

An issue for any soil assay is the longevity of the measured propagule (oospore or nematode). With manual counts, the presence of living nematodes is a prerequisite for accurate population estimates. If soils are stored at excessive temperatures, then a zero nematode count is highly likely – which obviously wouldn't reflect the actual field nematode population.

A key issue is the optimum storage conditions for soils sent for molecular (vs manual) assay. Questions that industry staff sometimes ask are: i. do I need to keep the samples cool?, ii how long can I store them before dispatch to the SARDI lab?, iii. Is it ok for the soils to be kept in vehicles for an extended time?

Method

Soil was collected from a suitably affected sugarcane crop at Mourilyan, northern Queensland (Spanos series soil, South Johnstone Mill area). Forty kilograms of soil were collected, sieved, mixed thoroughly and representative soils placed in labelled plastic bags (capacity = >500g soil). Treatments were randomly assigned to each bag, with three replicates per storage time / storage condition.

Some samples were immediately dispatched to SARDI for molecular assay and a paired set were immediately processed to manually count Pachymetra spores and parasitic nematodes. Other samples were stored for different time periods under a range of conditions (Table 1). Initial manual and molecular assessments were performed on just three samples to establish a base-line for each treatment. Samples were stored at 4^0C in a refrigerator, 25^0C in an incubator and 40^0C in a drying oven.

Storage conditions: representative samples were then placed in storage (dark) under the following conditions: -

TABLE 1: STORAGE CONDITIONS FOR SOILS TESTING THE EFFECT OF TEMPERATURE AND TIME ON ASSAY RESULTS

STORAGE TIME (DAYS) / TEMPERATURE	0	7	14	42	72
4^0C	✓				✓
25^0C	✓	✓	✓	✓	✓
40^0C	✓	✓	✓	✓	✓

Development of a new test

5.1.6. Pratylenchus parazeae

Introduction: in assessing the relationship between manual and molecular assays for *Pratylenchus zeae*, several outliers (discrepancies) were found in several data sets; manual counts suggested significant *P. zeae* populations were present in some paired samples, while molecular assays indicated very few or no *Pratylenchus zeae* populations present. At the same time, there was a report of *Pratylenchus parazeae* being present in the South Burnett by researchers working on other crops. This species is very hard to distinguish morphologically from *P. zeae*. This led the SARDI team to consider developing a specific molecular assay for *P. parazeae* and to test sugarcane soils for this different species.

Soils from several soil health projects (Burdekin and Herbert sites) late in the project were assayed for both *P. zeae* and *P. parazeae*.

5.1.7. Free-living nematodes

Introduction
At the September 2019 soil health meeting, the potential for assessing soil health via nematode community analyses was discussed. Nematode community analyses, taking into account nematode community structure / both parasitic and free-living nematode genera and groups, is one indicator of soil health known to be influenced by farming system. Preliminary research by Bhuiyan *et al.* (2020) suggests that this indeed may hold for the Australian sugarcane industry. Other research (Katherine Linsell) in cereals has also shown the value of nematode community analyses. If molecular assays for various nematode groups reflected manual population assessments for the same groups, then large numbers of samples could be processed and a soil health measure provided to industry, without the extraordinary resources needed in manual processing.

There has been no specific sugarcane research to develop molecular assays for sugarcane FLN groups. However, FLN research conducted in cereals (Katherine Linsell, SARDI) has seen the development of molecular assays for similar FLN groups and species. The opportunity arose therefore to compare assay results for manual FLN population assessments (cane industry) with molecular assays (cereals) and to undertake soil community analyses. Soil health project soils were dispatched for molecular FLN assay.

Method
The focus of the molecular assays was on the following FLN species and groups: i. *Pratylenchus zeae*, ii. *Meloidogyne* species, iii. Aphelenchoididae, iv. Aphelenchidae, v. Cephalobidae, vi. Dorylaimidae, vii. Mononchidae, viii. Mononchidae, ix. Panagrolaimidae, and x. Rhabditidae. From the analyses, 'enrichment' and 'structure' indices were calculated based on published work (reference).

Soil samples from both Herbert (Skocaj) and Mackay (Salter) regions were sent to both assay laboratories and soils assayed for PPN and FLN nematodes. Initially bulk soils were sent to the Tully assay laboratory, mixed thoroughly and dispatched to the two laboratories. Assays were undertaken on 61 samples. For the manual assays, the following FLN groups were identified and counted (Table 2).

TABLE 2: FLN GROUPINGS FOR MANUAL AND DNA ASSAYS.

MANUAL COUNT GROUPINGS (SRA)	DNA-BASED ASSAY GROUPINGS (SARDI)
Bacterivores	Cephalobidae, Mesorhabditidae, Panagrolaimidae, Rhabditidae, Rhabdolaimidae
Aphelenchida/Fungivores	Achoid / Achus
Dorylaimids/Omnivores	Dorylaimids
Mononchids/Predators	Mononchids

From the results, community analysis trends were examined (Ferris, 2010) with a small sub-set of treatments (Skocaj soil health experiment considered in the light of FLN present.

5.1.8. AMF

Introduction: Anders Claassen (USQ PhD student) has been researching the species and groups of AMF in Queensland and New South Wales sugarcane soils and has accessed the services of the SARDI laboratory. Project 2018009 provided a specific link between all three research teams – utilising some of the findings from Ander's research, plus the AMF assays developed by SARDI for temperate crops to provide feedback to soil health project leaders on AMF species / groups in their experiments.

Method
Soil samples from the two soil health projects were subjected to SARDI AMF assays, using archived DNA from previous assays for PPN and *P. chaunorhiza*. Ander's research suggested that the genera and species present in sugarcane soils were appropriately quantified via the SARDI molecular AMF assays (Anders Claassen, personal communication). The classification for each of the assayed AMF groups is outlined in Table 3.

TABLE 3: SPECIES AND GROUPS OF AMF DETECTED BY EACH MOLECULAR ASSAY DEVELOPED BY THE SARDI LABORATORY.

MYCORRHIZAL GROUP (DNA)	AMF SPECIES DETECTED IN EACH AMF GROUP
AMF group A	*Funneliformis mosseae, F. constrictum, F. coronatum, F. geosporum, F. verruculosum, F. caledonium F. fragilistratum*
AMF group A2	*Rhizophagus intraradices, Funneliformis vesiculiferum, F. vesiculiferum, R. clarus, R. proliferus, Sclerocystis coremioides, S. sinuosa, R. manihotis, R. fasciculatus*
AMF group B	*Claroideoglomus claroideum*, *C. etunicatum*
AMF group C2	*Scutellospora calopsora, Gigaspora margarita*, *G. gigantea*
AMF group D	*Diversispora spurca*, *Glomus* spp.
AMF group E	*Acaulospora laevis*, *Entrophospora* spp.

5.1.9. Sugarcane roots

Introduction
In a separate project (2018003), Johann Pierre (CSIRO) researched the development of a molecular assay for living sugarcane roots. Manual separation of roots from soil, and measuring root lengths via scanning equipment, is labour-intensive, tedious and very time consuming. This makes such work very costly and difficult. With the development of a molecular assay for sugarcane roots, the possibility now arises to undertake much more detailed assessment of sugarcane root growth across cane fields.

At a September 2019 soil health meeting, it was decided that project 2018009 provide funding for molecular assays for *P. chaunorhiza* and *Pratylenchus zeae* on a series of 200 soil cores extracted from a single cane field in the Herbert region. This would enable Johann to match the root density with the detected populations of the target pathogen / parasites.

Method
Following the harvest of a plant crop in the Herbert, 200 soil cores were extracted from across a sugarcane field at a distance of 30cm from the planting line. The cores were split into differing depths, rapidly dried to preserve the DNA and sent to the SARDI laboratories in Adelaide for both root, pathogen and parasitic nematode assay. The data were then spatially analysed and 'heat maps' developed to illustrate the distribution of roots, pathogen / parasite across the cropping area.

6. RESULTS AND DISCUSSION

6.1 Soil health projects:

The results for assay of soil health project samples is outlined and discussed in more detail in sections 6.2.2 and 6.2.3. The data provide more relevant outcomes to sections on AMF and free-living nematodes

6.1.1 Industry samples

The results from paired comparisons of molecular and manual assays for *Pachymetra chaunorhiza*, *Pratylenchus zeae* and *Meloidogyne* spp are outlined below.

Pachymetra root rot

Count and molecular assay data detected regional differences (Figure 1). However, individual samples results were relatively poorly correlated, not helped by several outliers which had a substantial effect (Figure 2).

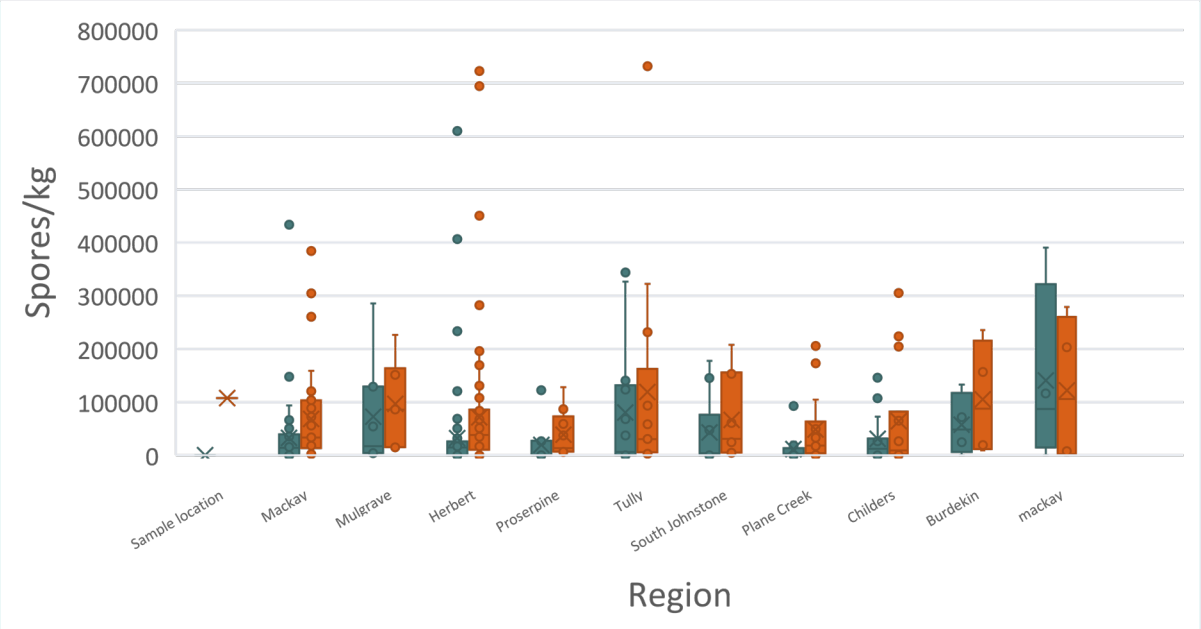

Figure 1
Box and Whisker plots of molecular (blue) vs count (red) data for Pachymetra root rot across the Queensland sugarcane industry.

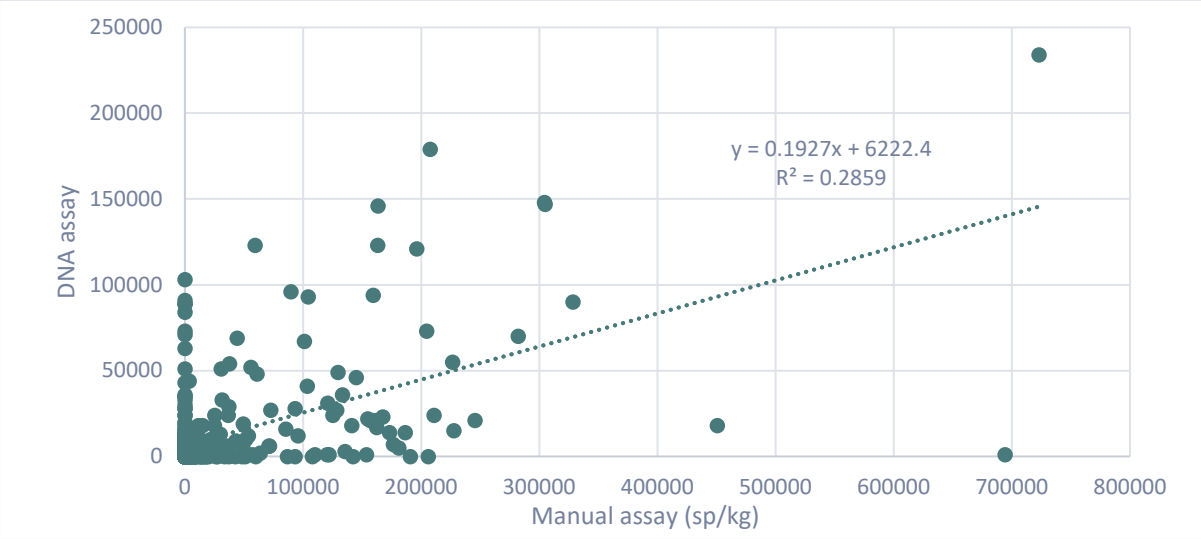

Figure 2
Comparison of P. chaunorhiza count and molecular data for samples from Burdekin, Central and Southern regions.

Root lesion nematode

The correlations between count and molecular data for industry soil samples was good and similar to that observed for root lesion nematodes in other industries (Figure 3).

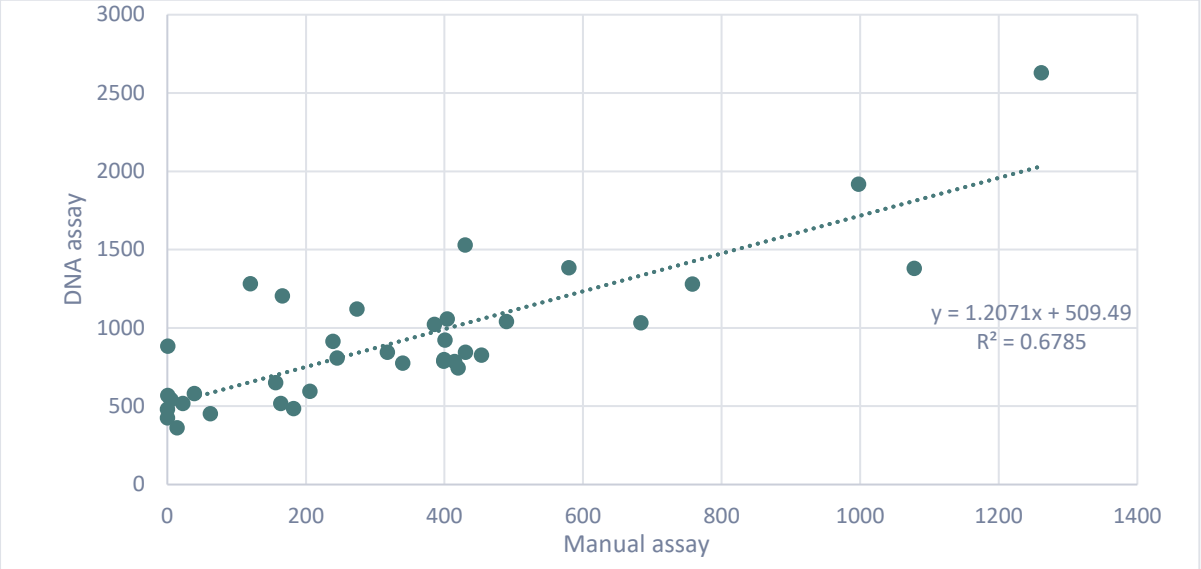

Figure 3
Correlation between count and molecular data for Pratylenchus zeae from industry nematology samples.

Root knot nematode

The correlation between count and molecular root knot nematode data for industry samples was relatively poor, due in part to several outlier samples (Figure 4). Both methods identified most infested samples however the molecular assay detected significantly greater levels of root knot nematode.

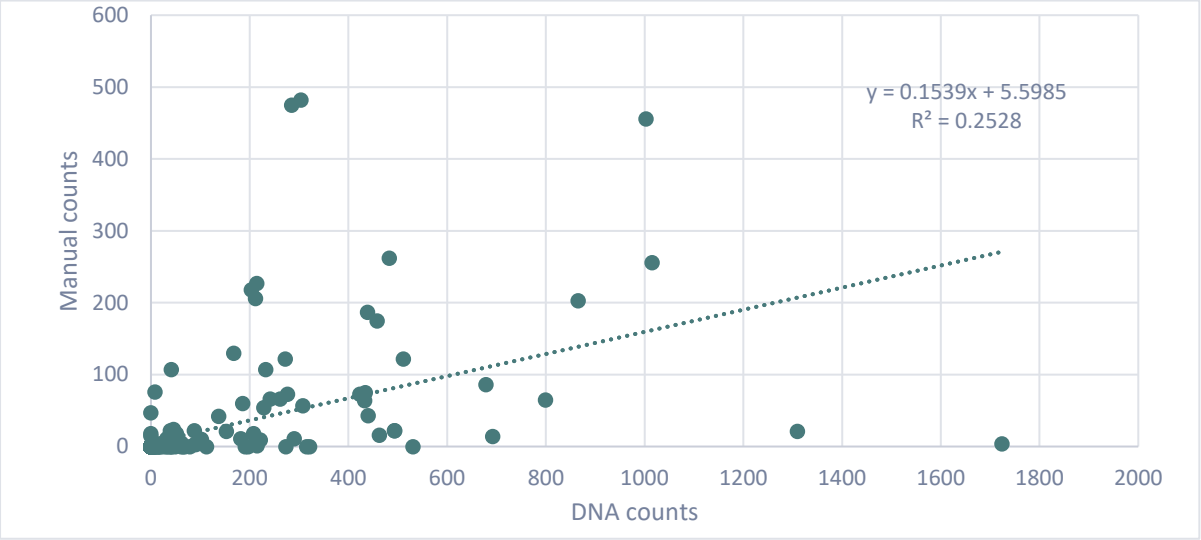

Figure 4
Comparison root knot nematode count and molecular data (expressed as nematodes / 200g soil) for all industry samples.

Discussion

The numbers of grower samples identified as infested with *Pachymetra*, *P. zeae* and root knot nematodes by count and molecular methods were similar, however the correlations between manual and molecular data for Pachymetra and root knot nematode were relatively poor, the relationship was much better when several outliers were excluded. The poor correlation for the *Pachymetra* data is discussed further in a later section.

For root knot nematode, the molecular assay detected far greater numbers than manual counts. This is probably due to the molecular assay detecting egg masses that are missed in manual counting. This is also discussed in section 6.1.4.

Tully plant breeding trial

DNA data shows that all plots contained *P. chaunorhiza* and *P. zeae*. Average populations across the site were *P. chaunorhiza:* 14,276 spores / kg: *P. zeae* 284 nematodes / 200g soil; and *Meloidogyne* spp 6 nematodes / 200g soil. These levels were relatively low and below the thresholds for yield loss.

Pachymetra root rot

The molecular assay data indicate that in most plots *P. chaunorhiza* populations were below 30,000 spore equivalents / kg soil, which is the threshold for yield loss (Figure 5). Levels were below detection in about 20 plots and some plots had up to 90,000 spores / kg soil, well above the yield loss threshold.

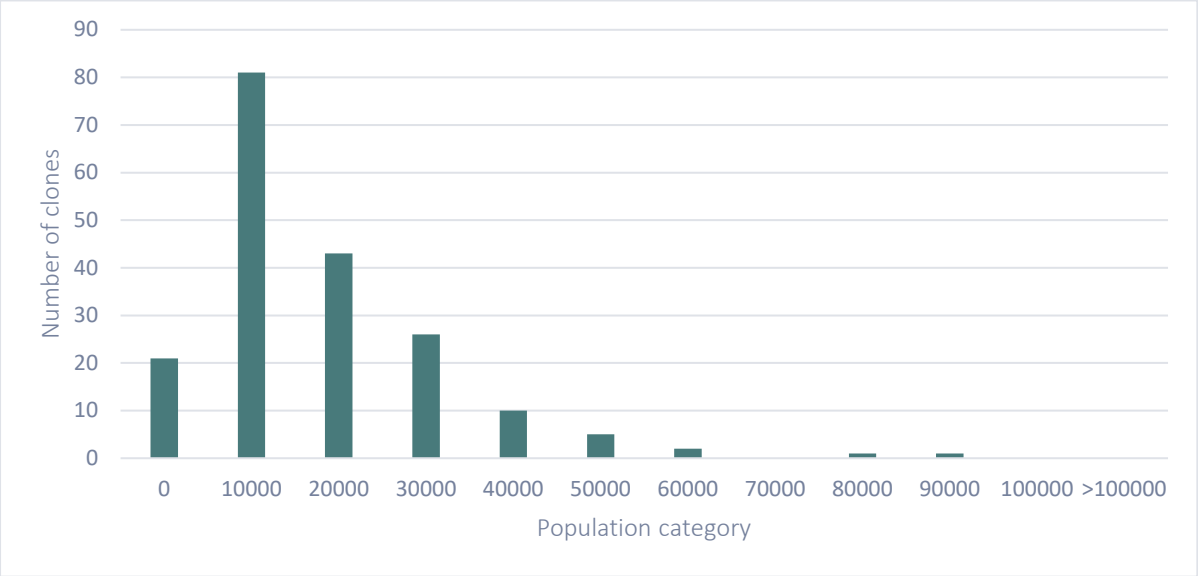

Figure 5
The number of clones in each Pachymetra population category (spore equivalents / kg soil), October 2018, Tully plant breeding selection trial (TUL16-35).

Pratylenchus zeae manual count vs molecular data

At the first sampling time, 10 paired samples were assessed by manual and molecular assays. The correlation was good if the regression was forced through zero (Figure 6). *Pratylenchus zeae* counts were generally low, except for six plots with over 1,000 / 200g soil (Figure 7).

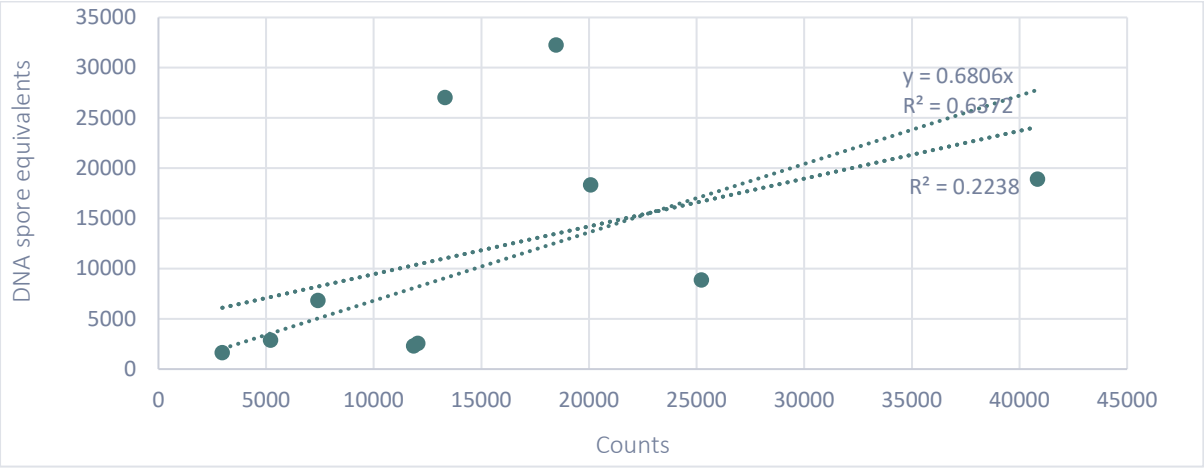

Figure 6
Manual vs molecular population equivalents in 10 plots randomly selected from the Tully FAT trial.

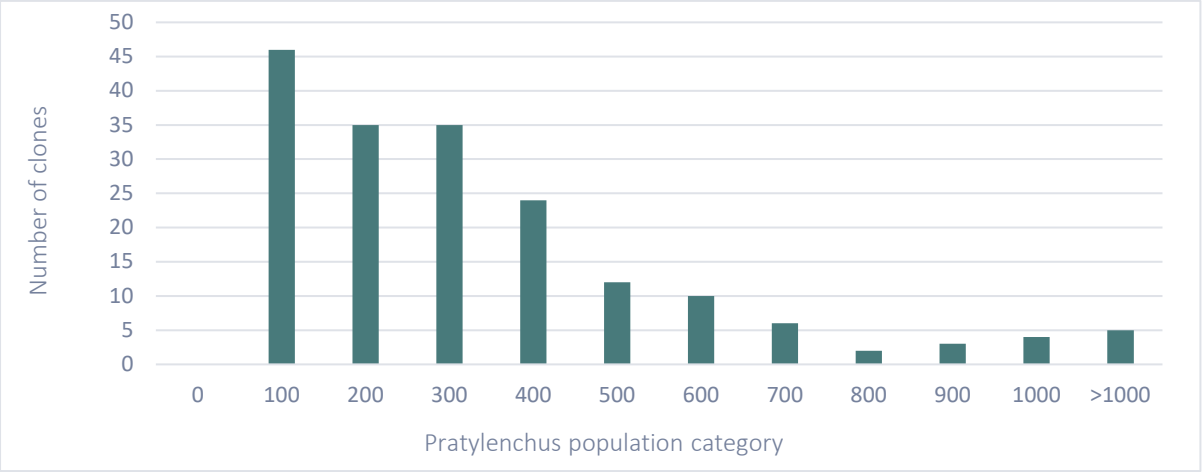

Figure 7: Clones in each Pratylenchus category (nematodes / 200g soil) in the Tully plant breeding trial (TUL16-35), October 2018.

Meloidogyne spp (root knot nematode)

Root knot nematode level were low – it was not detected in 120 plots and a large number where populations were below 100 / 200g soil (Figure 8). High populations generally only occur on sandy soils.

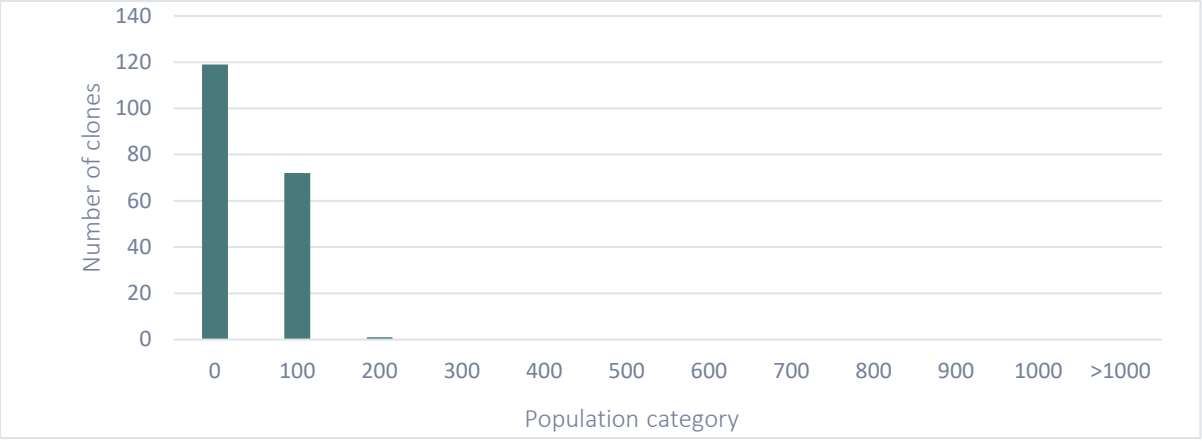

Figure 8: Clones in each Meloidogyne category (nematodes / 200g soil) in the Tully plant breeding trial (TUL16-35), October 2018.

Pathogen associations

Data for *P. chaunorhiza, P. zeae* and *Meloidogyne* Tully FAT trial were examined to see if there were any significant correlations between each other; none were detected and the data are not presented.

Second sampling

Average molecular assay populations of *P. chaunorhiza, P. zeae* and *Meloidogyne* spp in the Tully FAT trial at the second sampling time in 2020 were *P. chaunorhiza* 17,321 spores / kg; *P. zeae* 259 nematodes / 200g soil; and *Meloidogyne* spp 8 nematodes / 200g soil.

Pachymetra root rot

Changes in *Pachymetra* populations in each plot between 2018 and 2020 are outlined in Figure 9. The qPCR data indicates *Pachymetra* populations had increased slightly across most plots, with most still below the threshold for yield loss (30,000 spores / kg soil), and below detection in 50 plots (Figure 10). However, populations in some plots were high, and up to 100,000-200,000 spores / kg soil.

The correlation between manual and molecular assays for the 30 samples assessed in 2020 was far better than for the 10 samples assessed in the 2018. It should be noted that in this case that a single high spore count contributes significantly to the higher R^2 value (Figure 11).

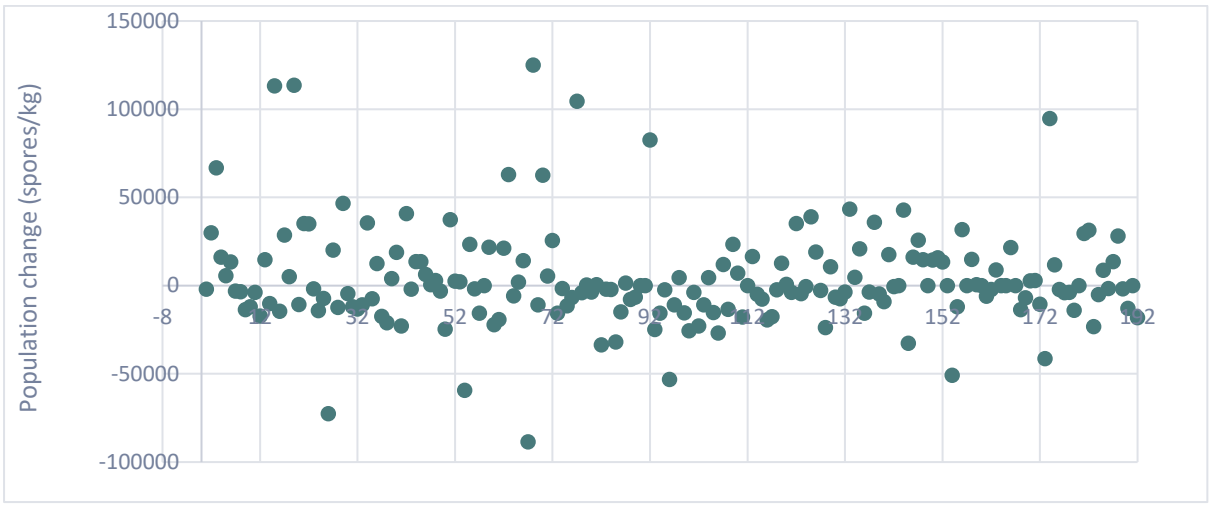

Figure 9:
Change in Pachymetra populations in each plot (molecular data): 2020 minus 2018.

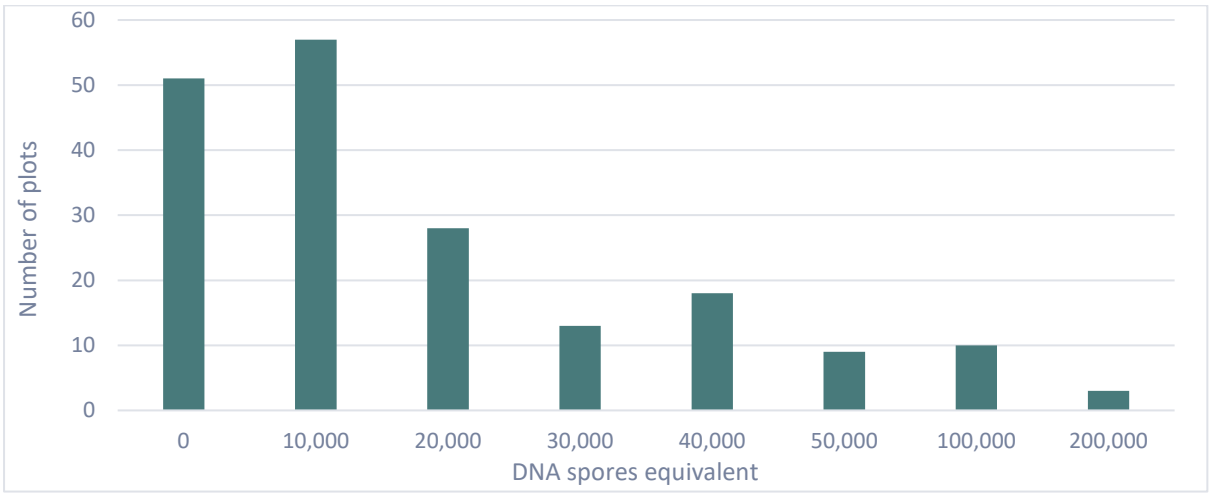

Figure 10:
The number of plots in each Pachymetra root rot population category (spores/kg soil) in soils sampled in November 2020 in the Tully plant breeding selection trial (TUL16-35).

Pachymetra manual vs molecular data

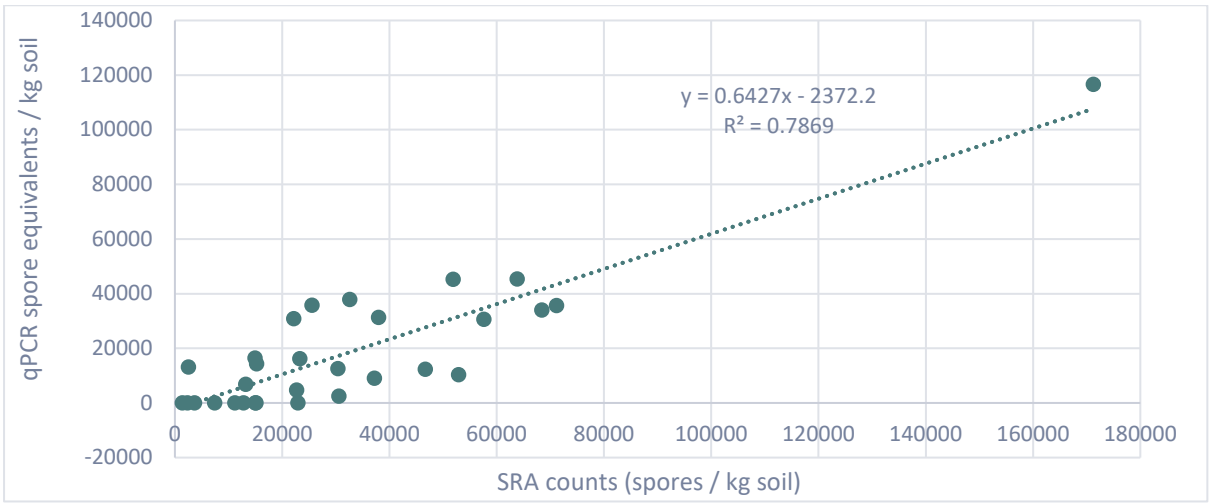

Figure 11:
Manual vs molecular assays in soils from 30 plots in soils sampled in October 2020 from the Tully FAT trial.

Pratylenchus zeae

Average *P. zeae* levels were similar to those detected in 2018 are remained relatively low, apart from six plots with high populations some over 1,000 individuals / 200g soil (Figures 12 and 13). Clones in plots where *P. zeae* levels and had decreased or increased most should be investigated further to identify possible sources of resistance,

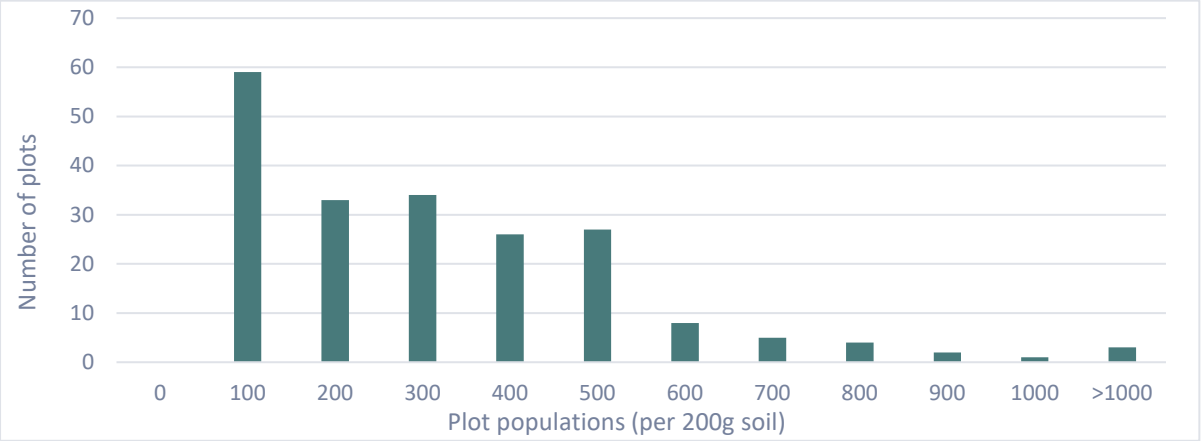

Figure 12:
The number of plots in each Pratylenchus population category (nematodes / 200g soil) in soils sampled in November 2020 in the Tully plant breeding selection trial (TUL16-35).

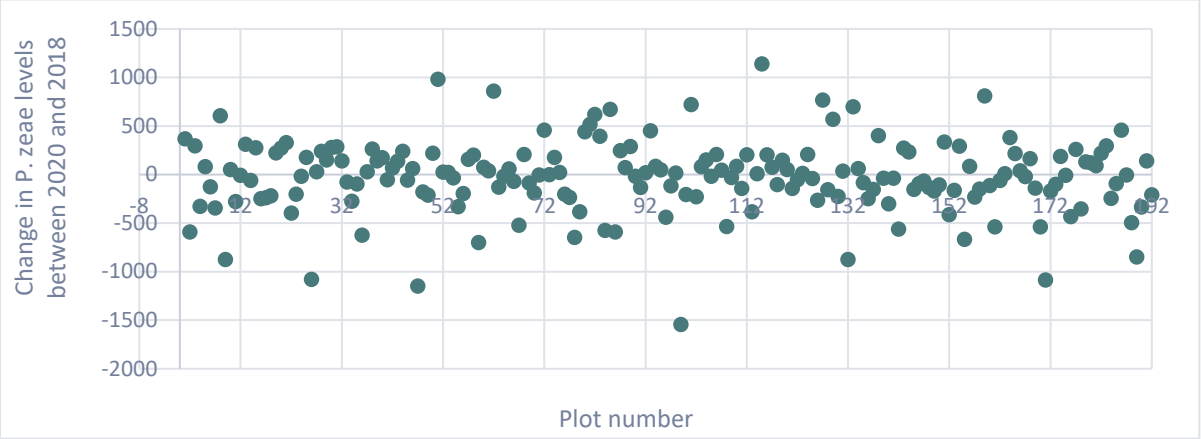

Figure 13:
Change in Pratylenchus zeae populations in plots between 2018 and 2020 (nematodes / kg soil).

***Meloidogyne* spp.**

Root knot populations in the Tully trial were below detection in 150 plots and <200 nematodes per 200g soil in 50 plots (Figure 14). The change in root knot nematode populations in each plot is illustrated in Figure 15.

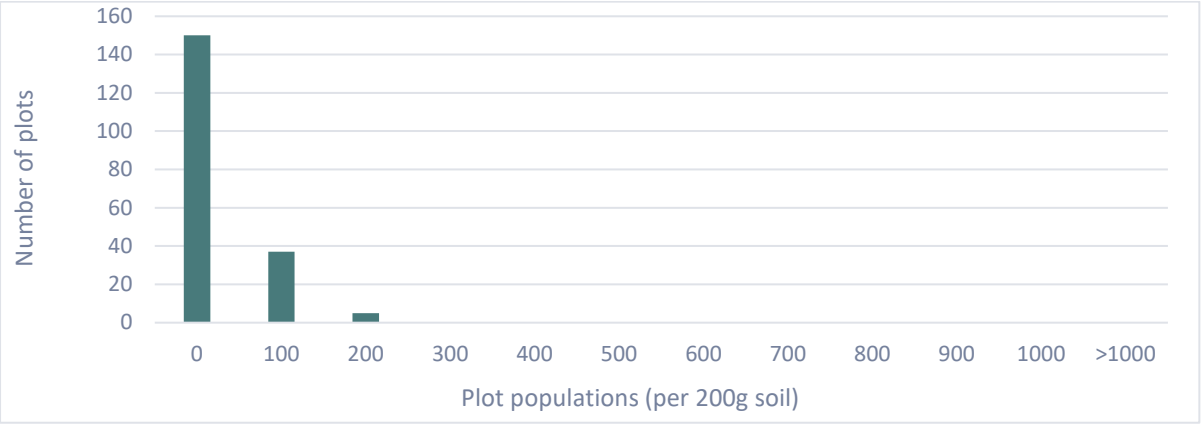

Figure 14:
Number of clones in each Meloidogyne population category (nematodes / 200g soil) in soils sampled in November 2020 in the Tully plant breeding selection trial (TUL16-35).

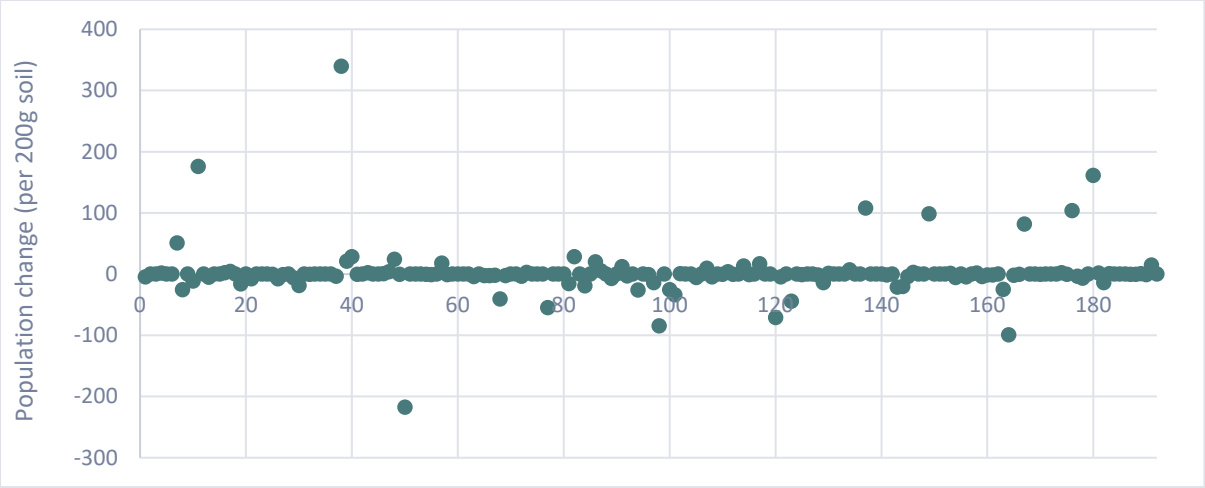

Figure 15:
The change in Meloidogyne population (nematodes / 200g soil) in each plot in soils sampled in November 2020 in the Tully plant breeding selection trial (TUL16-35).

Mackay plant breeding trial

First sampling
The site average levels (10 samples) for *P. chaunorhiza* was 32,517 spores / kg; *P. zeae* 231 nematodes / 200g soil; and *Meloidogyne spp* 43 nematodes / 200g soil

Pachymetra
Manual vs molecular assay data

Following the very poor detection of *P. chaunorhiza* by the molecular assay in many samples from Mackay (approximately half), 10 repeat assays on stored samples were dispatched to the SARDI laboratory and paired samples subjected to manual assay at the Tully laboratory. Manual assay data indicated all samples contained *P. chaunorhiza*, several with high populations (Table 4). The corresponding molecular data detected low *P. chaunorhiza* levels in the first assessment, and in only one in the repeat samples. This indicated there could be a sample integrity issue that needs to be managed.

TABLE 4: *P. CHAUNORHIZA* MANUAL AND MOLECULAR ASSAY RESULTS (SPORES / KG) FOR 10 RANDOM SAMPLES FROM THE MACKAY FAT TRIAL AT ROSELLA

SAMPLE ID	MANUAL	DNA
	6970	0
	10379	0
	15126	0
	18223	3000
	19210	0
	32506	0
	40304	0
	45144	0
	52118	0
	85193	0

Pratylenchus zeae

Root lesion nematodes were present in almost all soils assayed (Figure 16).

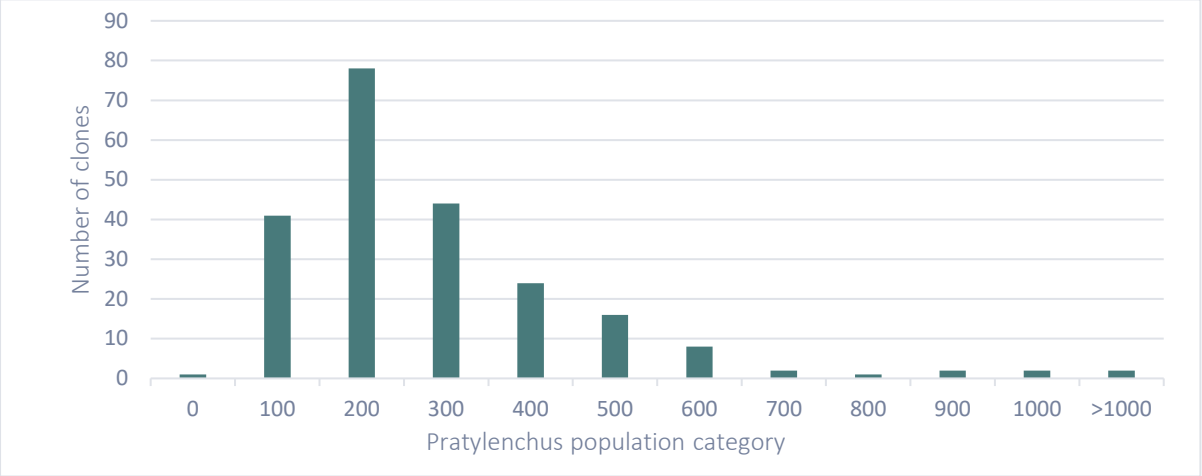

Figure 16:
Sugarcane clones in each P. zeae category (nematodes / 200g soil) in the Mackay plant breeding trial (RAC16-31) sampled in November 2018. The numbers represent plots with less than, or equal to, the stated population (and above the previous category).

Meloidogyne spp

Root knot nematode was more infrequent, as expected with this species (Figure 17).

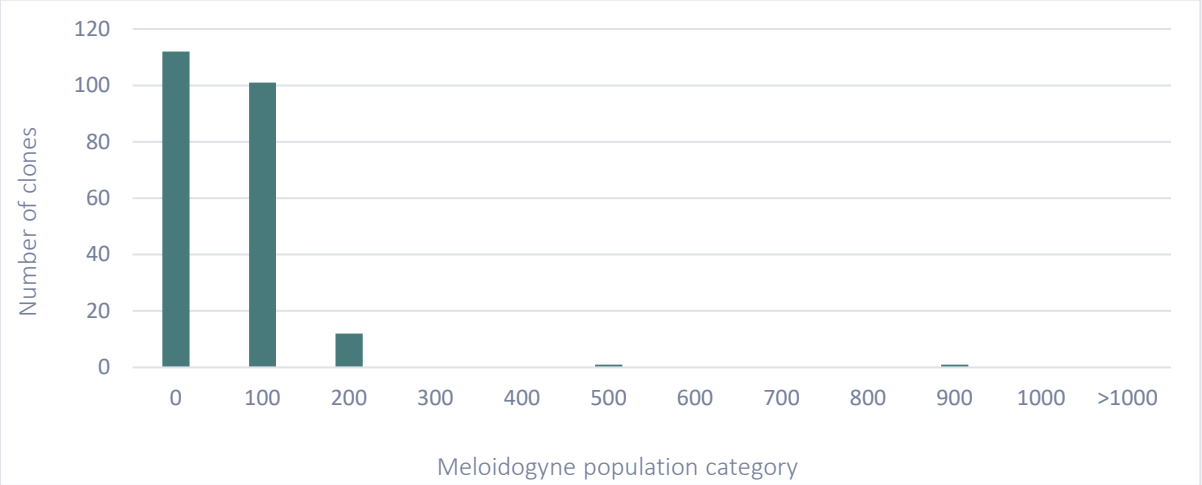

Figure 17:
The number of clones in each Meloidogyne population category (individuals / 200g soil) in soils sampled in November 2018 in the Mackay plant breeding selection trial (RAC16-31). The numbers represent plots less than, or equal to, the stated population (and above the previous category).

There was no relationship between populations of each nematode species at sampling times 1 or 2; data are not presented.

Second sampling

Mean populations over all plots for *P. chaunorhiza* was 25,752 sp/kg; *P. zeae* 231 nematodes / 200g soil; and *Meloidogyne* spp 12 nematodes / 200g soil.

Pachymetra root rot

P. chaunorhiza populations were greater in 2020 and not correlated with the populations in the same plots in 20180 (Figure 18). The net changes per plot are illustrated in Figure 19. The data indicate *Pachymetra* had increased in the two years between sampling events. These changes are further illustrated in Figure 20, with spore count data categorised into different categories for the 2018 and 2020 data.

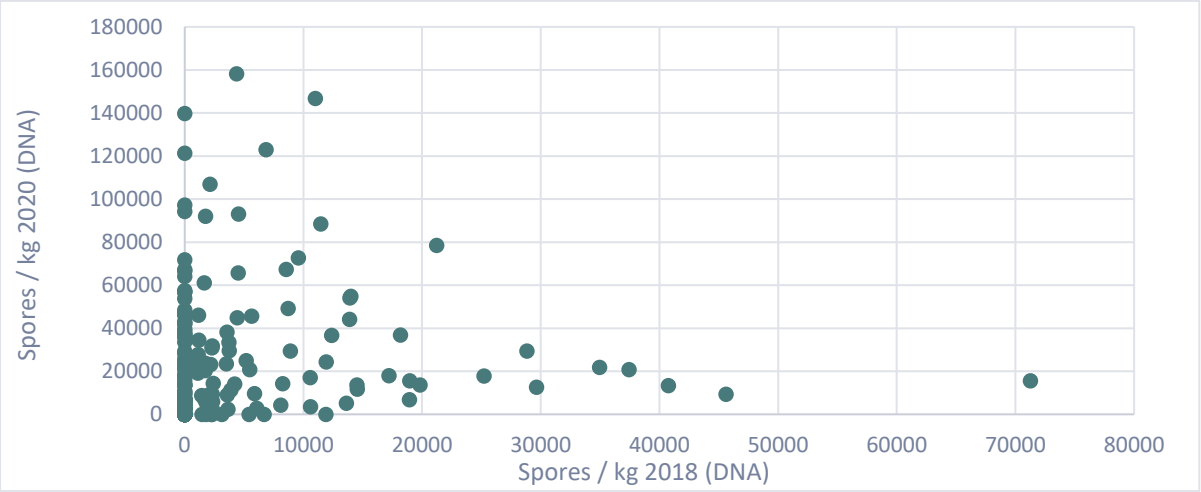

Figure 18:
There was no correlation between P. chaunorhiza populations in plots in the Mackay FAT trial between 2018 and 2020.

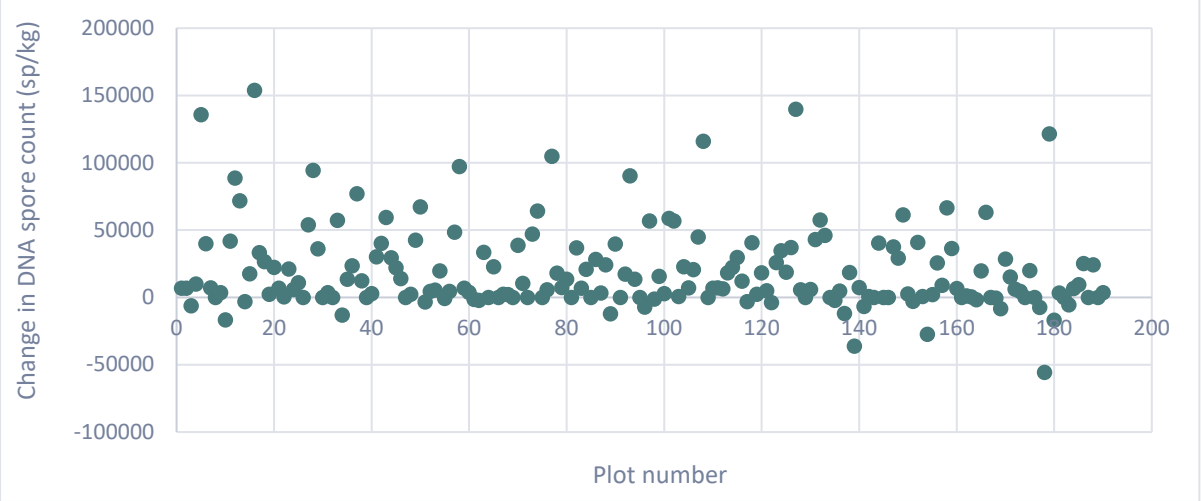

Figure 19:
Change in P. chaunorhiza populations in each plot (molecular assay results) in the Mackay FAT trial: data reflect the 2020 population minus the population present in 2018.

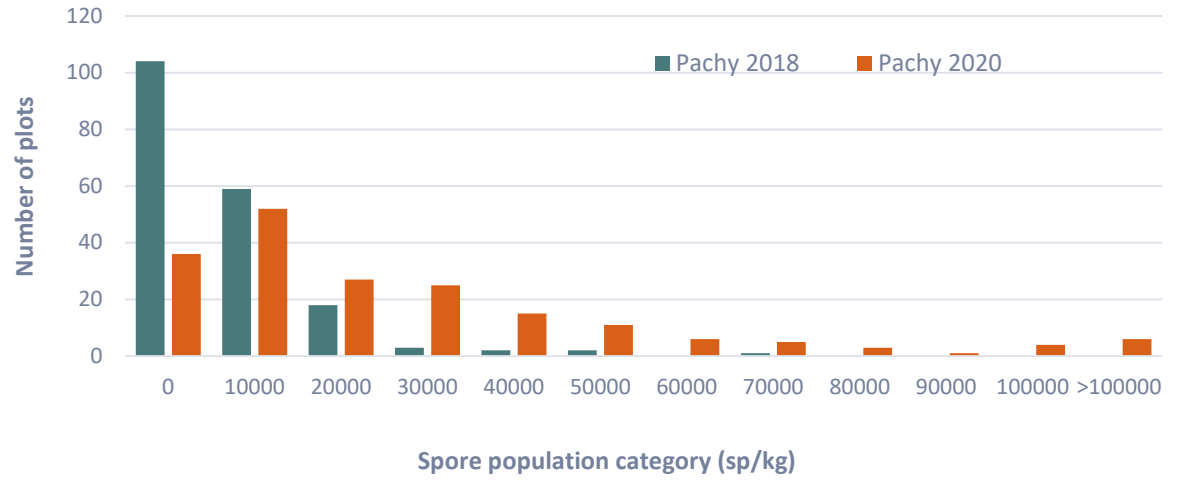

Figure 20:
Clones in each P. chaunorhiza category in 2018 (green) and 2020 (red) in the Mackay FAT trial.

Potential variants of *P. chaunorhiza*

The molecular assay detected each of the new *Pachymetra* isolates obtained from the Mackay FAT site (six in total). Next generation sequencing of soil DNA did not find any mutations (in sequences that the assay targeted) that would affect detection. This suggests that the poor detection in samples sourced in 2018 from the Mackay site was probably associated with DNA degradation resulting from sample storage conditions.

Manual vs molecular assay data

A comparison of manual v molecular assays for the soils collected from the Mackay FAT is illustrated in Figure 21.

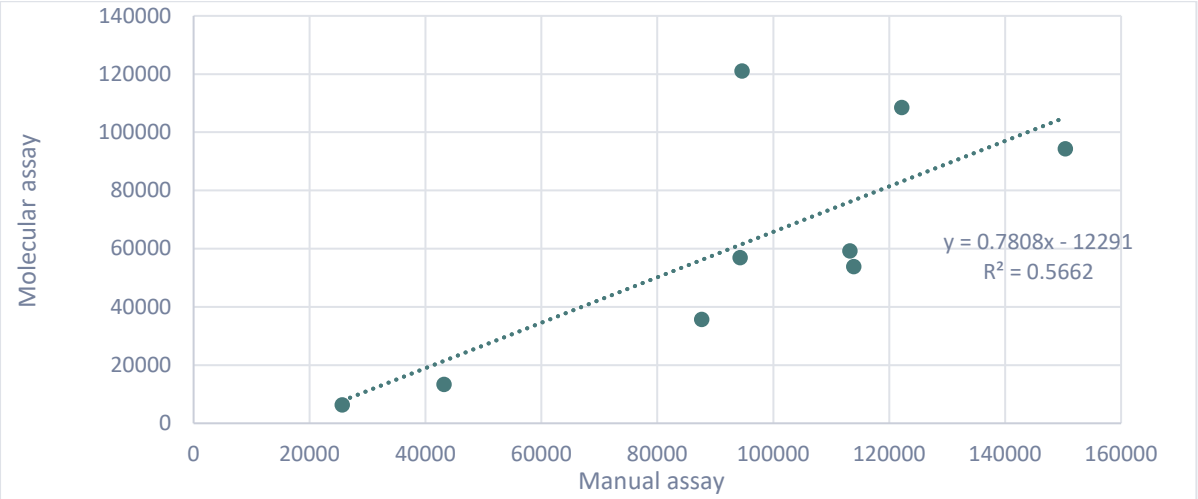

Figure 21:
Comparison of count and molecular data for selected plots from the Mackay FAT trial.

Inoculum v clone resistance rating

The relationship between clone resistance rating (arising from a glasshouse resistance screen) and the inoculum level in the early third ratoon crop (molecular assay) is illustrated in Figure 22

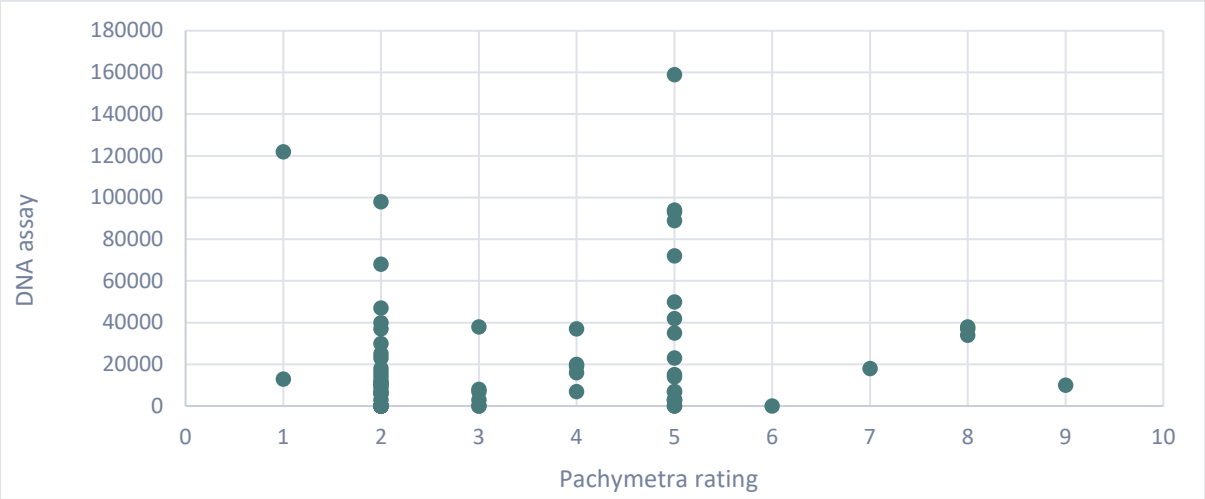

Figure 22:
Mackay FAT trial Pachymetra molecular assay data plotted against the clone glasshouse-derived resistance rating.

P. zeae

P. zeae populations in the Mackay FAT trial did not change substantially between 2018 and 2020 (Figure 23). Figure 24 illustrates that there were changes in root lesion populations in individual plots, even though average population levels did not change significantly.

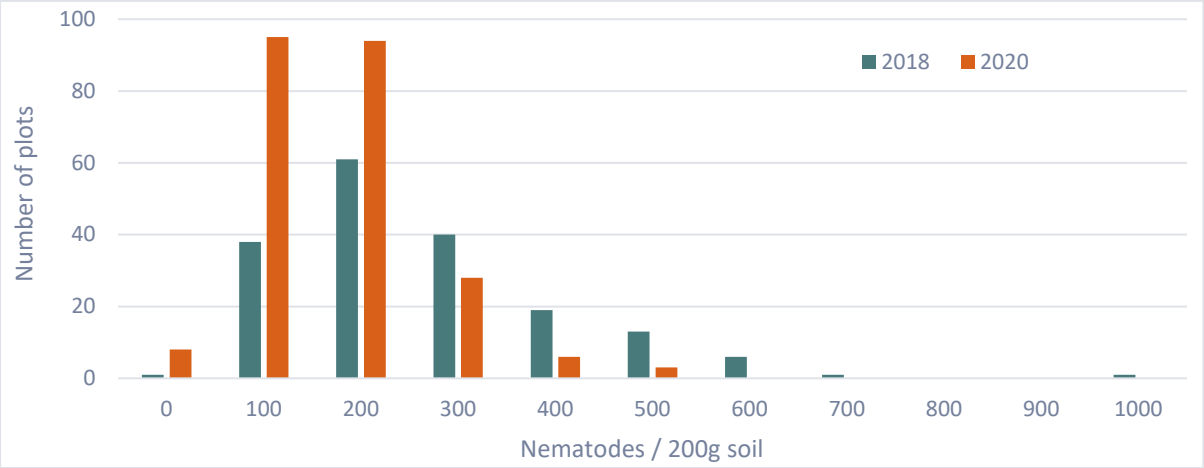

Figure 23:
Pratylenchus zeae levels in 2020 (red) and 2018 (blue); there was a gradual decline in nematode levels in the two years between samplings.

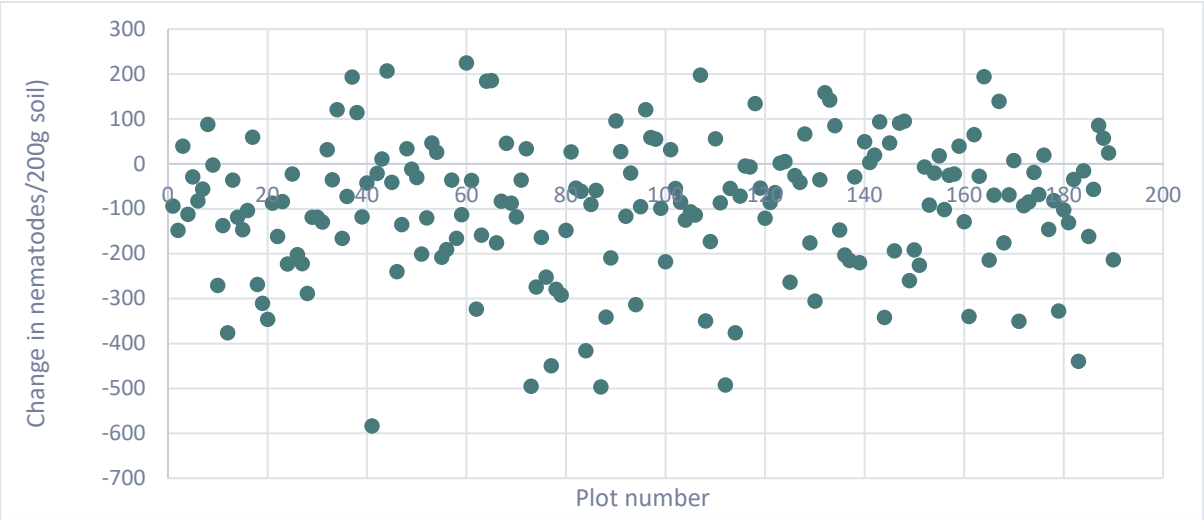

Figure 24:
Changes in Pratylenchus zeae levels in the Mackay FAT trial between 2018 and 2020.

Meloidogyne spp:
Root knot populations dropped significantly during the two-year sampling period (Figures 25, 26). The low and declining root knot populations illustrate the relatively poor hosting ability of sugarcane (compared to some other host crops) and the heavier soils present at the site (root knot nematodes proliferate in sandy soils).

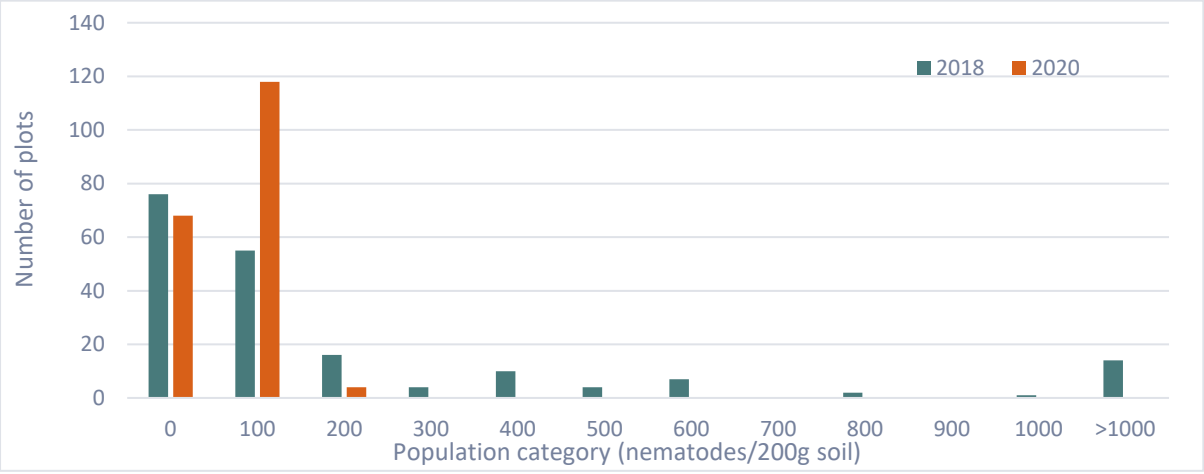

Figure 25:
Meloidogyne populations per plot in the Mackay FAT trial in 2018 (blue) and 2020 (red).

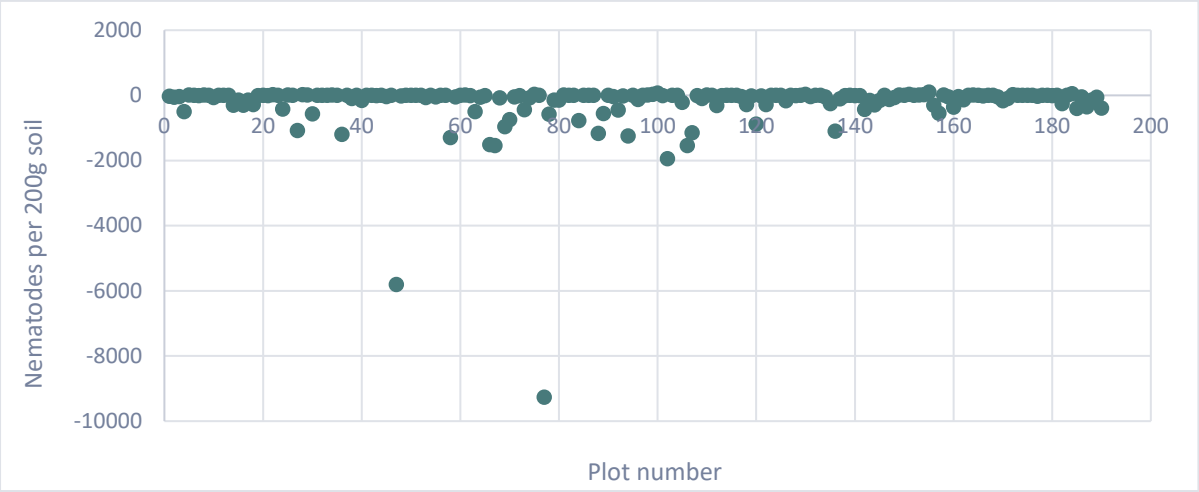

Figure 26:
Changes in Meloidogyne populations per plot at the Mackay FAT trial site between 2018 and 2020.

Discussion

Significant outcomes from this part of the research include variable correlation between manual and molecular assay data for *P. chaunorhiza*. The project investigated if there were *P. chaunorhiza* variants at this site that the molecular assay could not detect; none were found. Next generation sequencing of stored DNA of the aberrant samples also did not find sequence variations that would prevent detection. Problems with sample integrity is now the most likely explanation for the poor detection of *P. chaunorhiza* in Mackay.

Molecular assay results for soil samples collected at the second sampling two years later correlated better with manual counts.

Nematode molecular assay data reflected what was expected based on previous research, including that *Pratylenchus zeae* is very common across all sugarcane soils; that *Meloidogyne* populations are usually low and sporadic in soils other than sands, and that populations of both nematodes decline in farming systems that don't favour multiplication. Root knot nematode was only found in a relatively small proportion of breeding trial plots.

The molecular assay method enabled assays to be conducted for multiple organisms on large numbers of soil samples. This facilitated the monitoring of changes in individual pathogens/parasite populations associated with individual clones in different plant breeding trials over time.

6.1.2 Nematode laboratory comparisons

Pratylenchus zeae

Correlations between *P. zeae* manual counts obtained by five experienced nematologists and molecular assay data provided by the SARDI Molecular Diagnostic Centre were very good, varying between 0.73-0.94 (Table 5).

TABLE 5: CORRELATIONS (PEARSON) BETWEEN *PRATYLENCHUS ZEAE* COUNTS MADE BY FIVE NEMATOLOGISTS AND MOLECULAR ASSAY DATA BASED ON ASSAYS CONDUCTED ON REPLICATED SOIL SAMPLES FROM BUNDABERG.

Laboratory	SARDI	ESPJC	ESPON	SJRS	Tully	Woodford
SARDI	1.0000					
ESPJC	0.7374	1.0000				
ESPON	0.8698	0.9620	1.0000			
SJRS	0.9407	0.9069	0.9775	1.0000		
Tully	0.7984	0.9390	0.9530	0.9312	1.0000	
Woodford	0.8813	0.9474	0.9868	0.9847	0.9793	1.0000

***Meloidogyne* spp**
Correlation among the five nematologist root knot nematode counts and molecular assay results varied between 0.75-0.99 (Table 6).

TABLE 6: CORRELATIONS (PEARSON) BETWEEN *MELOIDOGYNE* COUNTS OF FIVE NEMATOLOGISTS AND MOLECULAR ASSAY RESULTS FOR SOIL SAMPLES FROM BUNDABERG

	SARDI	ESPJC	ESPON	SJRS	Tully	Woodford
SARDI	1.0000					
ESPJC	0.7574	1.0000				
ESPON	0.9681	0.8591	1.0000			
SJRS	0.8766	0.8619	0.9168	1.0000		
Tully	0.7981	0.8854	0.9153	0.7859	1.0000	
Woodford	0.9925	0.8215	0.9894	0.9175	0.8508	1.0000

Discussion
Correlation statistics between all nematologists and the molecular assay results shows there was good agreement amongst the different methods. These data confirm the findings obtained in the previous project (2016/047).

These analyses provide confidence in the molecular assays for *Pratylenchus zeae* and *Meloidogyne* species and show that with samples stored and transported appropriately, there is good agreement between manual counting and molecular assays.

6.1.3 Sample integrity

Effect of sample storage on *Pachymetra chaunorhiza*
The manual assay data for soils stored at different temperatures and times is summarised in Figure 27. The results show that manual assay of soils stored at 40^0C are stable for around 14 days; after this time, spore populations decline. After 84 days at 40^0C, *P. chaunorhiza* oospores are almost undetectable. On the other hand, storage at 4^0C or 25^0C, appears satisfactory for at least 84 days. While molecular assay data shows that DNA is stable for soil stored at 25^0C for up to 42 days (Figure 28).

Effect of sample storage on *Pratylenchus zeae*
Manual count data for *P. zeae* indicate that storage conditions are more important than for *P. chaunorhiza*. *P. zeae* DNA was not stable in samples stored at 25ºC for >7 days. Counts on samples stored at either 4 or 25ºC for 84 days would not provide meaningful assay results.

Molecular assays provided a much more stable indication of nematode populations at either 4 or 25ºC. However, sample storage at 40ºC for either manual or molecular assay led to much reduced (or zero) populations (Figures 29, 30).

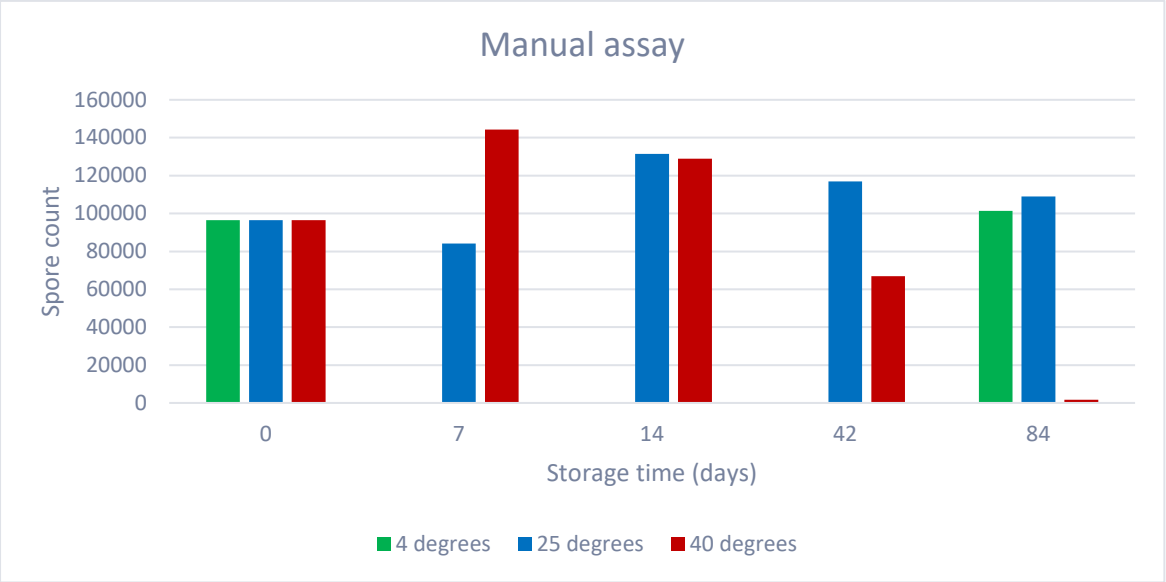

Figure 27:
The effect of storage temperature and time on P. chaunorhiza manual spore counts. No assays were conducted on soils stored for 7, 14 and 42 days at 40C.

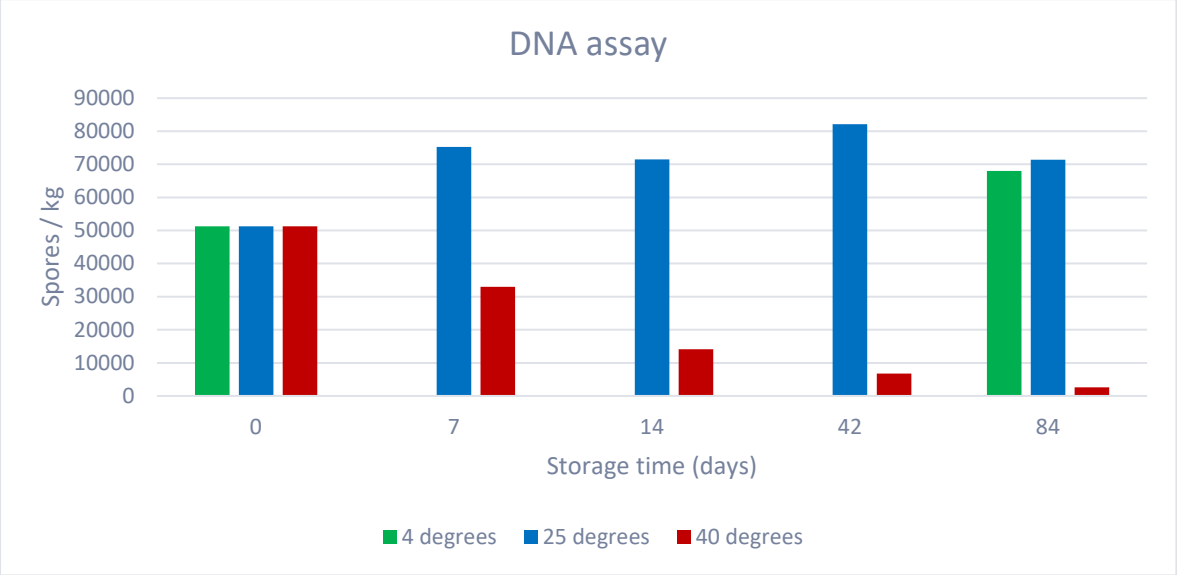

Figure 28:
The effect of storage temperature and time on P. chaunorhiza molecular assay results. No assays were conducted on soils stored for 7, 14 and 42 days at 4^{0}C.

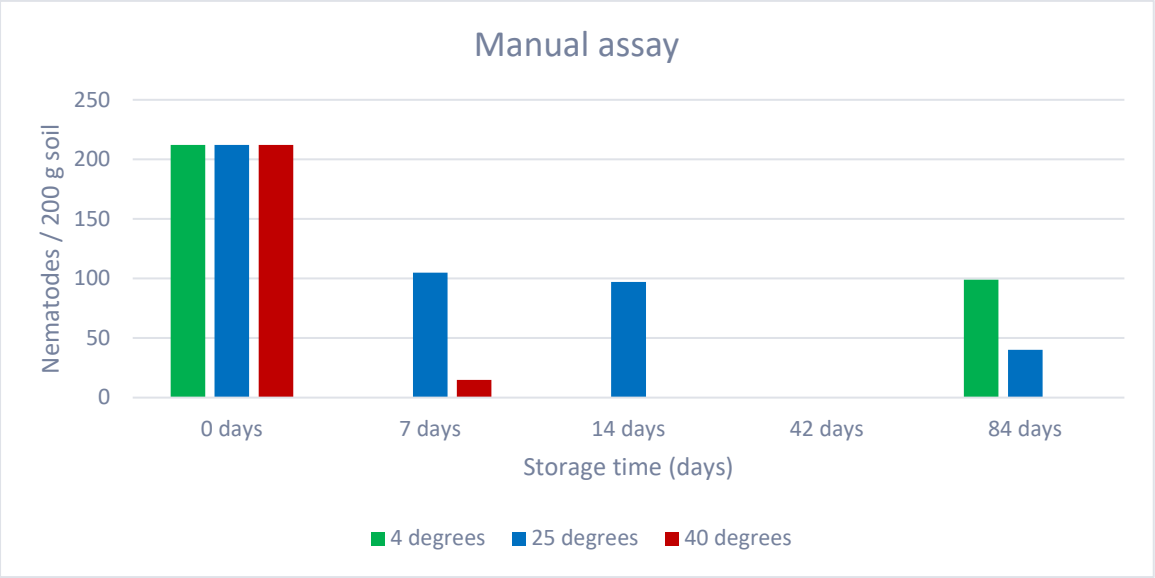

Figure 29:
The effect of storage time and temperature on P. zeae manual count results. No assays were conducted on soils stored for 7, 14 and 42 days at 4^{0}C.

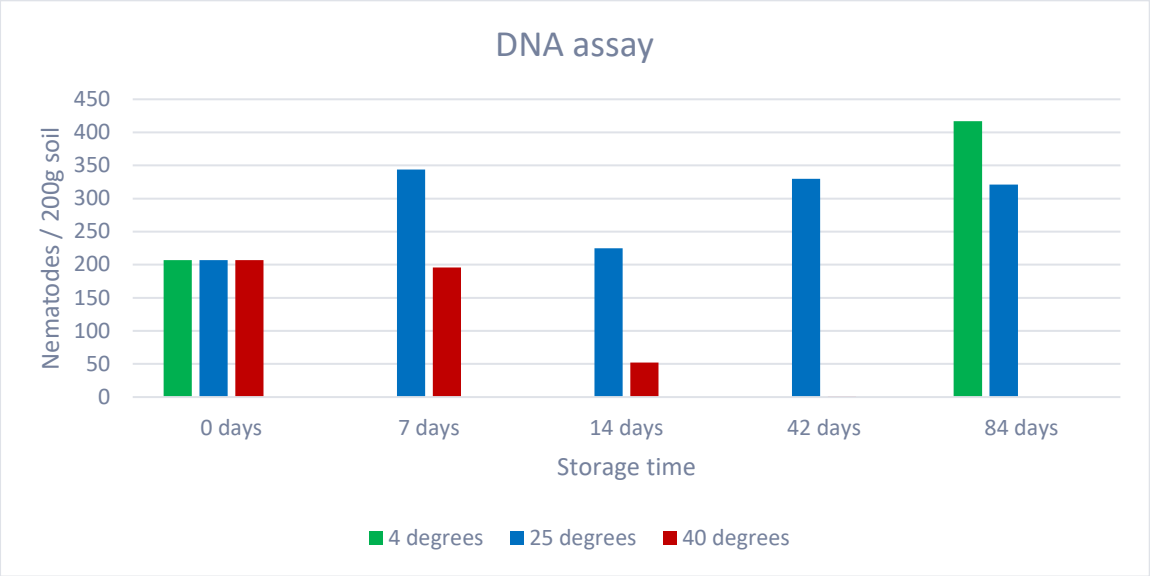

Figure 30:
The effect of storage time and temperature on P. zeae molecular assay results. No assays were conducted on soils stored for 7, 14 and 42 days at 4^{0}C.

Discussion

The storage data provided some very interesting results. Manual assays for *P. chaunorhiza* were not affected by storage temperatures of either 4^0 or 25^{0}C, but spore counts started dropping between 14 and 42 days when soils were stored at 40^{0}C; almost falling to zero at 84 days. This was a little surprising given that *P. chaunorhiza* oospores are considered robust. Molecular assays followed a similar trend, though *P. chaunorhiza* detection started decreasing even at 7 days at 40^{0}C. There was no decrease in detection over the 84-day period for soils stored at 4^0 or 25^{0}C. These data suggest that *P. chaunorhiza* storage conditions are not critical for manual counts at 4^0 and 25^{0}C (up to 84 days), but higher temperature storage is likely an issue, especially if samples are kept in vehicles for excessive periods.

The data for *Pratylenchus zeae* showed different trends; manual counts were affected by storage conditions more than molecular assays. Populations were reduced by storing samples at 25^{0}C over just a 7-day period; *P. zeae* populations were close to zero at 40^{0}C after the same period. Storage at 4^{0}C for 84 days, though completely satisfactory for *P. chaunorhiza*, gave low assay outcomes for *P. zeae*. For manual assays it is imperative that samples are processed as soon as possible after field sampling.

However, molecular assays were less subject to storage times (4^0C / 25^0C) and it appears that soils could be kept for as long as 84 days under these conditions without a significant reduction in the molecular assay results. However, storage of samples >25^0C is subject to a rapid loss of DNA, with population assessments declining after just 7 days storage (40^0C).

Manual assays require active nematodes; low temperature storage may 'deactivate' individual nematodes so they are not subject to extraction using a whitehead tray. However, low temperature appears to maintain the DNA integrity (nematodes may remain alive for the period) leading to a successful DNA extraction.

These data illustrate how basic information on sample storage is needed, if assay results are to reflect the field situation. Forty degrees was chosen as a storage temperature since keeping soil samples in vehicles after sampling is not uncommon. Even in relatively mild tropical weather conditions, temperatures may soar well-over 40^0C in vehicles. These data suggest that sample exposure time within vehicles must be minimised; storage in other places where temperature elevation occurs should also be avoided.

Nematode sampling processing within a short time after field sampling has been an agreed strategy for manual counts (SRA soil biology laboratory); these data reflect the need for this. Of interest, molecular assays appear less prone to storage time, if samples are kept between 4^0 and 25^0C. This bodes well for mass assay of samples from research experiments, where processing samples in batches may reduce individual assay charges. The same applies to *P. chaunorhiza*. Sample storage at higher temperatures, for both *P. chaunorhiza* and *P. zeae,* is likely to lead to aberrant outcomes.

6.2 Development of a new test

6.2.1. Pratylenchus parazeae

P. parazeae was first detected in the South Burnett region in 2016 by sequencing an individual nematode. This study was part of a national survey funded by GRDC. *P. parazeae* was subsequently detected by sequencing and by molecular assay in this project in the Burdekin region, as part of an investigation into possible discrepancies between manual and molecular assays for *P. zeae* (Figures 3, 36).

The test for *P. parazeae* was used to assay 284 samples from various sugarcane districts. This work was undertaken late in the project, and the specificity and sensitivity of the test has yet to be confirmed, however the sequence data indicate the assay should only detect *P. parazeae* and *P. haiduongensis*; the latter is associated with carrots in China.

Discussion

The results obtained reflecting the presence of *P. parazeae* in several regions in Queensland (Burdekin, South Burnett) are very significant. SRA is currently testing the sugarcane germplasm for breeding lines resistant to *P. zeae*, as a means for developing a resistance management strategy. With the finding of a closely related species, the question arises: 'how will lines potentially resistant to *P. zeae* react to infestation by *P. parazeae'?*. In addition, issues associated with symptomatology, yield effects and other management options also become relevant, with research on these topics an obvious need.

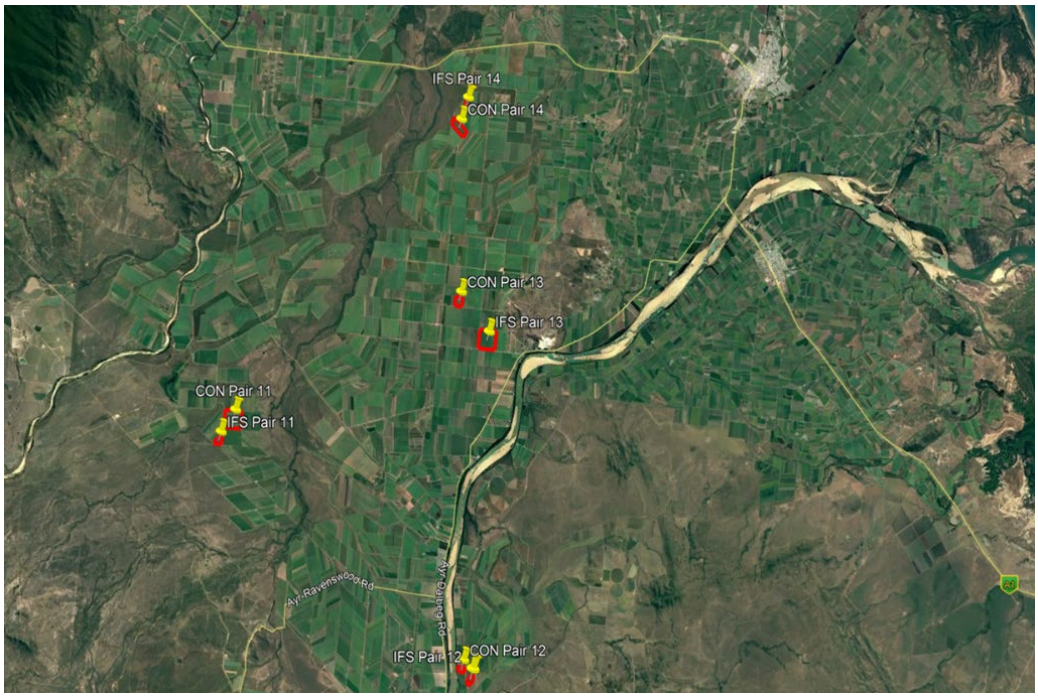

Figure 32:
Locations where Pratylenchus parazeae DNA had been detected in Burdekin.

6.2.2. Free-living nematodes

Nematode community analyses was undertaken on 83 samples from the Herbert and Mackay regions, where enrichment and structure indices were calculated and proposed soil biological health categories (1 – 4) generated (Figure 33). The suggested categories are:

- category 1 soils are stressed and degraded
- category 2 soils are nitrogen enriched and disturbed
- category 3 soils are nitrogen enriched and maturing
- category 4 soils are moderately enriched and structured

This analysis showed the 83 samples had a range of differing soil health conditions, likely driven by management practices and/or environmental differences.

To compare to manual and molecular assays which reflect the biomass of the target taxa, the biomass of the nematode counts was calculated according to Ferris (2010) using the formula, $W = (L^3/a^2)/(1.6*10^6)$, where (W) nematode biomass, (L) body length and (a) body diameter. Where appropriate multiple molecular data within a trophic group were summed to align with taxa count data.

Linear correlation analyses were used to assess the extent to which the estimates for biomass of each taxa obtained through manual counts correlated with the estimates obtained using the molecular assays (Table 7).

Correlated values illustrate consistency in measurement between manual and molecular estimations indicating that the molecular assays and manual count are providing similar information about samples. The notable exception is Dorylaimidae which appears to be un-correlated with the manual count data. The pattern of cross-correlations indicates quite strong agreement between the molecular and manual count data.

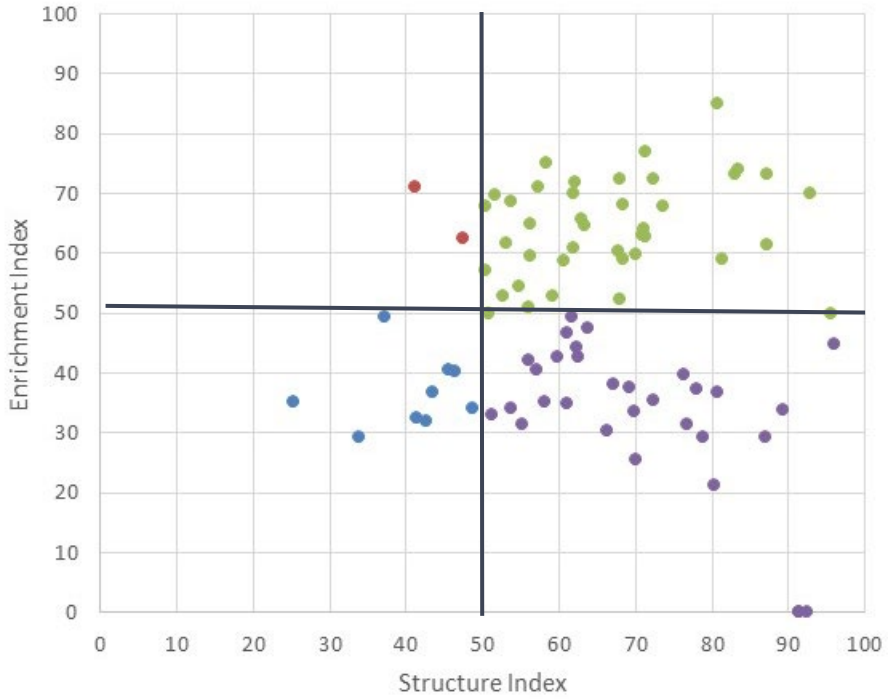

Figure 33:
Soil food web analysis (structure index versus enrichment index) and assigned soil biological health categories representing the structure and enrichment conditions of the soil food web using molecular nematode community data.

TABLE 7: CORRELATION MATRIX FOR MOLECULAR AND MANUAL COUNT DATA FOR EACH NEMATODE FEEDING GROUPS

qPCR	Counts				
	Pratylenchus	Aphelenchoida	Bacteriavore	Dorylaimidae	Mononchidae
Pratylenchus	0.33				
Aphelenchidae +Aphelenchidae		0.38			
Cephalobidae +Panagrolaimidae +Rhabditidae			0.40		
Dorylaimidae				-0.23	
Mononchidae					0.77

The subset of treatments from a single site in project 2017005 (Skocaj project) shows an increase in Mononchidae in 1.83 m dual and single row treatments (Figure 34). Mononchidae are the largest nematode species (predator) and easily killed by cultivation/traffic. Increase in these treatments suggests the controlled traffic is starting to result in changes in nematode community structure. Differences should increase with longer management times.

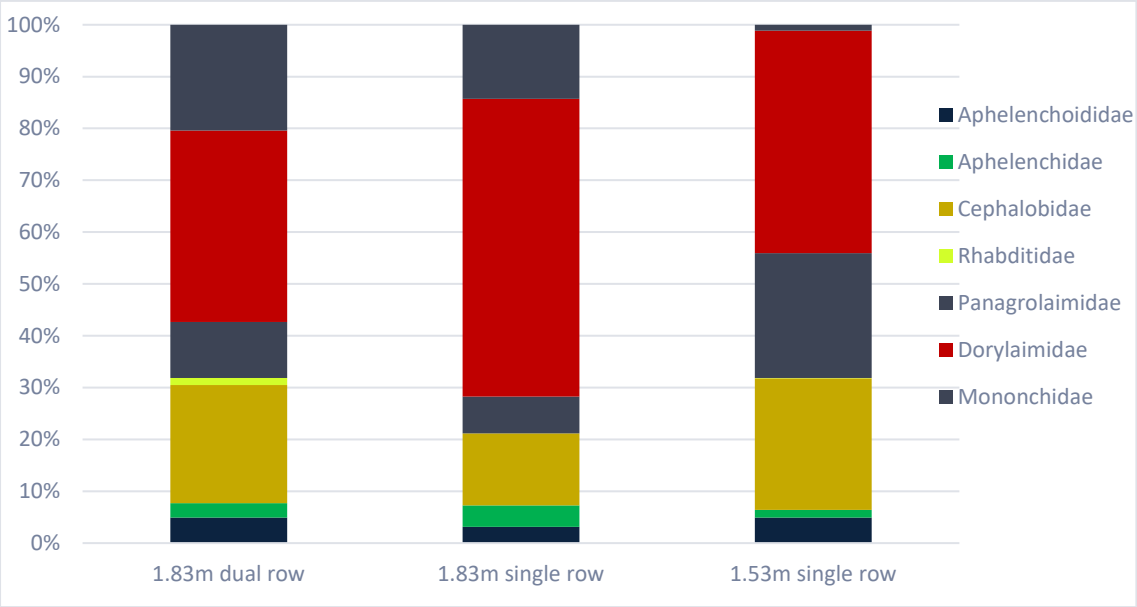

Figure 34:
Proportion of FLN groups as influenced by farming system.

Discussion

Nematode assays (both molecular and manual assays) have provided a wealth of information on soil samples collected from soil health project soils in the Herbert, Burdekin and Mackay regions. These data have been sent to soil health project leaders for discussion, interpretation and comment. The management practices applied (i.e. treatment) in each of the trials appears to have had a very significant influence on populations of FLN (and PPN). The significance of these differences can only be assessed once detailed examination of the relationship between management and populations has been explored by the project leaders.

There were clear differences between each site when the actual populations of both PPN and FLN were considered. This is not unexpected since nematode populations are reflective of such factors as soil type, local climate / weather, crop growth stage, crop yield and even variety.

There remains a need to compare the two assay systems on the basis of either nematode biomass or population estimates based on converted DNA copy numbers / actual individuals counted (manual assay system). The method for undertaking these analyses is still being investigated.

The analyses conducted on sugarcane FLN populations illustrate the potential for assessing the soil biological heath of sugarcane soils. The differences detected amongst farming system treatments shows that such analyses may be effective in highlighting how individual treatments affect the soil biological community. Further analyses are needed in association with soil health project leaders to identify which treatments provided positive biological community changes.

There was also good agreement between manual and FLN molecular assay data, except for the Dorylaimidae. This should be further investigated, but the general agreement bodes well for use of assays developed for the grains industry in the sugarcane industry.

6.2.3. AMF

The molecular assay results for each of the assayed AMF groups for the Herbert and Mackay soil heath trials are outlined in Table 4 and Table 13 in the Appendix. The data suggest that the assays developed by Anders in association with SARDI detected and quantified various AMF groups in soil health project soils in the Burdekin and Herbert River districts. The data also showed that management practices appear to have influenced soil AMF populations; as with FLN, further collaboration with soil health project leaders is needed to identify which farming systems favoured specific AMF groups.

The major AMF group in soils, from both Mackay and the Herbert, is a group which includes many species of *Funneliformis.* Very large differences in this group of species were noted with the different management practices applied in these trials.

The second largest populations in the Herbert were in the AMFc2 group (*Scutellospora calopsora, Gigaspora margarita*, *G. gigantea*), but AMFb (*Claroideoglomus claroideum*, *C. etunicatum*) were the second most common

group in the Mackay trial. There are obvious differences in AMF populations between the regions, with a greater number of groups represented in the Herbert. Only one group in the Herbert were poorly represented, the AMFe group. The reason for the fewer detections in other AMF groups in the Mackay soils is unknown but may among other things depend on the crops grown, and management practices applied, at the site.

Discussion

There have been no other AMF assays conducted on soil health project soils, so the data reported above are the first obtained.

There has been previous research into AMF in sugarcane (Magarey *et al.*., 2005; Kelly *et al.*, 2001). This illustrated the potential positive influence of AMF on sugarcane growth. In Tully research, the focus was on establishing cultures of the most common northern AMF species and to determine their influence on plant growth and nutrition. Cultures of *Glomus clarum* (AMF Group D) were isolated from northern Queensland soils; test plants were infected and planted into field plots deficient in P and Zn. Varying levels of P were applied to fumigated soils into which were planted mycorrhizal and non-mycorrhizal plants. This provided for assessing the influence of a single AMF species on plant growth and nutrition. Root colonisation was associated with improved P and Zn uptake, and reduced transplant shock in transplanted cane plants. Crop yield improved with mycorrhizal colonisation, as did both P and Zn uptake.

In conversations with Anders, a particularly important fact he highlighted was the difference between populations of AMF groups in the soil, versus the populations within roots. He suggested that two very different biomes may be found; this highlights the need to not simply quantify soil inoculum levels and rush to conclusions on the effect of specific AMF groups on crop growth. AMF will require research into population dynamics, the influence of groups of species on crop growth and the relationship between soil populations and the species that benefit sugarcane growth and root health.

6.2.4. Sugarcane roots

With spatial pattern information available, Johann was able to provide GIS maps to illustrate root surface area and pathogen / parasite variation both across the paddock and down the soil profile. Example data are illustrated in Figures 35 and 36.

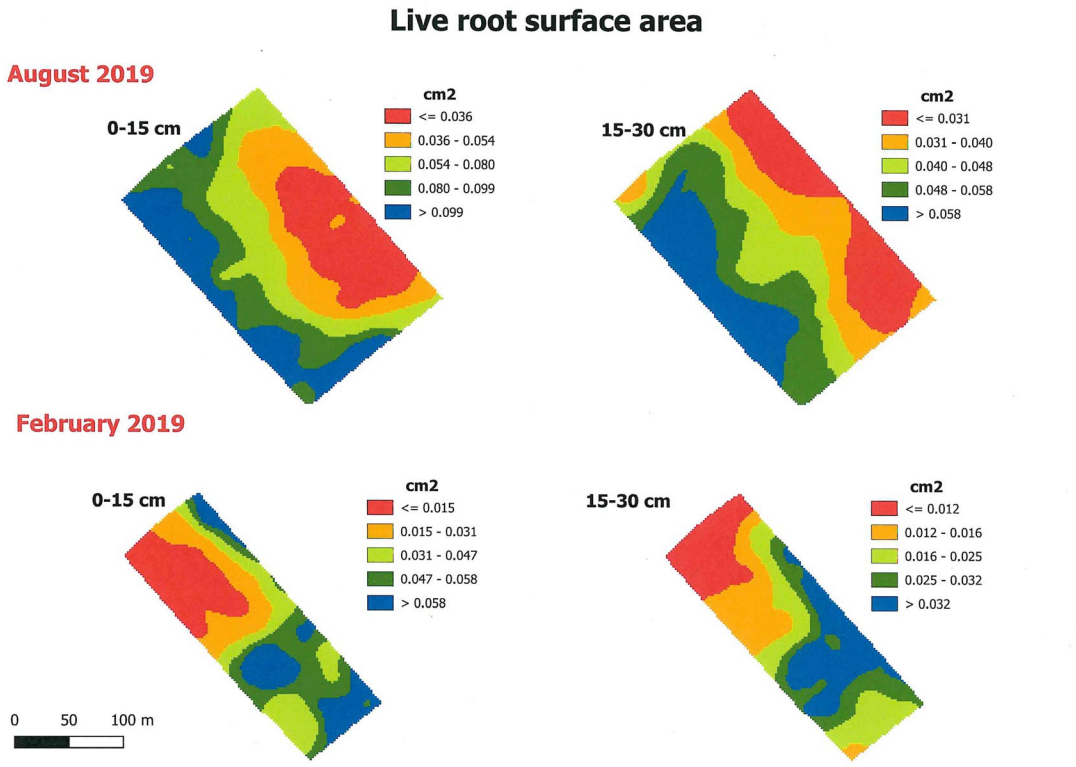

Figure 35:
Heat maps illustrating the distribution of roots across a commercial cane field during the crop growth period (February) immediately after harvest (August; data supplied by Johann Pierre).

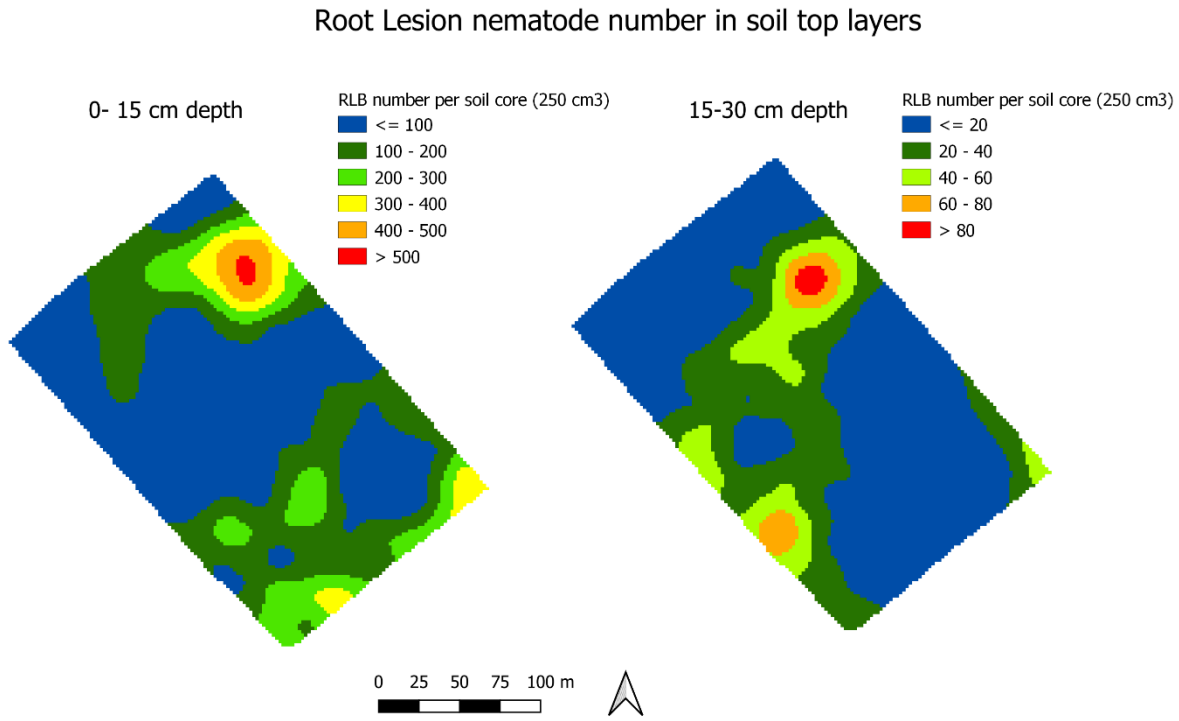

Figure 36:
Heat maps illustrating the distribution of Pratylenchus zeae across a commercial cane field immediately after harvest; maps represent two different soil depths (data supplied by Johann Pierre).

Discussion

The molecular assay for living sugarcane roots provides an excellent tool for further investigating the dynamics of sugarcane root growth and pathogen / parasitic nematode populations. Nematode populations are known to fluctuate markedly with weather conditions and as the root systems grow. Greater plant growth can lead to higher nematode populations, even though the parasites may only be having a limited impact on yield. Therefore there would be significant benefit in quantifying root growth at the same time as parasitic nematodes / pathogen populations are being assessed. The analyses undertaken in Johann's research provides a foundation for teasing apart the dynamics of root growth and root pathogen / root parasite interactions.

A factor to be considered in the review of these specific data are the conditions under which Johann extracted the samples. Root growth varies enormously through a growing season, starting off slowly at plant establishment, increasing with an increase in shoot biomass, peaking during the growing season (usually in cane, this is from December-April). Following the production of most of the plant biomass, root growth slows. Nematode sampling is recommended close to the sugarcane stool, at a point which corresponds with the extent of the sugarcane root system – where active root growth is occurring. On the other hand, *P. chaunorhiza* populations, and their influence, is greatest directly beneath the sugarcane stool - in the planting line. Johann extracted samples straight after crop harvest (August) at 30cm from the sugarcane stool. One would expect nematode populations to have declined compared to when peak growth was occurring; *P. chaunorhiza* populations would likely have been much higher underneath the sugarcane stool. These factors should be kept in mind when interpreting project 2018003 data.

The ability to assay for roots however is an exciting new tool and could be used throughout a growing season to highlight both the growth and development of the sugarcane root system and the concurrent variation in parasitic nematodes and *P. chaunorhiza* populations. This has not been practically possible before and would provide a unique slant on root growth and root constraints.

7. CONCLUSIONS

1 **Test reliability**: the research highlighted the ability of the molecular assays developed in project 2016047 to detect *Pachymetra chaunorhiza, Pratylenchus zeae* and *Meloidogyne* species populations in soil health project, plant breeding trials and industry samples sent to the Tully laboratory. Further research is needed to determine the reason for the failure to detect *P. chaunorhiza* under some circumstances in the Mackay region and for the lower-than-expected correlations between *P. chaunorhiza* manual v molecular assays under some circumstances.

2 **Nematode manual assays**: the molecular assays for two parasitic nematodes species were shown to be equally as reliable as manual assays performed by experienced nematologists under controlled conditions.

3 **Soil storage**: sample storage conditions are important for ensuring assay results reflect field populations.

4 **Bulk assays**: molecular assays offer the ability to assay bulk samples rapidly and hence the opportunity to relate the results from several different assays in many soils samples.

5 ***Pratylenchus parazeae test***: a new test was developed for *Pratylenchus parazeae*.

6 **Other molecular assays**: assays developed for other industries (free-living nematodes) and in other projects (mycorrhizae / living sugarcane roots) were shown to be of real value in samples obtained from soil health projects.

7 **Future**: further consideration is needed from SRA management on the extension of molecular assays to industry, both in terms of costings, databases, tests offered and the follow-up research needed to confirm the application of relatively untested assays to commercial industry (FLN community analyses / AMF / roots).

8. RECOMMENDATIONS FOR FURTHER RD&A

There is a need to determine the conditions under which some molecular assays did not correlate as well as expected with manual assays. Under certain circumstances, correlations were good while under others, the correlations were significantly worse. This could be related to sample storage conditions; this should be elucidated in a short follow-up research activity.

As highlighted above, further testing of assays for AMF, FLN and roots is needed to ensure exploitation to the full of the potential use of these assays for soil and root health indices. This would preferably be undertaken in the context of soil health-type research where the influence of farming systems on soil biology could be explored. A soil health assay based on DNA testing would have distinct advantages for the sugarcane industry and this opportunity should not be lost.

SRA management will need to decide a policy on commercialisation of the molecular assays; issues include the development of a pricing structure, soil submission procedures, guidelines on soil sample storage, result delivery procedures, databases for archive of test results and extension packages for the assay.

Our recommendation is that a review of the project outcomes be undertaken by relevant project staff (including soil health scientists) to assess how to progress molecular-based assays and their applicability to further sugarcane industry soil and root health assay applications.

9. ACKNOWLEDGEMENTS

The authors acknowledge the inputs from many across the Australian sugarcane industry, including soil health project leaders (Danielle Skocaj, Barry Salter and Lawrence DiBella), Cane Productivity Board managers (who sent soils to the Tully assay laboratory), SRA research Assistants (principally Laura MacGillycuddy, but also those associated with soil health projects) and various SARDI staff (including Daniele Giblot-Ducray, Nigel Percy, Katherine Lindsell) as well as SRA Management and Funding staff.

10. REFERENCES

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Magarey RC, Bull JI, Reghenzani JR (2005). Influence of vesicular arbuscular mycorrhizae (VAM) on sugarcane growth in the field. *Proceedings of the Australian Society of Sugar Cane Technologists*. **27**, 282-290.

11. APPENDIX 1 METADATA DISCLOSURE

TABLE 1 METADATA DISCLOSURE 1

Data	(Description)
Stored Location	(I.e. organisation and server)
Access	(I.e. publically accessible or restricted? Please provide details.)
Contact	(I.e. Details of person/position with access)

TABLE 2 METADATA DISCLOSURE 2

Data	(Description)
Stored Location	(I.e. organisation and server)
Access	(I.e. publically accessible or restricted? Please provide details.)
Contact	(I.e. Details of person/position with access)

12. APPENDIX 2 FREE-LIVING NEMATODE DATA

The tables below outline the free-living nematode data obtained during the course of the project.

TABLE 1: DNA-BASED BACTERIVORE POPULATIONS IN THE HERBERT SOIL HEALTH TRIAL. DATA FOR EACH BACTERIVORE GROUP ARE EXPRESSED AS A PERCENTAGE OF THE HIGHEST TREATMENT SCORE.

Treatment	Cephalobidae	Mesorhaditidae	Panagrolaimidae	Rhabditidae	Rhabdolaimidae
1	11.8	8.8	0.0	0	9.0
2	9.5	0.0	21.3	0	0.0
3	35.8	40.3	5.8	0	0.5
4	6.0	0.0	0.0	0	0.0
5	5.8	0.0	0.0	0	3.8
6	18.8	0.0	0.0	0	19.4
7	33.0	0.0	0.0	0	0.0
8	22.1	0.0	0.0	0	29.0
9	24.4	0.0	7.6	0	0.0
10	100.0	63.3	0.0	0	25.4
11	9.7	0.0	0.0	0	0.0
12	46.8	0.0	22.6	0	49.6
13	17.0	0.0	13.8	0	12.0
14	10.5	0.0	0.0	0	37.3
15	39.5	3.2	54.8	0	35.7
16	9.2	0.0	0.0	0	0.2
17	7.4	0.0	0.8	0	1.1
18	11.0	0.0	0.0	0	14.7
19	6.0	0.0	0.0	0	1.9
20	50.0	0.0	6.0	0	26.3
21	14.6	4.0	0.0	0	0.0
22	12.6	49.1	26.8	0	50.5
23	4.8	0.0	3.8	0	1.0
24	20.0	77.3	100.0	0	79.3
25	12.4	3.4	0.0	0	23.9
26	22.9	12.3	17.7	0	65.7
27	3.2	0.0	8.4	0	0.0
28	10.3	26.8	0.0	0	6.7
29	20.0	16.3	0.0	0	0.3
30	19.6	48.4	6.6	0	40.0
31	19.3	44.3	1.6	0	2.1
32	2.3	12.9	0.0	0	17.3
33	3.4	12.6	0.0	0	56.3
34	65.9	29.1	7.8	0	23.4
35	5.0	31.2	0.7	0	3.7
36	1.4	0.0	0.0	0	14.3
37	8.9	40.2	15.8	0	7.0
38	11.6	4.1	0.0	0	0.0
39	12.4	23.1	0.0	0	21.9
40	2.0	0.0	0.0	0	3.2
41	6.9	100.0	1.7	0	16.1
42	17.2	10.6	8.8	0	0.0
43	8.5	72.1	0.0	0	12.0
44	1.1	47.8	17.2	0	100.0

TABLE 2: DNA-BASED ASSAY RESULTS FOR BACTERIVORE POPULATIONS IN THE MACKAY SOIL HEALTH TRIAL. DATA FOR EACH BACTERIVORE GROUP ARE EXPRESSED AS A PERCENTAGE OF THE HIGHEST TREATMENT SCORE.

Treatment	Cephalobidae	Mesorhaditidae	Panagrolaimidae	Rhabditidae	Rhabdolaimidae
1	16.5	4.3	0.2	0.0	94.4
2	25.3	4.1	6.6	0.0	4.6
3	56.9	42.2	5.7	0.0	36.8
4	53.0	19.1	14.9	0.0	22.8
5	46.0	91.6	18.1	0.0	96.4
6	100.0	100.0	50.0	0.0	55.0
7	59.2	11.2	100.0	0.0	12.0
8	40.8	96.6	32.0	0.0	2.8
9	18.2	0.2	0.0	0.0	0.2
10	29.7	5.6	23.5	0.0	0.0
11	30.7	10.6	23.3	0.0	3.0
12	80.1	9.0	2.3	0.0	1.9
13	77.7	12.7	55.1	0.0	28.9
14	38.6	26.0	33.5	0.0	3.4
15	52.9	58.0	69.9	0.0	99.0
16	39.9	19.7	2.7	0.0	0.0
17	38.8	23.0	28.2	0.0	0.0

TABLE 3: RELATIVE ABUNDANCE OF FLN IN EACH DNA ASSAY GROUP FOR THE HERBERT SOIL HEALTH PROJECT, EXPRESSED AS A PERCENTAGE OF THE HIGHEST TREATMENT POPULATION IN EACH GROUP.

Treatment	Omnivores	Fungivores		Predators
	Dorylaimids	Achoid	Achus	Mononchids
1	22.1	6.2	10.4	4.4
2	5.6	5.7	7.3	0.0
3	2.1	25.3	0.7	0.0
4	19.9	12.7	21.9	0.0
5	4.3	1.4	9.2	5.4
6	15.5	48.9	4.6	0.0
7	19.6	13.1	18.0	0.4
8	10.4	19.6	4.8	1.2
9	22.1	24.9	1.1	3.8
10	19.8	32.8	8.3	0.0
11	9.2	52.6	9.1	2.6
12	16.6	10.3	5.7	0.3
13	6.3	19.9	37.1	0.0
14	16.0	10.5	3.0	0.1
15	9.0	48.3	9.5	0.9
16	7.4	4.7	8.4	0.0
17	3.8	5.9	0.3	6.7
18	13.7	8.0	1.5	0.0
19	12.8	22.1	20.8	4.4
20	35.3	70.9	2.9	4.3
21	3.3	19.3	17.1	5.3
22	35.5	28.1	38.2	62.7
23	8.1	3.1	13.6	0.0
24	4.2	75.5	87.3	0.0

Treatment	Omnivores	Fungivores		Predators
	Dorylaimids	Achoid	Achus	Mononchids
25	8.6	21.9	5.9	3.9
26	54.6	100.0	37.1	26.2
27	18.7	10.1	27.7	0.0
28	8.5	5.4	71.5	0.0
29	6.6	2.4	13.7	12.9
30	36.0	48.1	64.3	27.5
31	9.0	13.7	67.9	2.8
32	3.6	1.3	51.3	97.2
33	6.6	19.6	33.5	10.5
34	100.0	89.9	24.8	100.0
35	10.2	20.8	12.1	0.0
36	1.6	4.9	94.3	13.8
37	9.2	13.8	99.8	0.0
38	20.6	16.6	27.8	0.0
39	20.8	13.1	39.4	0.0
40	1.9	7.8	3.5	8.9
41	3.6	11.1	95.0	0.0
42	33.0	13.9	25.6	21.7
43	3.9	44.9	12.8	0.0
44	4.6	15.2	28.5	44.1

TABLE 4: AMF DNA DATA FOR SOIL SAMPLES RECEIVED FROM THE HERBERT SOIL HEALTH PROJECT.

Sample	AMFa kDNA copies/g Sample*	AMFa2 kDNA copies/g Sample*	AMFb kDNA copies/g Sample*	AMFc2 kDNA copies/g Sample*	AMFd kDNA copies/g Sample*	AMFe kDNA copies/g Sample*
1	0	0	0	0	0	0
2	5.88	0	0.97	1.32	0	0
3	7.19	2.07	1.18	0	0	0
4	0.23	0.63	0	0	0	0
5	10.32	0	1.23	0	0	0
6	37.02	0.16	0	7.60	0	0
7	1.74	0	0	1.15	0	0
8	0.33	0	0	6.14	0	0
9	14.07	0	0	25.42	2.75	0
10	1.14	0.27	0	4.04	0	0
11	9.10	0	0	1.11	0	0
12	7.74	0	0	2.85	0	0
13	11.29	0	0	0.48	0	0
14	12.03	4.75	0	0	0	0
15	0.91	0	0	2.31	0	0
16	17.15	2.99	0	0	0	0
17	7.58	0	0	0	2.42	0
18	1.29	0	0	5.05	0	0
19	28.70	6.04	0	0	0	0
20	2.72	14.98	0	0	0.82	0.22
21	21.68	0	0	0	1.25	0

22	33.90	0	2.81	0	3.85	0
23	4.25	2.71	0.08	0.57	0	0
24	18.50	0	0.34	0	0	0
25	12.82	0	0	2.24	0	0
26	23.87	0	0.06	0.05	9.75	0
27	10.08	0.03	1.78	1.79	4.44	0
28	5.62	0	2.32	0	0	0
29	8.40	0	0	1.12	1.60	0
30	37.4	0	0	0	3.91	0
31	52.06	0.76	2.98	0	0	0
32	10.99	0	0	0	0	0
33	18.42	0	0	1.16	0	0
34	50.80	0	0.06	0	1.82	0
35	19.34	0	0	0.63	0	0
36	20.59	0	0	0	0	0
37	2.83	1.16	0	1.27	0.52	0
38	3.07	0	0.11	9.62	1.32	0
39	18.11	4.10	0.42	0	0	0
40	0.62	0	0	0	0	0
41	4.71	0	0	0	0	0
42	8.22	0	0.83	3.24	5.51	0
43	17.46	0	1.29	0	1.39	0
44	26.05	0	0	0	5.72	0

TABLE 5: AMF DNA DATA FOR SOIL SAMPLES RECEIVED FROM THE MACKAY SOIL HEALTH PROJECT.

Sample	AMFa kDNA copies/g Sample*	AMFa2 kDNA copies/g Sample*	AMFb kDNA copies/g Sample*	AMFc2 kDNA copies/g Sample*	AMFd kDNA copies/g Sample*	AMFe kDNA copies/g Sample*
1	0.32	0	0	4.32	0	2.09
2	2.63	0	0	0	0	0
3	0.66	2.15	1.22	0.27	0	0
4	11.09	0.47	10.75	0	0	0
5	1.85	0.32	5.70	0	0	0
6	22.67	0	1.36	0	0	0
7	20.95	0	7.10	0	0	0
8	1.25	0	5.41	0	0	0
9	4.15	0	0	0	0	0
10	0.62	0	0	0.55	0	0
11	1.11	0	2.81	0	0	0
12	2.24	0	2.22	0	0	0
13	3.60	0.78	10.15	0	0	0
14	6.33	0	3.82	0	0	0
15	22.97	0	2.71	0	0	0
16	0.63	0	0	1.06	0	0
17	13.08	0	1.01	0	0	0

TABLE 6: RELATIVE ABUNDANCE OF FLN IN EACH DNA ASSAY GROUP FOR THE MACKAY SOIL HEALTH PROJECT, EXPRESSED AS A PERCENTAGE OF THE HIGHEST TREATMENT POPULATION IN EACH GROUP.

Treatment	Omnivores	Fungivores		Predators
	Dorylaimids	Achoid	Achus	Mononchids
1	8.0	6.4	5.5	21.6
2	71.2	17.4	33.3	11.6
3	58.8	14.8	44.0	0.0
4	73.0	41.9	39.1	12.1
5	71.4	42.9	100.0	17.3
6	76.6	54.9	30.7	94.4
7	93.1	35.8	29.9	22.6
8	64.4	30.3	39.0	31.2
9	22.2	5.1	10.7	24.9
10	54.2	26.8	22.6	7.8
11	35.8	11.6	22.1	0.0
12	48.7	15.1	24.3	25.7
13	86.8	40.8	96.5	100.0
14	52.6	29.9	31.7	0.0
15	74.1	100.0	91.2	91.4
16	100.0	45.3	18.2	0.0
17	62.0	34.1	34.6	0.0

TABLE 7: RELATIVE ABUNDANCE OF FLN IN EACH MANUAL ASSAY GROUP FOR THE HERBERT SOIL HEALTH PROJECT, EXPRESSED AS A PERCENTAGE OF THE HIGHEST TREATMENT POPULATION IN EACH GROUP.

Treatment	Omnivores	Bacterivores	Fungivores	Predators
	Dorylaimids			Mononchids
1	14.8	58.8	22.6	30.3
2	45.2	16.9	49.9	0.0
3	41.7	62.4	6.5	0.0
4	4.9	79.5	3.7	51.0
5	0.0	75.0	25.4	0.0
6	14.0	34.5	55.8	0.0
7	21.3	44.0	14.2	100.0
8	0.0	87.6	12.5	0.0
9	0.0	36.7	64.2	0.0
10	1.8	0.0	100.0	0.0
11	14.5	50.3	39.4	0.0
12	3.3	95.0	2.5	0.0
13	14.6	50.0	39.6	0.0
14	3.4	90.8	6.8	0.0
15	3.2	90.3	7.4	0.0
16	33.6	68.7	0.0	23.6
17	0.0	91.9	8.2	0.0
18	0.0	100.0	0.0	0.0
19	51.2	33.2	28.9	0.0

Treatment	Omnivores	Bacterivores	Fungivores	Predators
	Dorylaimids			Mononchids
20	11.0	66.8	8.3	64.5
21	24.9	66.7	14.9	0.0
22	45.9	38.2	17.4	39.4
23	17.1	24.4	63.7	0.0
24	0.0	30.7	70.3	0.0
25	45.9	38.2	17.4	39.4
26	0.0	0.0	0.0	0.0
27	48.6	27.3	36.9	0.0
28	6.5	51.3	44.5	0.0
29	77.7	16.9	17.1	31.2
30	0.0	0.0	0.0	0.0
31	6.7	30.0	63.6	8.9
32	24.0	72.7	9.5	0.0
33	42.0	47.6	21.3	0.0
34	0.0	0.0	0.0	0.0
35	0.0	50.0	50.7	0.0
36	56.0	33.1	17.1	31.2
37	7.3	16.6	79.0	0.0
38	100.0	8.4	17.0	0.0
39	16.8	81.4	6.1	0.0
40	60.1	34.8	10.3	38.9
41	15.0	38.7	50.8	0.0
42	99.0	11.3	14.9	0.0
43	0.0	0.0	0.0	0.0
44	0.0	0.0	0.0	0.0

TABLE 8: RELATIVE ABUNDANCE OF FLN IN EACH MANUAL ASSAY GROUP FOR THE MACKAY SOIL HEALTH PROJECT, EXPRESSED AS A PERCENTAGE OF THE HIGHEST TREATMENT POPULATION IN EACH GROUP.

Treatment	Omnivores	Bacterivores	Fungivores	Predators
	Dorylaimids			Mononchids
1[1]				
2	50.5	50.5	94.2	0.0
3	27.9	70.9	73.8	0.0
4	36.7	54.7	91.8	0.0
5	21.2	69.3	77.2	0.0
6	13.3	99.6	41.9	0.0
7	36.9	76.5	65.2	0.0
8	15.8	67.7	80.3	0.0
9	44.7	52.0	93.5	0.0
10	100.0	56.0	77.4	0.0
11	44.1	70.3	71.3	0.0
12	26.3	82.2	60.5	0.0
13	29.1	49.3	100.0	0.0
14	48.5	90.8	45.5	0.0

Treatment	Omnivores	Bacterivores	Fungivores	Predators
	Dorylaimids			Mononchids
15	26.4	59.6	87.9	0.0
16	83.5	51.2	86.6	0.0
17	62.1	100.0	31.5	0.0