

Final Report

Fusarium Wilt Tropical Race 4 – Biosecurity and Sustainable Solutions

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Fusarium Wilt Tropical Race 4 – Biosecurity and Sustainable Solutions – BA14013

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Summary

An incursion of the fungus *Fusarium oxysporum* f.sp. *cubense* Tropical Race 4 (*Foc* TR4) was identified on a farm in the Tully region in March 2015. The wet tropical coast region, including Tully, produces 96% of Australia's banana crop worth approximately \$600 million annually and sustains regional communities in coastal north Queensland. *Fusarium wilt of banana* – also known as Panama disease – is caused by the fungus *Fusarium oxysporum* f.sp. *cubense* and is regarded as one of the most destructive diseases of banana and has devastated banana production in many countries around the world. Very few varieties are resistant to the *Foc* TR4 strain of the pathogen and despite years of breeding no commercially acceptable varieties have proven to be fully resistant.

This project has aimed to deliver new science, information and practices to address key areas of need for the banana industry to:

- Successfully contain the disease and prevent further spread of the pathogen through the adoption of robust, science-based biosecurity practices and
- Identify and investigate options to facilitate the development of economically viable production systems, capable of minimising inoculum build up, that are suitable for use on infected or at risk farms.

The adoption of effective biosecurity practices on north Queensland banana farms has been significantly supported by project activities identifying risk pathways for spread of the pathogen, research identifying the most effective disinfectant products, research activities outlining how to monitor and manage their use and replenishment and how to effectively destroy inoculum in infected plants to minimise build up in the soil. The project has also significantly progressed development of methods for assessment of plant stress and its influence on *Foc* infection, identified possible rotation crops that suppress *Foc* populations in the soil and the influence of ground cover and nitrogen management practices on the soil microbiome and its capacity to suppress *Foc*.

Significant extension and communication efforts have been made to keep the banana industry informed of the latest results as they became available and a biosecurity Best Management Practice guide has been produced with input from banana producers and regulatory agency staff that can assist banana growers to benchmark their biosecurity practices and identify potential improvements. Evaluation activities have shown that the project has significantly influenced knowledge change and practice adoption in the banana industry.

Keywords

Fusarium wilt; Banana; Biosecurity; Disinfectants; Varieties; Plant stress; Soil micro-organisms

Introduction

An incursion of the fungus *Fusarium oxysporum* f.sp. *cubense* Tropical Race 4 (*Foc* TR4) was identified on a farm in the Tully region in March 2015. The wet tropical coast region, including Tully, produces 96% of Australia's banana crop worth approximately \$600 million annually and sustains regional communities in coastal north Queensland. There are few alternative cropping options and those available, such as sugar cane, provide significantly smaller economic returns and employment opportunities.

Fusarium wilt of banana – also known as Panama disease – is caused by the fungus *Fusarium oxysporum* f.sp. *cubense* and is regarded as one of the most destructive diseases of banana and has devastated banana production in many countries around the world. Very few varieties are resistant to the *Foc* TR4 strain of the pathogen and despite years of breeding no commercially acceptable varieties has proven to be resistant.

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- Identify and investigate options to facilitate the development of economically viable production systems, capable of minimising inoculum build up, that are suitable for use on infected or at risk farms.

This project focused on achieving objectives 1 and 2 in the Australian Banana Strategic Investment Plan 2017-21:

- New varieties introduced and improved pest and disease management that improve varietal diversity and biosecurity – Continue research to improve pest and disease management and biosecurity
- Increased adoption of the industry's BMP plan that improves industry sustainability, biosecurity and environmental stewardship – Continue to drive adoption of BMP for on-farm biosecurity to ensure biosecurity risks are minimised; Strategically communicate the industry's biosecurity and environmental BMP's to the community

Methodology

The project aimed to deliver new science, information and practices to address key areas of need in the banana industry. The identified priority areas were distilled from a Fusarium wilt R&D gap analysis workshop held in 2009 and from questions recorded at industry meetings held in north Queensland at the beginning of the incursion. These identified knowledge gaps were then allocated into 3 different scenarios:

- I don't have the disease, what do I need to do to avoid getting it?
- I have the disease on one part of the farm, how do I contain it and limit its spread?
- I have the disease across the farm, how can I continue to farm safely and economically?

These scenarios, which can also be perceived as a timeline progression, served to provide a structure with which to frame the RD&E response. In addressing the RD&E needs existing information and research work from Australia and overseas was accessed, particularly on disease epidemiology, but significant knowledge gaps were revealed that required research effort. This resulted in five activity areas for the project:

1. Conduct research to underpin improved biosecurity practices on farm

1.1. Rapidly identify solutions for early uptake in biosecurity practice: reducing inoculum movement from around banana plants, within plantations and between plantations – the project's initial focus was on providing the banana industry with the information and planning required to implement robust, science-based biosecurity measures to contain the disease and prevent further spread. Key components of this were to:

- rapidly establish a common level of understanding about Fusarium wilt Tropical Race 4 (TR4) in bananas (the disease biology, mechanisms of spread and potential impact);
- develop a planning process for landholders to identify the Fusarium wilt TR4 risk pathways associated with their production system to help identify the best biosecurity options for each;
- identify and refine a range of biosecurity practices (boot exchange, footbaths, vehicle washing facilities and processes, the application of differential access zoning to a property)

This resulted in support for the development of an interactive workshop process on farm biosecurity practices for Fusarium wilt TR4 which was then rolled out to industry through the ABGC-led biosecurity extension project from July 2015 to February 2016. The project contributed significant content to the workshop modules addressing disease biology, identification and reporting, identification of risk pathways and biosecurity practice options.

Research and development activities to support biosecurity practice implementation were also undertaken to address identified knowledge gaps around the efficacy and optimal use of sanitisers/disinfectants effective against the organism. Research activities were conducted in Queensland and the Northern Territory to screen available sanitiser products for their efficacy against the range of spores and fungal material produced by *Fusarium oxysporum* f.sp. *cubense* Race 1 (*Foc* R1) and TR4 (*Foc* TR4), the impact of soil quantity and type on product efficacy, product longevity, practical on-farm methods of monitoring concentration and the corrosion potential of the most effective products against a range of metals.

1.2. Develop tools that provide early indications of infection and allow for prompt interventions –

Research activities were undertaken by SARDI to develop a quantitative PCR assay for identifying *Foc* TR4 in soil and water samples. SARDI are internationally recognised for their development of techniques for DNA extraction from relatively large quantities of soil and the development of a reliable test provides the ability to check if inoculum reduction practices are successful. The TaqMan MGB qPCR assay was designed for *Foc* TR4 and assessed for sensitivity and specificity against more than 100 isolates including several *Foc* TR4, *Foc* subtropical race 4 (SR4) and *Foc* R1 isolates, and a range of *Fusarium* species/subspecies from Australia and overseas. Further specificity testing was conducted using a new DNA collection which included plant pathogenic/non-pathogenic *Fusarium* species and Australian/international isolates. Assay sensitivity was assessed against soil samples naturally and artificially infected with *Foc* TR4 to test the ability of the assay to detect the organism at a range of spore concentrations in soil.

This project activity also planned to update and streamline *Foc* diagnostics but discovered that there was an existing project activity already underway. An existing initiative of Plant Health Australia (PHA) encompassed the development, review and updating of National Diagnostic Protocols (NDP's) for high priority pests, including *Foc* TR4, as an identified action of the National Plant Biosecurity Diagnostics Strategy. To avoid duplication the project contacted the PHA project leader to establish and report on the status of the activity.

1.3. Improve monitoring, early detection and responses to disease incursion – activities planned under this objective were transferred to “ST15011 Child 10 DAF Multi-scale monitoring tools for managing Australian Tree Crops: Industry meets innovation”

1.4. Managing inoculum load in pseudostems, soil and water and alternative hosts – this objective aimed to develop practices that are effective at reducing the level of *Foc* in banana pseudostems, soil and water, and to reduce the risk that *Foc* TR4 populations persist on the root systems of alternative plant hosts. Laboratory and field trials were undertaken to identify effective chemical or biological treatments to destroy disease inoculum in infected plants and infested soil. Laboratory and field trials were also conducted to test chemical and biological treatments to enhance decomposition of banana pseudostem material to prevent saprophytic colonisation by *Foc*. The status of potential alternative host plants was tested with sampling of weeds from known *Foc* R1 sites in north Queensland. In conjunction with diagnostic staff from Biosecurity Queensland, the project identified and provided common weed and ground cover species from north Queensland to an inoculated alternative host glasshouse trial conducted at the Eco-sciences Precinct using *Foc* SR4 as a surrogate for *Foc* TR4.

2. Improve access to new cultivars and build capacity in propagation – this objective aimed to ensure that disease resistant/tolerant banana cultivars are available to the Australian industry and contribute to resilience in future banana production. Currently the Australian industry invests significantly in identifying, importing and screening banana cultivars with potential pest and disease resistance through ‘BA16001 Improved plant protection for the banana industry’ and in developing resistant varieties with improved agronomic and organoleptic qualities through mutagenesis and somaclonal selection in ‘BA14014 Fusarium wilt TR4 research program’. A review of the current global banana breeding activities, outlining their origins, objectives, methodologies, current status and progeny suitable for the Australian industry was undertaken to help ensure that these investments are focused in priority areas for the Australian banana industry.

3. Develop resilient crop management options

3.1. Assess the use of cover crops to reduce inoculum in infested soil – living groundcovers have been shown to reduce Fusarium wilt symptoms by 20% in fields heavily infected with *Foc* R1 and reduce water runoff that can be a pathway for inoculum transport by up to 85%. This activity will investigate the efficacy of cover crops in reducing inoculum in infested soils.

3.2. Assess how root exudates affect *Foc* TR4 populations – the role of root exudates such as carboxylic acids in controlling disease is unknown. The chlamydospores of *Foc* are known to germinate in the presence of root exudates. This activity will investigate the role of root exudate profiles under banana and cover crops to determine if they induce or reduce chlamydospore germination, and whether certain root exudates attract beneficial microorganisms to the roots.

3.3. Assess the use of microbes to suppress *Foc* – the activity investigated the characteristics of a suppressive soil microbiome by analysing the metagenics of soil organisms and assessing the influence of crop management practices on the banana microbiome. Next generation DNA sequencing technology has been used to characterise the microbial community around the rhizosphere of banana plants as well as identified *Foc*-suppressive microorganisms.

3.4. Assess the role of plant stress in susceptibility to *Foc* infection – plant stress can be a precursor to symptom development of Fusarium wilt. A higher incidence and severity of symptom development occurs at different times of the year and findings from the ACIAR funded project HORT/2008/040 found that periods of high/low infection were related to climatic conditions. This activity undertook both field and glasshouse trials to investigate how plant stress interacts with *Foc* infection and the role of plant stress in reducing plant defence mechanisms and increasing susceptibility to *Foc*. The study investigated the use of rapid plant physiological assessment methods (proline accumulation in leaf tissue, chlorophyll fluorescence, chlorophyll content, stomatal conductance and thermal imaging)

to determine the potential for effectively detecting plant stress (induced from abiotic factors) and disease (from *Foc* R1 infection) prior to the appearance of visual symptoms. The second objective was to use these tools for quantification of the severity of the stress and objectively measure leaf function and plant health. The investigation was carried out in three parts: 1) a glasshouse pot trial which involved applying stress treatments to 'Williams' (Musa AAA) plants; 2) a glasshouse pot trial in which Dwarf Ducasse (Musa ABB) plants were inoculated with *Foc* R1; 3) a field trial where seven varieties with various susceptibilities to *Foc* R1 were monitored on an infected property for their symptom expression.

4. Update biosecurity protocols for banana production to reflect project

4.1. *Research becomes biosecurity protocol* – it was anticipated the outcomes from objective 1.1 could be adopted into biosecurity practices within the life of the project. As project outcomes have become available they have been communicated with banana growers, industry service businesses and key members of the Biosecurity Queensland Panama Response program team at a range of regular program meetings, R&D update meetings, regular discussion groups and industry activities such as the Banana Industry Roadshows and industry field days.

4.2. *The economics of banana biosecurity* – knowledge of the cost of implementing farm biosecurity practices and/or implementing new production systems is essential to support banana growers to make informed decisions. This activity modelled the costs of implementing identified biosecurity practices as well as modelling the relative productivity of alternative Cavendish varieties in possible alternative production systems.

4.3. *Review industry practice to develop an industry-led code of practice* – a Best Management Practice (BMP) guide for on-farm biosecurity has been developed with input from banana growers and Biosecurity Queensland. Eight banana grower representatives and Biosecurity Queensland staff from the Panama Disease Response Program have actively contributed to the development of the guide. The structure of the guide is based on the successful banana industry environmental BMP guide to ensure continuity and familiarity for users. It consists of a self-assessment checklist to audit their adoption of effective biosecurity practices, a management plan template that can be populated with gaps identified from the audit and comprehensive resource material that can assist in identifying what improved practices are available.

4.4. *Link with international expertise* – the further spread of Fusarium wilt TR4 is recognized as a global threat and networking with scientist working on the disease allows us to benefit from international experiences. This activity supports networking and communication with international scientists working with Fusarium wilt TR4 at a range of industry and scientific conferences both internationally and domestically. Results from project activities will be shared with scientific peers at identified conferences such as the ISHS-ProMusa Conference '*Agro-ecological approaches to promote innovative banana production systems*', October 2016, Montpellier and the APPS-PHA '*Science protecting plant health*', September 2017, Brisbane.

5. Facilitate rapid adoption of the research findings

5.1. *Engagement, needs analysis and communication plan* – this activity has developed an engagement and communication plan that identifies the key stakeholders for project information and results, identifies the key messages to be relayed to the key stakeholders and describes the activities and channels for extension and communication to occur.

5.2. *Deliver education and training to deliver the Plan* – this activity has provided extension and communication activities to assist banana growers and other target audiences to prepare for the critical scenarios identified. This has been achieved by working closely with banana growers and key client groups, particularly the Australian Banana Growers' Council, in extension and training activities aimed at supporting the implementation of effective biosecurity practices. As the project progressed new results and scientifically validated practices were disseminated through participation in industry events coordinated by other projects such as the Banana Industry Roadshows, Australian Banana Industry Congresses and through the banana industry publications 'Australian Bananas' and the ABGC e-Newsletter.

Outputs

1. Conduct research to underpin improved biosecurity practices on farm

1.1. *Rapidly identify solutions for early uptake in biosecurity practice: reducing inoculum movement from around banana plants, within plantations and between plantations*

Movement pathways and associated risks identified

The HACCP (Hazard Analysis and Critical Control Point) process is a risk analysis tool and a modified HACCP process was used to identify the most likely movement pathways for *Foc* TR4 associated with north Queensland production systems. From this process a checklist for banana producers was produced and revised in collaboration with banana growers, culminating in the Panama disease risk assessment tool (RAT) (Appendix 1). The value of the Panama disease RAT was realised when it was used to provide input to the development of content and process for the ABGC Biosecurity Extension project workshops and activities. It was also considered by Biosecurity Queensland in their development of the Panama disease Standards and Guidelines which are intended to provide banana growers with guidance on their biosecurity requirements if they become infected and are quarantined.

This ABGC-led project used an interactive workshop process to take participants through a farm planning process, using aerial maps of their individual properties, to identify risk pathways for their properties and possible biosecurity management options. At the completion of the workshop process participants had developed some or all of a draft biosecurity plan for their farm (Appendix 1).

This ABGC Biosecurity Extension project ultimately delivered 37 workshops involving 246 growers, partners and farm managers representing 228 farms, representing 77% of farms and 82% of production area in north Queensland.

Improved biosecurity methods identified through soil, water and people movement

A range of effective biosecurity methods have been identified for the north Queensland banana industry although the ease of adoption is influenced by the financial and economic circumstances of individual properties. The foundation principle for effective biosecurity adoption is one of exclusion of all non-essential vehicles/machinery/tools/people/planting material from the property. Where exclusion is not practical or feasible, such as delivery of farm inputs, staff access and packed fruit dispatch, then access should only occur subject to practices to manage the associated risk. Management of movement or access is simplified by the application of differential access zones on a property with movement across zones boundaries subject to the risk management practices.

Practices that have been proposed to manage these risks include:

- Use of dedicated vehicles, footwear and tools within specific zones
- Procedures and facilities for footwear change at zone boundaries
- Use of footbaths and vehicle washing and disinfection procedures
- Physical barriers such as fencing to minimise people and animal movement across zones, drainage to intercept surface water movement from external sources onto the farm zone where practical
- Use of certified clean planting material

These biosecurity practices and their planned implementation were an integral part of the aforementioned ABGC Biosecurity Extension project workshop process (Appendix 2). They were also communicated at industry events and extension activities such as the Panama Field Day (November 2015 – 140 attendees), Panama R&D Open Day (May 2017 – 109 attendees), Banana Industry Roadshows (June/July 2016 – 147 attendees)

Identifying the most effective disinfectants for each situation

The use of disinfectants to decontaminate machinery, tools and footwear was identified as a key practice to manage the risk of spreading *Foc* between infected and uninfected farms. However, published information or data on the efficacy of disinfectant products against *Foc* was not available and there was uncertainty on the efficacy of products in the presence of soil, rates of application and the longevity of products being offered to the banana industry.

As a result research trials were undertaken in Queensland and the Northern Territory to determine the effectiveness of

a range of disinfectants or sanitisers against both *Foc* R1 and TR4 that could be used in footbaths, vehicle dips, or to treat waste water held in tanks and washing down farm equipment that would reduce the survival of spores of *Foc* to a 'zero' detectable level.

Due to the urgent need for efficacy data the initial trials were conducted in Queensland using *Foc* R1 isolates (VCG 0124) as quarantine restrictions prevented the use of *Foc* TR4. Products with identified activity against *Foc* R1 were then tested against *Foc* TR4 by NTDPIR staff following the same protocols to confirm the validity of the initial results.

In the Queensland trials a total of 31 products were received for testing which could be classified into five main categories: quaternary ammonium products, miscellaneous disinfectants, bioflavonoids, oxidising agents and an undefined group that were predominantly detergent based (Appendix 3). From this forty-five disinfectant treatments, consisting of different products at various concentrations with and without soil, and controls consisting of water inoculated and water alone, were tested at 4 sampling times (0, 5 and 30 min and 24 hr).

The key findings were that the quaternary ammonium compounds (>12% a.i.) and Evo Tech 213 (bioflavonoid) were effective at achieving a 'zero detectable' level of *Foc* R1 at rates of 1% across the range of contact times and in the presence of soil. The results also showed that the longer the contact time, the greater the reduction in colony formation and that generally the presence of soil decreased the efficacy of the treatment, especially at the weakest dilution. Of particular interest was that detergent based products, which are the primary products used by the cotton industry for management of Fusarium wilt of cotton, appeared to only suppress or delay colony development.

In the Northern Territory trials a total of 31 disinfectant products were used for testing (Appendix 4), and included 19 quaternary ammonium compounds, 3 bioflavonoids products, 1 oxidising agent, 6 detergent-based and 2 miscellaneous products. All products were tested at the rates of 1:10, 1:100 and 1:1000, with and without soil, and compared to controls consisting of water inoculated and water alone, at 4 sampling times (0, 5 and 30 min and 24 h).

In general, the results achieved from all disinfectant treatments were consistent between *Foc* R1 and TR4. Of the five categories of disinfectants assessed, the quaternary ammonium (QA) compounds were the most effective against the survival of propagules of both *Foc* TR4 with no colony detected in treatments at 1% dilution, both with and without soil added, across all the four contact times for products with >10% active ingredient.

From these results clear recommendations have been made for industry regarding effective disinfectant use and presented at the Panama R&D Open day, Banana Industry Roadshow 2016 and local producer association meetings, as well as published in the 'Australian Bananas' magazine and as a fact sheet.

Managing QA disinfectant products – monitoring tools, monitoring tools and corrosion potential

Quaternary ammonium (QA) compound based disinfectants such as didecyl dimethyl ammonium chloride (120 g/L DDAC) applied at the recommended label rate of 1% solution (1200ppm) are effective in killing the fungal spores of *Foc* R1 and TR4. Concentration of the product is crucial to effective control so trials were conducted to identify rapid and effective test methods to monitor and manage effective concentrations of QA compounds in dips, sprays and footbaths for banana growers and other stakeholders. Various QA compound test kits which measure the active ingredient concentration were tested against three QA products containing 120 g/L DDAC – Sporekill®, Steri-max® and Path-X™. Although a number of different QA compound test kits were trialled, the Precision Laboratories High Level 0-1500ppm test strips (0-1500ppm) provided consistent and accurate results, and are proving to be an effective tool for routine monitoring of QA compounds (Appendix 5). A sample QA compound test kit was developed for banana growers to test their QA's and evaluate the Precision Laboratories High Level 0-1500ppm test strips in a real situation. Approximately 90 test kits have been supplied to banana growers and industry stakeholders through industry events including; the Panama R&D Open Day, Australian Banana Industry Congress, Panama Tropical Race 4 update meetings and farm visits.

The longevity of 3 QA products (Sporekill®, Steri-max® and Path-X™) made at 1% (1200ppm) solution with and without the addition of soil, and exposed to field conditions was investigated (Appendix 5). After 8 months exposure to field conditions, including sunlight, humidity and diurnal temperature fluctuations, the inoculation results had shown that all three QA products without the addition of soil maintained complete efficacy, with zero colony growth of *Foc* R1 detected. At the 8 month mark, the Precision Laboratories High Level QAC 0-1500ppm test strips started to show a reduction in concentration for some of the QA products. However, despite the reduction in concentration, the QA products without the addition of soil remained at an effective concentration after 8 months exposure to field conditions. The addition of 5% soil gave contrasting results early on in the experiment. Using the test strips as

indicators, it appeared that the concentration of active ingredient was reducing over time. Inoculation results of the QA products with the addition of soil at the 4 month mark had shown that colony growth was detected at an instant contact time for all QA's, and a contact time of up to 30 minutes was required to reach zero colony growth for the QA's with the presence of soil.

Two experiments were conducted to investigate the corrosive abilities of various cleaning and disinfecting products on different types of metals, commonly used for biosecurity infrastructure, when submerged in solution or dipped frequently (Appendix 5). The experiments compared 3 QA products, a detergent-based product and water against unpainted steel, painted steel, galvanised steel, aluminium and stainless steel (grade 304). In the first experiment the disinfectant products did not corrode the metals significantly more than water alone when submerged for 2 months. Painted and unpainted steel were the most susceptible metals to rust development and would be the least suitable metals for constructing long term on-farm biosecurity infrastructure. The second experiment involved frequent dipping (2-3 times per week) of the metals in the cleaning and disinfecting products, followed by exposure to field conditions. From the results unpainted steel was the most susceptible metal to rust development, and the three QA products – Sporekill®, Steri-max® and Path-X™ appeared to be more corrosive on unpainted steel than the water, Farmcleanse and control treatments. All three QA's were more corrosive on unpainted steel with the frequent dipping application method as opposed to the submerged in solution method, where all treatments including water had similar corrosive abilities. The painted steel, stainless steel, aluminium and galvanised plates remained in good condition throughout the experiment with the frequent dipping application method.

Results from these trials have been presented to the banana industry at the Panama R&D Open Day, May 2017 and the Australian Banana Industry Congress 2017.

Managing contaminated waste and effective decontamination of tools

Results from the disinfectant trials have identified options for managing wash down water. The best options show that a number of disinfectant products can be used to treat waste water containing soil for 24 hours at the lowest effective chemical concentration that achieves zero colony counts (Appendix 3). Wash down/vehicle dip/footbath waste treated in this fashion can then be disposed of following the recommended method for that disinfectant treatment.

The treatment of contaminated waste, including protective clothing from surveillance staff, is being treated/ handled commercially under the supervision of Biosecurity Queensland, and as such is not being researched. This activity was planned with the expectation that *Foc* TR4 would be identified on more properties and that there would be large quantities of material generated in a small period of time requiring treatment. This situation has not eventuated so research capacity has been focused in the managing wash down waste and sanitising tools.

Three disinfectants were tested for their ability to eliminate chlamydozoospores added to soil and present on field sampling equipment such as cane knives (Appendix 3). Products included: Diggers Violet Methylated Spirits at four rates (neat product, 90%, 80% and 70%), 1% Bleach (100g/L active) and 1% Steri-Max (120g/L DDAC). Chlamydozoospores of *Foc* R1 were applied to PDA plates and allowed to dry for different lengths of time, '0' (instant), 5 minutes or 1 hour, prior to being sprayed with the disinfectant treatments listed above. This technique was designed to simulate as close as possible field sampling activities and decontamination procedures. The final colony counts indicate that both Bleach and Steri-Max at 1% were effective at eliminating colony development across all drying times. For the methylated spirits treatments, colony numbers reduced as the product rate increased, however complete elimination was not observed. It needs to be noted that this experiment replicates a 'worse case' scenario.

1.2. Develop tools that provide early indications of infection and allow for prompt interventions

Development and validation of a *Foc* TR4 DNA soil detection test

Based on sequence information available in GenBank, a TaqMan MGB qPCR assay targeting the Intergenic Spacer (IGS) of ribosomal DNA was designed for the detection of *Foc* TR4. The assay was assessed for sensitivity and specificity against a collection of more than a 100 isolates including several *Foc* TR4, *Foc* Subtropical Race 4 and *Foc* R1 isolates and a range of *Fusarium* species/subspecies from Australia and overseas (Appendix 6). The assay detected most *Foc* TR4 with a high sensitivity but cross reacted at a low level with most other *Foc* races and many *F. oxysporum* subspecies from some collections. It was suspected that these issues could be explained by a combination of contamination and/or misidentification and required further investigation.

A new collection including pathogenic/non-pathogenic and Australian/international isolates was received from DAF Queensland and used to further assess the specificity of the assay. The results, together with those reported previously indicate that the qPCR assay preferentially detects *Foc* TR4, but some isolates are detected well (VCG 1213/1216), some less well (VCG 0122) and others are hardly detected (VCG 0121). The new results confirm that some isolates previously tested may have been contaminated or misidentified. Further investigation is needed to understand the poor detection of VCG 0121 isolates and, if required, determine if the assay can be modified to improve specificity.

The assay consistently detected *Foc* TR4 in spiked soil samples provided from the NT and the results indicate that the limit of detection of the new assay is about 2.5 spores/g of soil. Overall the results show that the assay is sensitive and specific enough for *Foc* TR4 detection in soil, however where a new incursion is detected it is recommended that this be confirmed by sequencing to confirm identification. The assay is now commercially available through SARDI Molecular Diagnostics Centre.

Update of *Foc* manual completed with streamlined diagnostics

This project activity has offered support if required to an existing initiative of Plant Health Australia (PHA) that encompassed the development, review and updating of National Diagnostic Protocols (NDP's) for high priority pests, including *Foc* TR4, as an identified action of the National Plant Biosecurity Diagnostics Strategy (<http://www.planthealthaustralia.com.au/wp-content/uploads/2013/03/National-Plant-Biosecurity-Diagnostic-Strategy.pdf>).

Reviewing and updating the "National Diagnostic Protocol for *Fusarium oxysporum* f.sp. *cabense*, the cause of Panama disease of bananas", has been undertaken as a project funded by Plant Health Australia and led by Dr Lucas Shuttleworth, NSW DPI. Diagnosticians from QAAFI and DAF BQ have contributed to the review and Dr Shuttleworth submitted the revised manual to SPHDS for review in May 2017. The review may take from 3-12 months to finalise the document.

1.3. Improve monitoring, early detection and responses to disease incursion

Activities planned under this objective were transferred to "ST15011 Child 10 DAF Multi-scale monitoring tools for managing Australian Tree Crops: Industry meets innovation".

1.4. Managing inoculum load in pseudostems, soil and water and alternative hosts

Banana pseudostem destruction to manage *Foc* inoculum

The current method for dealing with contaminated pseudostems is to cut all plant material from the infected plants and place into large plastic bags with 1kg of urea/m³ of material to hasten the breakdown of the fungal and plant tissue. Laboratory and field trials have been conducted to test the effectiveness of urea as a fungicide against *Foc* R1 (VCG 0124). Using a soil bioassay test (Appendix 7) to test *Foc* R1 recovery from soil that had received different treatments (control, urea, urea + lime, lime) at 3 different levels of inoculum the results showed that *Foc* R1 was recovered at all inoculum levels from the control and lime treatments, but no samples from the urea or urea with lime treatments were positive for the pathogen. The results suggested that urea applied at a rate of 5 t/ha was an effective treatment for contaminated banana pseudostems.

Further trials to improve the understanding of the control mechanism and refine the application rates were conducted. Laboratory trials were conducted to test the effectiveness of lower rates of urea and investigate chemical alternatives (potassium chlorate, sodium nitrite, nitrite) potentially toxic to *Foc*. The trial results showed urea at rates greater than 0.031 kg/m² were effective at preventing *Foc* from being recovered from the soil, whereas nitrite forms and potassium chlorate (a nitrogen cycle disruptor) were ineffective at reducing *Foc* recovery (Appendix 7). Having confirmed that ammonia was the effective component of the urea breakdown cycle, an additional experiment was conducted to establish the lowest effective concentration of ammonia from urea and other sources to prevent *Foc* recovery. The results showed that ammonia (NH₃) from any source that produced a concentration equal to or greater than 2500 ppm was effective at preventing *Foc* from being recovered from the soil. These data have helped to inform field trials undertaken by Biosecurity Queensland to confirm the efficacy of their destruction protocol (Appendix 7). The results from the field trials confirmed that the standard destruction protocol is effective at destroying *Foc* in infected pseudostem material and can reduce the level of inoculum in soils associated with the infected plant to very low levels.

Injection of banana pseudostems with either herbicide (Basta®- glufosinate ammonium) or urea treatments was tested to explore their suitability for use in the destruction of infected plants. Three different application rates were used to apply both treatments which were injected into freshly harvested pseudostems. The results demonstrated that neither Basta® nor urea were suitable as stand-alone treatments for infected plants at the rates trialled, as they did not kill the treated plants.

Field testing of banana pseudostem destruction and decomposition methods completed

Trichoderma spp isolates have been tested for their ability to suppress the production of *Foc* R1 inoculum and enhance the decomposition of banana pseudostems in laboratory and field trials.

A number of *Trichoderma* spp isolates recovered from a *Foc* soil bioassay test were tested for their ability to suppress/compete with *Foc* R1. In-vitro tests showed that the isolate Trich-005 consistently outperformed all other isolates when averaged over all of the measurements, demonstrating the highest percentage inhibition of the pathogen and the most rapid growth in relation to the pathogen. The effect of the addition of this isolate to banana pseudostems that were either un-inoculated or inoculated with *Foc* R1 (VCG 0124) was then tested. While the Tri-005 treatments showed very little reduction in overall pseudostem weight when compared to either the control or *Foc* treatment, the numbers of total *Foc* R1 conidia/mL and chlamydozoospores/mL recovered from pseudostem pieces were dramatically reduced by the Tri-005 treatment (Appendix 7).

Field trials were then conducted to observe and measure the effect of injecting *Trichoderma* spp. (Tri-005) into standing harvested banana pseudostems on the rate of plant breakdown. Analysis of measurements taken at time zero and then monthly until the pseudostems toppled over have shown that there were no significant differences in sucker height, leaf emergence or stem integrity (penetrometer, kg/m²) reading or the change in these parameters, between the treated and untreated plants. Destructive sampling has shown a major difference in the degradation of pseudostem material between treated and untreated plants and *Trichoderma* sp. has been consistently recovered from inner tissue of the treated but not untreated pseudostems.

Weed host status to *Foc* determined.

The identification of weed and ground cover species that allow *Foc* to survive and persist in banana farms is important for inoculum control, identifying species that may assist in suppressing the pathogen, reducing the spread of the pathogen and long-term management of Fusarium wilt of banana.

To establish likely hosts for *Foc* surveys of north Queensland banana farms were conducted to determine the most common weed and groundcover species co-habiting banana farms (Appendix 8). The survey results established that the six most common species found on banana farms were Sour Grass (*Paspalum conjugatum*), Crowsfoot Grass (*Eleusine indica*), Mullumbimby Couch (*Cyperus brevifolius*), Cinderella Weed (*Synedrella nodiflora*), Broadleaf Carpet Grass (*Axonopus compressus*), and Pennywort (*Centella asiatica*). Sampling of weed, grass and ground cover species was then conducted from 3 banana farms with known *Foc* R1 (VCG0124/5) infection (Appendix 8). A total of 115 plants from 20 different species were subjected to root isolations to determine the presence of *Foc* R1. From this sampling *Foc* R1 was isolated from the roots of only four species (5 plants in total) – *Cleome aculeata* (2 isolates), *Youngia japonica* (1 isolate) *Eleusine indica* (1 isolate) and *Digitaria ciliaris* (1 isolate).

To overcome the low incidence of natural infection an inoculated glasshouse pot trial was conducted in collaboration with Biosecurity Queensland staff to more clearly determine the host status of the identified weed and ground cover species (Appendix 8). In this trial *Foc* SR4 (VCG 0120) was used as a surrogate for *Foc* TR4 (VCG 01213/16) to overcome quarantine restrictions and due to their genetic similarity. Eighteen weed and ground cover species identified from on-farm surveys, as well as the *Foc* TR4 infected property in Tully, were tested to determine their ability to 'host' *Foc* SR4 (VCG 0120). The results showed that the pathogen was recovered from all eighteen species tested, although it was more consistent from some species than others. For example, the pathogen was recovered from 80% of Mullumbimby Couch replicates, whereas the pathogen was only recovered from 20% of Green Amaranth replicates. Although the experiment showed that all of the species had the potential to host *Foc* SR4 (VCG 0120), the differences in the frequency of isolation may suggest that some species more readily host the pathogen than others.

2. Improve access to new cultivars and build capacity in propagation

Production and dissemination of a comprehensive review of international banana breeding programs

A review of international banana breeding programs has been undertaken and a report produced outlining the breeding objectives, the breeding methodologies being used, the likelihood of accessing progeny for testing in Australia and a list of identified varieties that we wish to import into Australia for testing. The full report is included in Appendix 9.

In summary, there are relatively few breeding programs actively breeding or selecting Cavendish style replacements. Most programs developing hybrid crosses are focusing on Lady Finger (AAB Pome subgroup) or Silk (AAB Silk subgroup) types with only limited success in developing replacement varieties with *Foc* resistance and market acceptable fruit quality. Improvement in Cavendish varieties has been almost exclusively limited to somaclonal selection due to their inherent sterility, although recently there has been privately funded hybridisation with Cavendish undertaken by the FHIA program in Honduras.

Access to breeding outputs of many of the programs is increasingly restricted or unavailable and ready access to varieties suitable for the Australian marketplace is not assured in the future. To improve access to new varieties options for co-investment in some programs has been examined, particularly the Cavendish improvement program at the Taiwan Banana Research Institute. The review document will form the basis of an options paper for consideration by the Variety Committee of BA16001 'Improved Plant Protection for the Australian Banana Industry' in setting a strategic focus for variety importation and improvement.

3. Develop resilient crop management options

3.1. Assess the use of cover crops to reduce inoculum in infested soil

Suitable groundcover species for suppressing *Foc* determined

Nineteen potential tropical rotation crops and groundcovers growing in the pasture collection at Walkamin Research Station were tested for their microbial profile and ability to host a complex soil microbial community, to determine differences from bare soil (Appendix 10). Soil from the different crops was evaluated using MicroResp™, soil nematode community assays and *Foc* baiting bioassays. The differing pasture species demonstrated differences in soil microbial communities, using both the soil nematode community and substrate utilization profiles (Appendix 10). While 6 species had a similarity in nematode community composition less than 90% compared to the bare soil plots, only *Leucaena leucocephala* and *Heteropogon contortus* had a mean similarity less than 90% for utilization of carbon substrates relative to the bare soil. Four pasture plot soils completely suppressed the recovery of *Foc* R1 from a baiting bioassay – *Leucaena leucocephala*, *Dismanthus bicornutus*, *Arachis pintoii* and *Chloris gayana* – although there was some variation in the recovery of *Foc* from pasture species that had multiple entries, such as *Chloris gayana* which ranged from zero to 50% recovery and *Arachis pintoii* which ranged from zero to 90%.

Results from the three analyses indicate that *Leucaena leucocephala* is a tropical pasture species worthy of further investigation. Soil taken from *Leucaena* plots completely suppressed the recovery of *Foc* from soil and altered the microbial community based on nematode community structure and MicroResp™ substrate utilisation profiles. Soil from other pasture species also suppressed recovery of *Foc* however their microbial activity under these species was greater than 90% similar to bare soil, which indicates that factors other than just the pasture species were important to the recovery of *Foc*.

The survey of pasture species for suppression of *Foc* and changes in soil microbial activity should be viewed as a rapid screening and requires further validation by more thorough experimentation. However, the survey was able to find novel rotation crops such as *Leucaena leucocephala* with potential to be used in rotation with bananas for further study.

3.2. Assess how root exudates affect *Foc* TR4 populations

Influence of root exudates on chlamydospore germination determined

Initial experiments on root exudates characterised the utilisation by *Foc* R1 (VCG0124) on known exudate compounds using the MicroResp™ system (Appendix 10). The carboxylic acids, which are the most common root exudates, resulted in the greatest activity of *Foc* R1, with citric acid being utilised the most. Fumaric acid, which has been found previously to promote *Foc* growth on bananas, was utilised significantly less than citric acid ($p < 0.05$). The sugars tended to be

utilised less than the carboxylic acids, except glucose which had a microbial utilisation equivalent to citric acid. The amino acids, as a group of exudates, tended to be utilised less than the other carbon sources, particularly L-arginine, which tended to inhibit the growth of *Foc* R1.

3.3. Assess the use of microbes to suppress *Foc*

Characterization of suppressive microbiome components identified

The development of banana production systems to suppress *Foc*, focusing on the use of vegetated groundcovers and nitrogen fertiliser management are continuing. Results from two field trials indicate that soil type and time are important factors in microbial community activity (Appendix 10). Indications are that it may take up to two years for microbial community activity to increase, as measured using β -glucosidase as an indicator. In current trials the vegetated groundcovers tended to enhance β -glucosidase activity after about 20 months. The impact of soil type is demonstrated by site variations in the current experiments with site 1, which is a ferrosol (red volcanic, medium clay) on a commercial farm, demonstrating a greater response to different management than site 2 which is a dermosol (alluvial clay loam).

The suppression of *Foc* R1 (VCG 0124), determined using a bioassay of the trial site soil, indicated that less *Foc* was recovered from soil with increasing time, particularly at site 1. The reduction in *Foc* recovery tended to correspond with increased microbial activity. Further, characterisation of the soil microbiome using MicroResp™ community level physiological profiling also demonstrated there was a shift in the utilisation patterns of different carbon substrates over time. This “maturity” of the microbial community appears to be linked with increased suppression of *Foc*.

The use of Next Generation Sequencing (NGS) to investigate the soil microbiome could significantly distinguish between banana soil management, with Site 1 groundcover and nitrogen treatments distinguishable due to bacterial and archaea taxa associated nitrogen cycling (Appendix 10). A *Pseudomonas* sp., which are putative antagonistic bacteria was found to be associated with the vegetated groundcover but not the bare soil treatment in Site 1. At Site 2 the analyses could distinguish between nitrogen treatments but not vegetated and bare groundcover treatments.

Due to the large volume of data generated using NGS Miseq analysis further analysis and correlations are required to link disease suppression with changes in the soil microbial community.

3.4. Assess the role of plant stress in susceptibility to *Foc* infection

Abiotic plant stress on wilt symptom development validated

A number of methods were identified as potentially useful for quantitative assessment of stress in bananas via a thorough literature review and discussions with experienced plant physiologists. The methods identified were:

- Leaf chlorophyll content
- Chlorophyll fluorescence
- Proline accumulation
- Cell membrane stability
- Thermography
- Gas exchange parameters

Not all of these methods have been applied to bananas previously and considerable time has been spent trying to optimise the protocols for each method. Initially working in a field variety trial in a *Foc* R1 (0124/5) infected farm, a glasshouse experiment was undertaken at DAF South Johnstone to assess these methods further against deliberately stressed plants in controlled conditions (see Appendix 11). This multicomponent investigation examined several proximal sensing methods which scrutinised the early physiological responses of banana plants to stress and disease. Measuring the concentration of plant pigments (particularly chlorophyll) assisted in determining the severity of stress or *Foc* infection, due to the relationship this pigment has with leaf function. Considerations need to be made for inherent differences in chlorophyll concentrations across the different banana varieties when using this variable as an indicator of plant health. Measuring chlorophyll fluorescence may also be useful for quantifying stress severity, however the instrument and method used in the present trials need to be reconsidered and improved for future work if this is to be a tool for pre-symptomatic disease detection. In this study, stomatal conductance, levels of proline accumulation and thermography could not be verified as useful measurements for stress quantification or disease

detection in banana plants, and further work is required before they are worth incorporating into early detection protocols. In addition, future trials involving inoculation treatments may wish to sample plants throughout the investigation to ensure infection has occurred so that the results are able to meet the experimental objective.

4. Update biosecurity protocols for banana production to reflect project outcomes

4.1. *Research becomes biosecurity protocol*

Best management biosecurity plan for keeping farms disease free and reducing on-farm spread

A Best Management Practice (BMP) guide for on-farm biosecurity has been developed with input from banana growers and Biosecurity Queensland (Appendix 12). Eight banana grower representatives and Biosecurity Queensland staff aligned to the Panama Disease Response Program actively contributed to the development of the guide.

The structure of the guide is based on the successful banana industry environmental BMP guide to ensure continuity and familiarity for users. It consists of a self-assessment checklist to audit their adoption of effective biosecurity practices, a management plan template that can be populated with gaps identified from the audit and comprehensive resource material that can assist in identifying what improved practices are available. The resource material has lots of pictures as examples on-farm biosecurity practices and systems that banana growers have already implemented and also links to the currently available resource material (e.g. factsheets, videos, Biosecurity Queensland resources).

The hard copy resource was launched at the Panama R & D Open Day held on the 12th of May 2017. Three hundred hard copies were produced in the initial print run and to date 115 of these have distributed at the Open day, Australian Banana Industry Congress 2017, and various other industry events. The low res pdf of the resource can be found at:

<http://horticulture.com.au/wp-content/uploads/2017/06/On-farm-Biosecurity-Manual.pdf>

Plans are progressing to develop an on-line based system based on the guide, similar to the environmental BMP system, as part of BA14014 'Fusarium wilt Tropical Race 4 Research Program'.

4.2. *The economics of banana biosecurity*

Economic scenarios for Panama disease established and disseminated

Comparisons of modelled scenarios for a hypothetical small-medium banana farm in north Queensland (56 ha) have shown that key characteristics of properties have a major influence on the ability to effectively implement exclusion and zoning to manage the spread of *Foc* TR4 (Appendix 13). Based on the application of identified effective practices the cost of capital investment ranged from \$3070 to \$8500 per hectare for a contiguous and non-contiguous scenario respectively. Estimates of operating costs for crossing zone boundaries safely (washing vehicles/machinery, changing boots, provision and maintenance of disinfectant products) ranged from \$134 to \$546 per hectare per year for the modelled contiguous and non-contiguous scenarios respectively.

Comparison of modelled productivity outputs for alternative Cavendish production systems show that none of the alternative systems yields more than 50% of the industry standard 'Williams' Cavendish in a disease-free situation based on the currently available data. The value of the productivity modelling is to allow the manipulation of key variables such as bunch mass, crop cycle times and population mortality to identify the key requirements and productivity drivers that any potential production system must achieve (Appendix 13).

4.3. *Link with international expertise*

Project staff have hosted a range of international banana scientists and producers, sharing project activities and results. Professor Altus Viljoen, Stellenbosch University, and Dr Chi-Ping Chao, Director of the Taiwan Banana Research Institute, were hosted in north Queensland after the Australian Banana Industry Congress 2015. During this visit discussion and presentations were arranged to share project activities, methodologies and results. Visits occurred to farms in Tully and Atherton Tablelands to inspect biosecurity practices and Cavendish variety trials. Visits were also received from Dr Franz Wielemaker, a consultant plant pathologist based in Costa Rica (2015) and Dr Roberto Young, a pineapple and banana breeder with Dole based in Honduras (2017).

Project staff travelled to Taiwan in February 2016 to discuss collaborative research opportunities and access to cultivars that are being developed. The Taiwan Banana Research Institute (TBRI) is mostly self-funded from the sale of tissue

culture plantlets and the development of their varieties. As the banana industry in Taiwan is currently in decline, due to competition with the Philippines, TBRI is looking at a greater global focus for its varieties. TBRI are interested in developing greater links with Australia and exchange of personnel and ideas. The greater global focus for its cultivars is seen as a priority for the Taiwanese government.

Project team members presented 2 papers at the 5th ISHS-ProMusa Conference *Agroecological approaches to promote innovative banana production systems*, at Montpellier, France in October 2016 on aspects of microbial diversity and soil suppression, and on the concept of integrated crop management approaches to managing Fusarium wilt in bananas (Appendix 14). Around 90 scientists attended the 3 day conference and the full papers will be published in *Acta Horticulturae* following the completion of the review process. (<http://www.promusa.org/article142-2016-France-symposium>)

A paper on the development of an integrated management approach to Fusarium wilt of bananas was also presented at the 9th Australasian Soil-borne Disease Symposium, held at Lincoln University, Christchurch in November 2016. Approximately 60 domestic and international researchers attended the symposium and the abstracts are published in the symposium proceedings.

(<https://conferences.lincoln.ac.nz/9th-australasian-soilborne-diseases-symposium>)

The costs of travel and attendance at these events were funded from BA14014 'Fusarium wilt Tropical Race 4 Research Program'.

The project leader was also invited to present an overview of the current situation with the R&D and regulatory response to the *Foc* TR4 incursion in Australia at the World Banana Forum Steering Committee global webinar in June 2017. Aspects of the RD&E activities undertaken in BA14013 and BA14014 were presented to an audience of global R&D organisations, major banana production companies and national and FAO policymakers.

5. Facilitate rapid adoption of the research findings

5.1. Engagement, needs analysis and communication plan

A communication and engagement plan was developed for the project that identified the key target audiences, the key messages and the best activities and communication channels to use (Appendix 15). This plan particularly identified existing communication and extension activities and products from both the industry extension project (BA13004) and the industry communication project (BA15005) that could be used to disseminate project results.

5.2. Deliver education and training to deliver the Plan

The project used a range of existing and project specific extension and communication activities and channels to disseminate project outputs (Appendix 16). A key activity was the development of improved networking with R&D providers working on Fusarium wilt projects in bananas across a range of agencies and funding bodies. The table below outlines the networking, communication and extension activities.

Date	Activity	Stakeholder Group	Attendance
Group activities – R&D coordination			
6/4/16	UQ Fusarium Focus video-conference	Panama disease R&D staff; ABGC staff; BQ staff	30
13/5/16	UQ Fusarium Focus video-conference	Panama disease R&D staff; ABGC staff; BQ staff	40
16/8/16	DAF Panama disease R&D update seminar	Panama disease R&D staff; ABGC staff; BQ staff, NT DPI staff	40
20/9/16	Meeting with ABGC TR4 R&D Manager – update for ABGC board	ABGC Board and staff	N/A

	and CEO on project progress		
7/12/16	Biosecurity Queensland Panama Response Program Planning Manager – update on project progress and activities	BQ staff	N/A
10/1/17	Dr E Aitken, UQ, seminar on <i>Foc</i> TR4 resistance gene marker development, South Johnstone	DAF RD&E staff; BQ staff; ABGC staff	15
14-15/2/17	Panama disease R&D Update seminar, Brisbane	DAF R&D staff; ABGC staff and board members; BQ staff; NTDPPIR staff; UQ staff; QAAFI staff; NSW DPI; ACIAR; HIA Ltd; JCU; UNE	50
6/3/17	BQ response and epidemiological review, Prof A Viljoen, Stellenbosch University	DAF project staff; BQ staff	15
25/9/17	Panama disease R&D Update seminar, Brisbane	DAF project staff; ABGC staff; 3 grower representatives; BQ staff; NTDPPIR staff; NSW DPI staff; UQ staff; JCU staff; UNE PhD student; Hort Innovation staff	35
Group activities - industry			
13/11/15	DAF/ABGC Panama disease industry field day	Banana growers, consultants, agricultural retailers, chemical company representatives	100
10/3/16	Banana agribusiness managers discussion group – sanitiser R&D presentation	Consultants, agricultural retailers, chemical company representatives	25
13/4/16	Mareeba Banana Growers Association meeting – sanitiser R&D presentation	Banana growers	5
8/6/16 Mareeba 9/6/16 Innisfail 16/6/16 Tully 23/6/16 Carnarvon 5/7/16 Coffs Harbour	National Banana Industry Roadshow – presentations on project results: <ul style="list-style-type: none"> • Sanitiser trials – K Grice • Early detection/remote sensing – K Ferro • Soil health – T Pattison 	Banana growers, consultants, agricultural retailers, chemical company representatives	165 total

7/7/16 Murwillumbah			
30/6 & 1/7/16	ABGC Panama TR4 industry meetings	Banana growers, industry service providers	50 total
July 2016	ABGC Chairman project progress briefing – summary of project activities for his panel discussion at PHA seminar, Melbourne	ABGC Board chairman, PHA seminar attendees	N/A
22/9/16	Banana agribusiness managers discussion group – supplementary sanitiser R&D presentation on corrosion and effective concentration monitoring	Consultants, agricultural retailers, chemical company representatives	22
4/4/17	ABGC TR4 R&D manager – discussions on new sanitiser screening results including assessment against TR4	ABGC TR4 R&D manager, and ABGC CEO and Board by extension	N/A
12/5/17	DAF Panama R&D Open Day, South Johnstone	Banana growers, consultants, agricultural retailers, chemical company representatives	109
22/6/17	Presentation at Australian Banana Industry Congress 2017 on project activities and results	Banana growers, consultants, agricultural retailers, chemical company representatives, supply chain businesses, R&D agencies	200+
6/7/17	Cassowary Coast Banana Growers Association meeting – update on project activities	Banana growers, ABGC staff	18
1-2/8/17	ABGC Panama TR4 industry meetings – update on new incursion (Tully, Innisfail, Mareeba)	Banana growers, industry service providers	130 total
Conference presentations			
10-14/10/16	5 th ISHS-ProMusa Symposium: Agroecological approaches to promote innovative banana production systems, Montpellier, France <ul style="list-style-type: none"> Integrating management practices to support banana production in the presence of Fusarium wilt (T Pattison) 	International banana researchers	90

	<ul style="list-style-type: none"> Monitoring microbial functional and structural diversity for management of disease suppressive soil (A McBeath) 		
14-17/11/16	<p>9th Australasian Soilborne Disease Symposium, Lincoln University, Christchurch</p> <ul style="list-style-type: none"> Development of an integrated management system to suppress Fusarium wilt of bananas (T Pattison) 	Domestic and international researchers	60
25-28/9/17	<p>Australasian Plant Pathology Society/CRC Plant Biosecurity - Science Protecting Plant Health Conference, Brisbane (pres.)</p> <ul style="list-style-type: none"> Engineering banana cropping systems to suppress soil borne diseases QA based disinfectants for effective on-farm biosecurity management of Panama disease in bananas <p>Posters:</p> <ul style="list-style-type: none"> Effects of commercial disinfectants on the survival of <i>Foc</i> Race 1 and TR4 propagules The survival of <i>Foc</i> in plants co-habiting Australian banana farms Testing the efficacy of urea as a treatment for the destruction of <i>Foc</i> in infected soil The assessment of physiological methods for early, quantifiable stress detection in banana plants Using <i>Trichoderma</i> to suppress Fusarium wilt (<i>Foc</i>) in banana cropping systems 	Domestic and international researchers; biosecurity agency staff and policymakers	523
Written material			
Australian Bananas – Issue 45, Spring 2015	<ul style="list-style-type: none"> Zoning out bad habits – making the change to effective biosecurity 	Banana growers, allied service providers, R&D staff	1000

Australian Bananas – Issue 46, Autumn 2016	<ul style="list-style-type: none"> Fielding ideas – growers share their TR4 innovations Ammonium compounds clean up 	Banana growers, allied service providers, R&D staff	1000
Australian Bananas – Issue 47, Spring 2016	<ul style="list-style-type: none"> Early detection trials DDAC test kits Panama disease R&D overview 	Banana growers, allied service providers, R&D staff	1000
Australian Bananas – Issue 48, Autumn 2017	<ul style="list-style-type: none"> Panama R&D update summary 	Banana growers, allied service providers, R&D staff	1000
Australian Bananas – Issue 49, April 2017	<ul style="list-style-type: none"> Meeting of TR4 Minds – the latest update on TR4 research 	Banana growers, allied service providers, R&D staff	1000
Australian Bananas – Issue 50, September 2017	<ul style="list-style-type: none"> Panama Open Day report 	Banana growers, allied service providers, R&D staff	1000
BQ Panama TR4 Program Update newsletter	<p>Mar/Apr 2016</p> <ul style="list-style-type: none"> Groundcover trials, reducing inoculum, weed host status survey, development of biosecurity BMP <p>May/Jun 2016</p> <ul style="list-style-type: none"> Weed host status survey, early detection, pseudostem destruction, soil ecology trials <p>Jul/Aug 2016</p> <ul style="list-style-type: none"> Panama R&D update seminar <p>Sept/Oct 2016</p> <ul style="list-style-type: none"> Disinfectant trials against TR4 from NT, new sanitiser testing in Qld, weed host trials, inoculum reduction <p>Jan/Feb 2017</p> <ul style="list-style-type: none"> Testing concentration of disinfectant solutions, disinfectant corrosion testing, detecting stressed banana plants 	Banana growers, ABGC staff, local government, utilities	400 recipients (116 growers)

DAF Fact sheets	<p>Panama Disease Tropical Race 4 Research Update series:</p> <ul style="list-style-type: none"> • Quaternary Ammonium products aid in the management of <i>Foc</i> • Testing the efficacy of urea as a treatment for the destruction of <i>Foc</i> in infected soil • Proximal sensing tools for early, quantifiable stress and disease detection • Quaternary ammonium (QA) products: How can you monitor them? How long are they effective for and are they corrosive? • Soil management, organic matter, biological activity and disease suppression • Multi-scale monitoring tools for managing Australian tree crops 	Banana growers, allied service providers, R&D staff	N/A
Banana best management practices guide	On-farm biosecurity	Banana growers, allied service providers, R&D staff	142 (as at 29/9/17)
International TR4 networks/visits			
23/2/17	3 Israeli banana R&D staff visited DAF South Johnstone to discuss <i>Foc</i> TR4 R,D&E activities	DAF R,D&E staff; BQ response program staff; ABGC staff	N/A
20/6/17	Prof R Ploetz (University of Florida) & Dr F Bakry (CIRAD, France) visited DAF South Johnstone to discuss <i>Foc</i> TR4 R,D&E activities	DAF R,D&E staff; BQ response program staff; ABGC staff	N/A
27/6/17	Dr Roberto Young, Dole Honduras, visited DAF South Johnstone to discuss <i>Foc</i> TR4 R,D&E activities	DAF R,D&E staff; BQ response program staff; ABGC staff	N/A

Outcomes

The project has been very successful in achieving the objective of providing new science, information and practices to address the priority needs for robust, science-based biosecurity measures to contain the disease and prevent further spread, and investigate measures for detecting and managing new incursions. The project has also made progress in the investigation of different elements of an integrated production system based on tolerant or resistant varieties and management practices to limit inoculum production. The project has been very successful in informing key stakeholder groups in the banana industry and RD&E community of the project results and activities throughout its duration.

1. Conduct research to underpin improved biosecurity practices on farm

1.1. Rapidly identify solutions for early uptake in biosecurity practice: reducing inoculum movement from around banana plants, within plantations and between plantations

The project has achieved all its intended outcomes to contain the disease and prevent further spread by developing and communicating practices and information that support the understanding and implementation of effective biosecurity practices. A systemic review of banana production systems with respect to the biology of *Foc* TR4 resulted in the identification of risk pathways associated with north Queensland banana production systems and this knowledge contributed to the development of an effective extension and communication project for biosecurity adoption.

The project also produced a major outcome in defining the effective use of sanitiser products for farm biosecurity. The project produced the most comprehensive screening of sanitiser products against *Foc* R1 and TR4 ever undertaken and the additional research that has supported their monitoring and management has provided the banana industry with the tools to effectively implement their use. Additional outcomes from this work include identification of methods for decontaminating tools and soil and water waste collected during farm biosecurity practices. The information has also been requested by both Nursery and Garden Industry Australia and Sugar Research Australia to assist their industries' efforts at maintaining effective biosecurity practices.

1.2. Develop tools that provide early indications of infection and allow for prompt interventions

The ability to detect and quantify *Foc* TR4 in large soil samples (200g) at levels as low as 2.5 spores/g of soil is a significant outcome for the project. While the test is not promoted for commercial use by industry due to the intricacies of appropriate sampling, the assay offers the significant opportunity for research activities to quantify the impact of management practices on disease inoculum. This capacity will accelerate the investigation and development of management practices fundamental to the development of an integrated crop management system for banana production in the presence of the disease.

1.3. Improve monitoring, early detection and responses to disease incursion

Outcomes for this objective have transferred to "ST15011 Child 10 DAF Multi-scale monitoring tools for managing Australian tree crops: Industry meets innovation"

1.4. Managing inoculum load in pseudostems, soil and water and alternative hosts

There is now a definitive recommendation for destruction of infected plants and the associated *Foc* inoculum so that spread of the disease is minimised. Biosecurity Queensland has implemented an effective and reliable destruction protocol for pseudostems infected with *Foc* TR4 using very high rates of urea in plastic bags. The protocol was confirmed as highly effective in destroying disease inoculum from laboratory trials undertaken by the project and field trials with *Foc* R1 undertaken by Biosecurity Queensland with funding support from the project. While there are currently only 2 infected properties in north Queensland, the practice is available for future scenarios where the disease has spread significantly and individual landholders are responsible for destruction.

The use of *Trichoderma sp.* for pseudostem destruction has also been successful and the treatment offers the opportunity to significantly reduce the production of chlamydospores within infected pseudostems while reducing the opportunity for saprophytic colonisation by *Foc* due to the rapid decay. While the urea destruction protocol is the preferred method for destroying infected plants, the inclusion of the *Trichoderma sp.* treatment with herbicide injection for crop destruction offers a more labour efficient method of inoculum management through prophylactic

treatment of non-symptomatic plants in blocks under destruction. A small scale application of this approach (100 plants) has been trialled by the second infected property during the destruction of the first infected block.

The investigation of weeds and ground covers as hosts of *Foc* R1 and SR4 has not revealed an effective non-host crop that can be used to reduce inoculum in the soil. Recovery rates from field infection were very low but all the plants inoculated in the glasshouse pot trial with *Foc* SR4 hosted the fungus. The work has however informed further trial work with *Foc* TR4 being conducted in the Northern Territory within the project 'BA14014 Fusarium wilt TR4: Research program'.

2. Improve access to new cultivars and build capacity in propagation

The review of global banana breeding programs is a major outcome that will assist future decisions regarding banana variety importation, screening and development for *Foc* TR4 resistance. The review has identified issues such as the long time frames and high costs associated with banana breeding. Additionally issues of infertility have made it difficult to breed from some commercially important varieties like Cavendish. The need for breeding programs to recoup some of the investment in breeding will almost certainly mean that future use of some varieties will be restricted and licenced with royalties due to the variety owner.

The review identified that the best prospects of delivering *Foc* TR4 resistance in a Cavendish banana in the near future appears to lie with somaclonal variants and mutagenesis, although the limited screening capacity in Australia will likely hamper on-going efforts using mutagenesis. If the current mutagenesis program in 'BA14014 Fusarium wilt TR4: Research program' is successful there could be sufficient interest in applying this same approach to obtaining a *Foc* R1 resistant Lady Finger type if some variation in resistance could be found.

The review will provide a basis for the Variety Committee of 'BA16001 Improved plant protection for the banana industry' to develop recommendations around importation and screening and variety development and commercialisation.

3. Develop resilient crop management options

3.1. Assess the use of cover crops to reduce inoculum in infested soil

The investigation of a range of tropical pasture and groundcover species has shown that some species, particularly *Leucaena leucocephala*, significantly change the soil microbial community, and that these changes can confer some suppression of *Foc*. This outcome is significant as it informs further research in rotation crops in BA14014 and identifies a rapid screening technique using the 3 analyses to refine the selection of candidate species for further validation by more thorough experimentation.

3.2. Assess how root exudates affect *Foc* TR4 populations

This activity showed that the most common root exudates, carboxylic acids, resulted in the greatest *Foc* activity while the amino acids tended to be used less than the other carbon sources, particularly L-arginine, which tended to inhibit the growth of *Foc* R1. This outcome significantly assists future screening of potential rotation crops and alternative varieties by allowing assessment of the propensity for their root exudates to support/deter *Foc* activity. Further investigation of this relationship will be undertaken in BA14014.

3.3. Assess the use of microbes to suppress *Foc*

A significant outcome from this research has been that increases in soil microbial activity, measured by β -glucosidase levels, corresponded to reduced *Foc* recovery using a baiting bioassay. Significantly the field trial results indicate that it may take up to two years for groundcover and nitrogen management practices to increase microbial community activity. Characterisation of the soil microbiome using MicroResp™ community level physiological profiling was also able to demonstrate changes in carbon substrate utilisation over time that link with increased suppression of *Foc*. The use of Next Generation Sequencing (NGS) to investigate the soil microbiome could also significantly distinguish between banana soil management. Overall these outcomes reinforce the value of continued research into the influence of management practices on the soil microbiome and the influence that has on suppression of *Foc*. Additionally it has shown that β -glucosidase activity and MicroResp™ community level physiological profiling are valuable tools for measuring the changes required for increased suppression of *Foc*.

3.4. Assess the role of plant stress in susceptibility to *Foc* infection

This objective has identified that measuring the concentration of plant pigments, particularly chlorophyll in leaves, assisted in determining the severity of stress or *Foc* infection in banana plants. However, other variables studied such as stomatal conductance, levels of proline accumulation and thermography could not be verified as useful measurements for stress quantification or disease detection in banana plants. These outcomes are informing more detailed early detection work in “ST15011 Child 10 DAF Multi-scale monitoring tools for managing Australian tree crops: Industry meets innovation”.

The outcome of this study highlights the importance of having well-trained biosecurity staff for surveying properties at-risk of contracting Panama disease, and having farm staff who are on the lookout for suspicious plants. At this stage, visual observations of external disease symptoms, primarily lower leaf yellowing and pseudostem splitting, remains the earliest way of detecting plants infected with *Foc*.

4. Update biosecurity protocols for banana production to reflect project outcomes

4.1. Research becomes biosecurity protocol

Major outcomes for the project have been the integration of key project findings into on-farm biosecurity practices in the banana industry. The research outputs on identifying and managing the *Foc* TR4 movement risks and the identification and implementation of on-farm biosecurity practices, particularly the effective use of sanitiser products, decontamination of tools and soil, and effective destruction of infected plants have been adopted by banana growers and regulatory authorities. Outcomes on the influence of stress on *Foc* infection and alternative hosts are informing activities in other projects so that they can direct their efforts into more promising lines of investigation.

4.2. The economics of banana biosecurity

The modelled data on the implementation cost for on-farm biosecurity practices and the productivity of potential alternative production systems have informed policymakers and managers at both ABGC and the Queensland Government on the relative value of different investment scenarios. The modelled cost of biosecurity practice adoption reinforced the evaluation data showing cost was a major barrier to adoption, particularly for farms with non-contiguous parcels of land. The implications reinforce RD&E investment in the identification of productive, resistant banana varieties with market acceptance. Data from this activity is now contributing to a more detailed investigation of the cost of biosecurity to the banana industry via the industry benchmarking project.

4.3. Review industry practice to develop an industry-led code of practice

The development of the biosecurity BMP for bananas has provided the banana industry with a framework for benchmarking and reviewing farm biosecurity practices and providing ready access to the resource information to assist implementation of improved practices. The biosecurity BMP guide is also providing the basis for the development of a web-based system via ‘BA14014 Fusarium wilt TR4: Research program’ that will integrate with the existing web-based environmental BMP system.

4.4. Link with international expertise

The project built significant links with international researchers working in *Foc* TR4, particularly in Taiwan and South Africa. The visits and discussions with the international visitors have significantly raised the international profile of the current RD&E activities underway in Australia on *Foc* TR4. Project activities in sanitiser screening and management, identifying effective destruction methods for infected plants with urea, the influence and management of soil microbial communities and the development of the biosecurity BMP have been world-leading outcomes for *Foc* management. As a consequence project staff have been presenters at both national and international conferences.

5. Facilitate rapid adoption of the research findings

5.1. Engagement, needs analysis and communication plan

The development of the communication and engagement plan resulted in a coordinated and effective extension and communication effort for the project findings that delivered both written and oral information material to a range of

identified target audiences. A key component for this was the identification of opportunities to disseminate project results via existing communication delivery mechanisms such as the industry roadshows, banana industry congresses and the Australian Bananas magazine, as well as specific Panama disease field days that highlighted key research findings.

5.2. Deliver education and training to deliver the Plan

The project has been successful in supporting the implementation of effective biosecurity practices by assisting extension and training activities. As the project progressed new research results and scientifically validated practices were disseminated through participation in a range of industry extension activities and the production of written material. The banana industry now has access to a framework and significant information resources for benchmarking and improving biosecurity practices through the biosecurity BMP guide and extension materials developed during the project. A high level of participation by banana industry members has been achieved in the project extension activities.

Evaluation and discussion

Evaluation of project impacts has largely occurred as part of the extension and communication activities undertaken by the project.

The research activities to underpin improved biosecurity practices on farm have contributed to significant change in industry knowledge and practice and have contributed significantly to the suite of industry extension activities. The project contributed significantly to the extension and communication of biosecurity practices through risk pathway assessment and the validation of biosecurity practices. The ABGC-led biosecurity extension project incorporated much of this material into its workshop modules. The workshops engaged 219 participants from 228 farms, representing 77% of the NQ grower population and 82% of the production area. Assessments conducted during the workshops showed that 91% of workshop participants improved their knowledge of Fusarium wilt 'quite a lot' or better (4 or 5/5 rating), 81% understood the risk pathways associated with the disease 'quite a lot' or better (4 or 5/5 rating) and 84% understood suitable, effective on-farm biosecurity practices for their farms 'quite a lot' or better (4 or 5/5 rating). Follow up evaluation conducted in the first half of February 2016 investigated the implementation status of 97 workshop participants, finding that 92% rated their current biosecurity practice implementation as moderate to high (4 or 5/5 rating). Where practices were not at the level desired by the respondent the most common reason given was cost (38%) followed by time (31%), with only 4% of respondents stating that they lacked sufficient knowledge to implement practices.

The project has also made significant contributions to the adoption of effective sanitiser practices for treatment of vehicles, tools and footwear. The project research into sanitisers has resulted in 100% adoption of quaternary ammonium products for disinfectant treatment by the industry. The management and monitoring of sanitiser solutions has been heavily influenced by project research with one major banana producer (400 ha) reporting use of the high range test strips has refined their replenishment program and reduced the product use and labour inputs for changing solution by 75%.

A range of project outputs have informed and contributed to the R&D direction and activities in projects such as BA14014, BA16001 and ST15011 in the aspects of variety screening, early detection and surveillance research and soil health/suppressive soils research. The project has also confirmed results for the standard destruction protocol used by Biosecurity Queensland and affected landholders, providing confidence to the banana industry that the treatment of infected plants minimises the risk of offsite movement of disease by reducing the contribution to inoculum build-up in the soil. While the disease remains contained and under active management the destruction protocol available for use should the disease become more widespread. In the Philippines where this scenario already exists the urea destruction protocol has been adopted by the Lapanday Food Corporation to manage inoculum loads in infected blocks.

The Banana Industry Roadshows 2016 and Panama R&D Open day 2017 were significant events that showcased the latest results and recommendations for the banana industry from the project. Evaluation activities undertaken for each show that participants had improved knowledge of the R&D activities being undertaken in the industry, including the outputs of this project (Appendices 17 & 18). The 2016 Roadshow series was held 15 months after the detection of *Foc* TR4 in Tully and the topic featured in more than a third of the program as significant R&D project investment was

targeted to address knowledge gaps. Participant responses during evaluation at the 2016 Roadshow series indicated a 45% increase in knowledge of industry R&D projects with 79% of participants indicating they would possibly or definitely consider making changes on their farm due to their attendance. For the Panama R&D Open day in May 2017 an evaluation activity was undertaken at the end of the event which was attended by 109 people. The participant responses show that 96% indicated they would change something in their business as a result of attending the event.

An additional significant impact of the project has been the development and distribution of the biosecurity BMP guide within the banana industry. More than 140 copies have been distributed to key industry stakeholders providing the opportunity to benchmark current biosecurity practices and identify opportunities for improvement. The BMP guide is also serving as the basis for further development of an on-line system to support improved biosecurity practice adoption as a part of BA14014.

Overall these assessments show that the project has been very successful in effecting practice change in the north Queensland production region with project activities contributing to the adoption of effective biosecurity practices and contributing to the planning and development of activities in other high priority projects focusing on *Foc* TR4.

Recommendations

Conduct research to underpin improved biosecurity practices on farm

- Consider options for future R&D to support biosecurity practices - at the completion of this project there is no R&D investment to support the development or refinement of improved biosecurity practices for the banana industry. Knowledge gaps will become apparent with time and the industry will need a means to address these new priority needs.
- There is value in a more detailed understanding of the motivations behind the non-adoption of biosecurity practices by some producers and other industry sectors. A deeper understanding of the motivations can help in the development of new approaches and policies to address the barriers to implementation.
- The potential use of *Trichoderma sp.* as a prophylactic treatment of non-symptomatic plants during crop destruction needs to be pursued further for its efficacy and practical application. This technique is a novel approach to the use of biological control agents and offers the opportunity to minimise the risk of inoculum development in an infested property scenario.
- Further investigation of the host status of alternative hosts is required as the inoculated glasshouse trial results did not help to refine the field sampling results. More detailed investigation of whether the alternative hosts are permitting the production of chlamydo-spores should be undertaken. BA14014 is supporting alternative host status research directly with *Foc* TR4 in the Northern Territory.

Improve access to new cultivars and build capacity in propagation

- The banana breeding review provide the basis for discussion and consideration of decisions on variety importation and screening and commercialisation pathways for the Variety Committee of BA16001.

Develop resilient crop management options

- Pursue further investigation of the impact of rotation crops, ground covers and nitrogen management practices on the soil microbiome and its potential to suppress *Foc*. BA14014 has significant R&D activity underway in understanding the nature of the soil microbiome and the ability to positively influence its composition to suppress *Foc*.
- There is need for continued testing of early detection methodologies and the influence of plant stress on *Foc* infection and symptom expression. Success in this research can significantly assist the cost and resourcing required for early detection and surveillance activities currently underway.

Update biosecurity protocols for banana production to reflect project outcomes

- There is an obvious need for continued extension and communication support to the banana industry for biosecurity practice implementation. The project BA16007 has identified this requirement as a project objective.
- The cost of implementing biosecurity practices needs to be more thoroughly investigated to help inform future industry policy development. The contracted industry benchmarking project has this activity as a project objective and the outputs from this project are assisting the development of that evaluation.

Facilitate rapid adoption of the research findings

- Continued development of digital tools to support biosecurity practice adoption by the banana industry, particularly using the biosecurity BMP guide as the basis for an on-line system. Improving record-keeping to support demonstration of biosecurity practices is also an identified need, and both these issues can be carried forward via BA14014.
- Facilitate more contact and linkage with international R&D programs and activities in *Foc* TR4. Australian RD&E providers are currently undertaking world-leading R,D&E and have much to offer international agencies and policymakers but can also benefit from greater collaboration with activities globally like the FAO Global TR4 program strategy, and varietal and integrated R&D activities in places such as Taiwan, France (CIRAD) and South Africa.

Scientific refereed publications

Journal article

Nguyen, V.T., Trevorrow, P., Grice, K., Tran-Nguyen, L.T.T. Evaluation of the efficacy of commercial disinfectants against *Fusarium oxysporum* f.sp. *cubense* race 1 and tropical race 4 propagules. (manuscript in preparation)

Papers in conference proceedings

McBeath, A.V. et al, 2016. Monitoring microbial functional and structural diversity for management of disease suppressive soils. In: Proceedings of 5th ISHS-ProMusa symposium: Agroecological approaches to promote innovative banana production systems, Montpellier, France, 10 - 14 October 2016.

Pattison, A.B. et al, 2016. Development of an integrated management system to suppress Fusarium wilt of bananas. In: 9th Australasian Soil-borne Disease Symposium, Christchurch, New Zealand, November 2016.

Pattison, A.B. et al, 2016. Integrating management practices to support banana production in the presence of Fusarium wilt. In: Proceedings of 5th ISHS-ProMusa symposium: Agroecological approaches to promote innovative banana production systems, Montpellier, France, 10 - 14 October 2016.

Intellectual property/commercialisation

No commercial IP generated.

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Appendix 1 – Rapidly identify solutions for early uptake in biosecurity practice – Movement pathways and associated risks identified

RISK ASSESSMENT TOOL - Panama disease prevention checklist – Banana farm practices

How to use this checklist

By considering the various practices associated with banana growing, this checklist will help to assess the risk of Panama disease infection to your property, and identify what are the most significant infection pathways to your business.

- Banana production practices change over time, therefore it is important that you complete this checklist each time you change farm practices to assess your risk.
- Keeping a copy of previously completed checklists is useful as a farm management tool to track progress

How to complete this checklist

Answer each statement by placing a tick or cross in the column that best fits your current practice or knowledge. For example:

Aspects of banana farming and associated Panama disease risk	Yes	No	Don't know	N/A
Before you start your farm (including land selection)				
You and your staff can identify the typical symptoms of Panama disease infection	X			
You and your staff clearly understand the biology of Panama disease and how it spreads			X	

Answers in the **boxes shaded red**, identify the practices that pose the **greatest threat** for potential Panama disease infection. If your answer to any of these questions falls in a red shaded box then the **risk of Panama disease infection is significantly increased** and changes to your farm practices are urgently needed.

Aspects of banana farming and associated Panama disease risk	Yes	No	Don't know	N/A
Before you start your farm – cropping history, knowledge of disease, financial resources, quarantine requirements				
Cropping history of the site is known, including any record of Panama disease				
You know if Panama disease has been identified in the past on neighbouring properties or properties above you in the catchment				
You are aware of any quarantine regulations and requirements that relate to Panama disease				
You can identify the typical symptoms of Panama disease infection				
Your key staff can identify the typical symptoms of panama disease infection				
You clearly understand the biology of Panama disease and how it spreads				
Your key staff clearly understand the biology of Panama disease and how it spreads				
The business has an established procedure with staff for reporting possibly infected plants				
<i>The business has the financial resources and knowledge to undertake essential practices such as:</i>				
<ul style="list-style-type: none"> Implementing and enforcing a strict farm quarantine policy for the movement of all people (staff, visitors, contractors) on to your farm 				
<ul style="list-style-type: none"> Establishing quarantine features such as bunded vehicle wash-down facilities at every entrance to the farm 				
<ul style="list-style-type: none"> Using only certified tissue-cultured planting material for each and every planting that will be made on your farm 				

Aspects of banana farming and associated Panama disease risk	Yes	No	Don't know	N/A
<ul style="list-style-type: none"> Establishing a plantation layout to manage the movement of runoff water on to and within the farm 				
Sourcing planting material				
Only certified Panama disease free tissue-cultured planting material is used for each and every planting that will be made on your farm				
Machinery, equipment and people visiting your farm				
An education program, and training in effective decontamination & on-farm biosecurity, is active on the property. Decontamination procedures and facilities, for vehicles, machinery and people that enter your property are in place.				
<i>Prior to entry, decontamination is carried out on these identified pathways:</i>				
<ul style="list-style-type: none"> All staff 				
<ul style="list-style-type: none"> All visitors – local, interstate & international 				
<ul style="list-style-type: none"> Earth-moving equipment 				
<ul style="list-style-type: none"> Contract cultivation equipment 				
<ul style="list-style-type: none"> Laser levelling equipment 				
<ul style="list-style-type: none"> Vehicles applying lime or soil amendments 				
<ul style="list-style-type: none"> Contract mounding equipment 				
<ul style="list-style-type: none"> Machinery used for irrigation installation 				



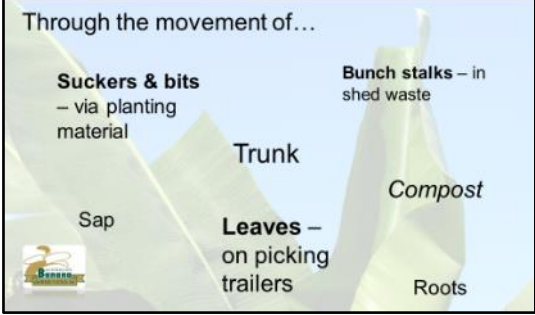
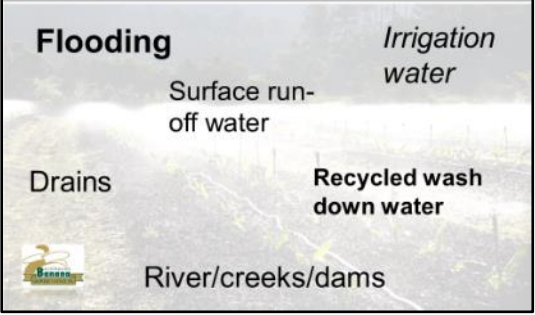

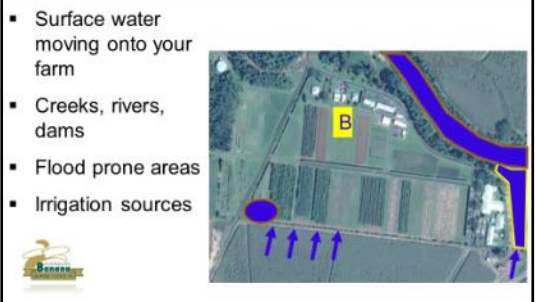
Aspects of banana farming and associated Panama disease risk	Yes	No	Don't know	N/A
• Bore drilling vehicles				
• Irrigation contractors/delivery vehicles				
• Fertiliser contractors/spreading machinery				
• Fertiliser delivery vehicles				
• Agronomists/consultants				
• Government agencies				
• Bell injection contractors				
• Deleafing contractors				
• Bagging contractors				
• Desuckering contractors				
• Harvest contractors				
• Harvest trailers and trucks moving between farms				
• Packaging delivery vehicles				
• Freight vehicles				

Aspects of banana farming and associated Panama disease risk	Yes	No	Don't know	N/A
<ul style="list-style-type: none"> Pallets contaminated with soil or banana material 				
<ul style="list-style-type: none"> Rubbish collection 				
<ul style="list-style-type: none"> Electricity companies 				
<ul style="list-style-type: none"> Fuel distributors 				
<ul style="list-style-type: none"> Pig hunters & their vehicles 				
Soil movement				
Crop removal practices that minimise the movement of soil and machinery are implemented				
The farm layout is planned and implemented for Panama disease prevention and management – the farm is surveyed for optimum placement of roads, drainage, water storage, runoff etc				
Water movement				
Land is selected that is free from flooding or major overland flow that can move soil or plants contaminated with Panama disease				
Crop nutrition				
Fertiliser products and amendments used are free of Panama disease (consider products like manures, composts, mill mud, mill ash etc)				
Irrigation				
<i>Your water source for irrigation and packing is:</i>				

Aspects of banana farming and associated Panama disease risk	Yes	No	Don't know	N/A
<ul style="list-style-type: none"> Free from Panama disease spores (eg. deep bore source) 				
<ul style="list-style-type: none"> Is managed so that it cannot be contaminated by infected runoff 				
<ul style="list-style-type: none"> Is sanitised appropriately to kill Panama disease spores 				
Re-used irrigation equipment is effectively decontaminated				
Pest, disease & weed management				
Other pests, diseases and weeds that may mask Panama disease symptoms are managed (eg Bacterial corm rot)				
Animals that move potentially contaminated soil or plant material are managed or excluded				

Rapidly identify solutions for early uptake in biosecurity practice – Movement pathways and associated risks identified

A modified HACCP process was used to identify the most likely movement pathways associated with banana production systems used in north Queensland. This information was then included in the biosecurity extension program delivered by DAF and ABGC to improve industry knowledge of Panama disease TR4, identification of effective biosecurity practices and development of individual farm biosecurity plans in a workshop format. Below is an excerpt of slides from the ABGC/DAF grower workshop modules incorporating the information outlining Panama disease TR4 movement pathways as part of an overall biosecurity farm planning process.

<p>Plant material</p>  <p>Identify the ways in which Plant Material can spread TR4</p>	<p>Water</p>  <p>Identify the ways in which Water can spread TR4</p>
<p>Plant material capable of spreading</p> <p>Through the movement of...</p>  <p>Suckers & bits – via planting material</p> <p>Bunch stalks – in shed waste</p> <p>Trunk</p> <p>Sap</p> <p>Leaves – on picking trailers</p> <p>Compost</p> <p>Roots</p>	<p>Water pathways capable of spreading</p>  <p>Flooding</p> <p><i>Irrigation water</i></p> <p>Surface run-off water</p> <p>Drains</p> <p>Recycled wash down water</p> <p>River/creeks/dams</p>
<p>Mark these areas on your farm map</p>  <p>▪ Blocks to be planted</p> <p>▪ Where shed waste is placed</p>	<p>Mark these areas on your farm map</p> <ul style="list-style-type: none"> ▪ Surface water moving onto your farm ▪ Creeks, rivers, dams ▪ Flood prone areas ▪ Irrigation sources 

Soil



Soil pathways capable of spreading

People	Field Equipment	Assoc. services	Goods
You	<i>Earth moving</i>	Utilities (eg. Ergon)	Pallets
Staff	<i>Picking trailers</i>	Fruit pick-up trucks	Delivered products
Visitors	<i>Laser levelling</i>	Delivery vehicles	Potting mix
Sales reps	<i>Belt spreaders</i>	Rubbish collection	Soil amendments
Agronomists	<i>Lime spreaders</i>	Fuel trucks	
Government personnel	<i>Contractor's Bore drilling</i>	Smoko van	
Shoes	<i>Trenchers for irrigation</i>	Wash down waste	Unauthorised
Clothing	<i>Contract mounding</i>	Animals	Pig hunters & motorbikes
	<i>Cultivation</i>	<i>Feral, domestic & native</i>	
	<i>Tractors</i>		
	<i>Tools & Equipment</i>		

Mark these areas on your farm map

- Property boundary
- Public roads
- Blocks separated by public roads
- Entry points
- Rail/mill lines
- Animal movement
- Utilities
- Houses



Appendix 2 – Improved biosecurity methods identified through soil, water and people movement



The development of improved biosecurity practices has been predicated on the identification of the key aspects and risk pathways for Panama disease TR4 movement associated with banana production systems in north Queensland. Emphasising exclusion of all non-essential visitors, vehicles or plant material from farms has been the underlying principle for effective farm biosecurity practices. This has been based on the concept of exclusion as the primary defence with treatment as a secondary option to reduce the chance of treatment/cleaning procedures being ignored or incorrectly applied.

Where essential commercial operations mean that exclusion is not possible (staff entry/exit, input supplies, packed product dispatch, accessing specialised machinery) then access should only occur subject to practices to manage the associated risk. Applying the concept of differential access zoning to farms assists in managing people and vehicle access and is a concept demonstrated best in the application of security and access at major airports. For banana farms the concept of 3 key zones is proposed as the best working solution:

- Exclusion – place for vehicles that don't need to enter the farm
- Separation – essential vehicles that are low risk like delivery trucks (not associated with field production) are designated to enter this zone, usually subject to treatment
- Farming – designated for farming activities; physical separation for staff, vehicles and drainage from other zones to manage risk of cross-contamination

Practices that have been proposed to manage the risk of essential movement of people, vehicles and equipment across zone boundaries include use of dedicated vehicles, footwear and tools in zones, procedures and facilities for footwear change at zone boundaries, use of footbaths, vehicle wash-down facilities and procedures, physical barriers such as fencing to minimise people and animal movements across zones and use of certified clean planting material. The financial and geographic circumstances of individual properties heavily influences the adoption of these proposed practices, with vehicle and staff movements between non-contiguous parcels of land remaining problematic because the need for duplicating facilities or machinery. In these instances the quickly becomes prohibitive.

The communication of the planning and implementation of biosecurity practices has occurred as a key component of the ABGC/DAF biosecurity workshop program as well as at the banana industry Roadshows, production of fact sheets and as short videos. Included below is an excerpt of slides from the ABGC/DAF grower workshop modules outlining the application of farm zoning and biosecurity practices for management of Panama disease TR4.

<p>MODULE 4: On-farm biosecurity</p> <ul style="list-style-type: none">■ Exclude, exclude, exclude■ "Come Clean Leave Clean"■ Minimise movement of soil, water, plant material 	<p>Managing zones</p> <p>Three Zones:</p> <ul style="list-style-type: none">■ Exclusion Zone – Vehicles that don't need to come onto your farm park here■ Separation Zone – Essential vehicles (e.g. delivery vehicles) are designated to drive in this area.■ Farming Zone – Farming activities 
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Planning for implementation of biosecurity practices

Ask yourself this question:

"To conduct my banana farming business and keep my farm free of Panama disease, does this:

- ◆ Person ◆ Vehicle ◆ Machinery
- ◆ Equipment ◆ Planting material

absolutely have to come onto my farm?"



Managing zones



Footwear

- Farm shoes only to be used on farm
- Supply visitors with shoes, rather than treating
- Colour code shed shoes and paddock shoes



Summing up zones

- Exclude first – treat second
- Build layers as no single practice is 100% effective
- There isn't a "one size fits all" approach – make it work for your business!



Footbath management



- Hard surface/gravel approach and exit
- Firm bristled brushed to **remove dirt** before footbath
- Use barriers to direct visitors/staff through footbath
- Long enough so both feet are treated
- Change disinfectant daily

Washdown design

- | Dos | Don'ts |
|---|--|
| <ul style="list-style-type: none"> ■ Hard surface pad ■ High pressure water ■ Manage waste water/soil ■ Fresh/treated water | <ul style="list-style-type: none"> ■ Dirt pad ■ Low pressure (e.g. garden hose) ■ Waste runoff unmanaged ■ Recycle water |



Plant material

How are you going to minimise the risk of introducing TR4 with planting material?



Fencing



- Emergency vehicles may still require access so don't fence off all entrance points



Appendix 3 – Effect of Commercial Disinfectants on Survival of Propagules of *Fusarium oxysporum* f.sp. *cubense* (Race 1)

Summary

The tropical race 4 strain of *Fusarium* wilt of banana or Panama disease was identified for the first time in a commercial Cavendish located in the Tully Valley, north Queensland. Disinfectants were available to decontaminate machinery and footwear, however, information or data on the efficacy of these products against the causal organism was not available. Forty five disinfectant treatments have been assessed for their ability to manage the survival of chlamydospores of the fungus (*Fusarium oxysporum* f.sp. *cubense* - *Foc* Race 1). Some products were suited for use in footbaths and drive-through dips, where others were suitable for treatment of potentially contaminated run-off water in a holding tank.

We can report that:

- Soil decreased the efficacy of the disinfectant treatments.
- The quaternary ammonium compounds (>12% a.i.) and Evo Tech 213 (bioflavonoid) were effective at achieving 'zero detectable' *Foc* colonies at the dilution rate of 1%. The same results were achieved in the presence of soil and at contact times ranging from 0 to 24 hours.
- Detergent based products such as Farmcleanse[®] suppressed or delayed *Foc* colony development.
- On-farm testing of drive-through dips indicated that the quaternary ammonium products (Steri-Max[®] and Path-X[®]) were stable (at least 3-4 weeks) and effective at reducing *Foc* colonies to a 'zero detectable' level.

Introduction

Panama disease (caused by the fungus *Fusarium oxysporum* f.sp. *cubense* - *Foc*) is considered to be the most devastating soil-borne disease to affect banana worldwide. There are three races which affect bananas: Race 1 infects Lady Finger, Sugar and Ducasse, Race 2 predominantly attacks cooking banana (ABB types) and Race 4 infects most cultivars including Cavendish. Race 4 is further subdivided into subtropical Race 4 which develops after periods of environmental stress and has been found in south east Queensland, northern New South Wales and Western Australia. Tropical Race 4 (TR4) was first recorded in Australia in the Northern Territory in 1997 and two further detections were made in different growing regions in the Darwin area between 1998 and 1999 (Conde and Pitkethley, 2001). However, the *Foc* race categories are cumbersome and other methods such as vegetative compatibility groupings (VCG's) (Ploetz and Correll, 1988) have been used to further classify the diversity that exists in *Foc* isolates (Ordonez *et al.* 2015)

In March 2015, Biosecurity Queensland confirmed that the TR4 strain had been recovered and identified from Cavendish plants on a property in the Tully Valley of north Queensland (ABGC, 2015). The property was placed under strict quarantine, restricting the movement of vehicles, equipment, machinery and workers both to and from the infected property and surrounding farms.

Decontamination procedures were implemented to minimise the risk of movement of the disease in soil, however, products had not been tested against the survival spores (chlamydospores) of the fungus. Even though TR4 can be found in many banana producing countries, the published information available on the effect of disinfectants on the management of TR4 was limited.

Furthermore, there was uncertainty on the efficacy of products in the presence of soil, rates of application and the longevity of products being offered to the banana industry. Previous work had been conducted with *Fusarium* wilt in cotton (Bennett *et al.*, 2011) caused by the organism *Fusarium oxysporum* f.sp. *vasinfectum* (*Fov*). In the case of *Fov*, the cotton industry recommended the use of the product Farmcleanse[®] to disinfect vehicles, machinery and boots. Castrol Farmcleanse[®], along

with Septone Truckwash® and Virkon® (PHA, 2009) had also been suggested as potential cleaning and disinfectant products for the banana industry. The aim of our studies was to ascertain the effectiveness of a range of disinfectants or sanitisers that could be used in footbaths, drive throughs dips, or to treat waste water held in tanks and washing down farm equipment that would reduce the survival of spores of *Foc* to a 'zero' detectable level. Due to quarantine restrictions in relation to TR4, our laboratory experiments were conducted with *Foc* Race 1 isolates.

Materials and Methods

Isolates

A total of 6 freeze dried isolates of *Foc* R1 were revived from long term storage (Table 1) at EcoSciences Precinct (ESP) and forwarded to Mareeba under Biosecurity Queensland Inspector's Approval 1.16. In addition, fresh material was collected from a previously known *Foc* R1 site on the Atherton Tablelands with the assistance of Biosecurity Queensland (BQ) staff (Table 1). Isolations from the fresh material consistently recovered cultures of *Fusarium* sp., which were forwarded to ESP for confirmation of the species and VCG.

Table 1 Collection details of isolates of *Fusarium oxysporum* f.sp. *cabense* Race 1.

Accession no.	Date	District	State	Variety	VCG
23760	22-Apr-91	Ingham	QLD	Ducasse (ABB)	0124
23970	19-Aug-92	Tully	QLD	Ducasse (ABB)	0125
24258	30-Aug-93	Yarrabah	QLD	Ducasse (ABB)	0125
24261	06-Sep-93	Mossman	QLD	Ducasse (ABB)	0125
24295	23-Feb-94	Atherton	QLD	Lady Finger (AAB)	0124
BRIP 63370	14-May-15	Rocky Creek	QLD	Ducasse (ABB)	0124

Experiment 1. Soil broth and chlamyospore production

The soil broth technique used by Bennett and Davis (2013) was modified to produce chlamyospores using two commercially available Searles potting mixes (Kickalong® Organic Premium Potting Mix and Superior Garden Soil Mix) together with our standard potting medium used for glasshouse experiments (UC Mix II-University of California, Riverside 2005).

Broths were prepared from each potting mix type, with the following modifications. Once the potting mix solution had been agitated for 1 hour, a kitchen sieve lined with 8 layers of cheesecloth was used to remove the large particles. A further filtration process using Whatman No. 4 filter papers was included to remove finer particles to produce a clearer broth. The solution was allowed to sit until a further sediment layer could be seen at the bottom of the flask. At this point 50 ml of the solution was decanted into 100 ml Schott bottles, with and without the addition of glucose, as per the protocol used by Bennett and Davis (2013). The broth solutions were autoclave once, prior to being inoculated with 400 µl of conidial suspension (10^6) prepared from 8 day old cultures of *Foc* R1 grown on carnation leaf agar (CLA) under blacklight. Broth cultures were placed on a bench-top orbital shaker located near a window and set to 90 rpm. After 6 days, a piece of mycelial mat was taken from each broth culture and assessed microscopically for the presence or absence of chlamyospores and conidia (micro and macro) of each isolate.

Experiment 2. Disinfectant Treatment

Experimental design

Forty-five disinfectant treatments and controls consisting of water inoculated and water alone, with and without soil were tested. Disinfectant treatments consisted of different products at various concentrations. Each treatment was trialled at 4 sampling times (0, 5 and 30 min and 24 h) and between 2 and 4 repeated colony counts recorded. Three replicates of each product, concentration, soil, time and repeated measure were included. Due to the number of treatments, testing was carried out in three experiments. The controls (water, water plus inoculum, water plus soil and a combination of water, soil and inoculum) and Castrol Farmcleanse[®] (1:10 dilution) treatments were tested in all experiments allowing them to be combined into a single multi-experiment analysis. Each individual experiment has been analysed using a generalised linear model (GLM) assuming a negative binomial distribution and log link function.

Soil

Soil was sourced from a residential property on the Atherton Tablelands (typical of a ferrosol) and used for each of the experiments. The soil was air dried, sieved (2 mm) to remove any large rocks or clods, placed into aluminium trays, moistened, mixed and autoclaved twice before use.

Disinfectant solutions

A total of twenty four products were received for testing from chemical companies, resellers and local banana producers. Products were classified into five main categories: quaternary ammonium products, miscellaneous disinfectants, bioflavonoids, oxidising agents and an undefined group that were predominantly detergent based (Table 2).

The number of products tested in each experiment varied, as did the range of dilutions tested. Stock solutions of the disinfectants were made up fresh prior to the start of each experiment. The dilutions ranged from 1:10 to 1:1000 and were prepared using sterile distilled water.

Table 2 List of product groups, active ingredients, rates and tradenames.

Category	Active ingredient	Commercial examples
Quaternary ammonium	Didecyl dimethyl ammonium chloride	Steri-Max - 120g/L (Agricrop) Sporekill - 120g/L (NuFarm) Algacide 1200/9/2015 - 120g/L (Minehan Agencies) BananaVehicle/Truckwash Cleaner - <20% ai (Taipan Traders)
	Didecyl dimethyl ammonium chloride (<40% ai) + alkyl benzyl ammonium chloride (<40% ai)	Anti-Fungal SS No. 1 Cleaner (Taipan Traders)
	Benzalkonium chloride (10% ai)	Bactex CF (Whiteley Industrial)
	Benzalkonium chloride (54g/L) + polyhexamethylene biguanide hydrochloride (4g/L)	F10SC (Health and Hygiene)
	Alkyl benzyl dimethyl ammonium chloride	Banana Shed/Equipment Cleaner No. 1 (30-60% ai) Super Cleaner Anti-Fungal - <60% ai (Taipan Traders)
	Didecyl dimethyl ammonium chloride (<20% ai) + alkyl benzyl dimethyl ammonium chloride (<20% ai)	Organic Food Fungal Cleaner (Taipan Traders)
	Alkyl benzyl dimethyl ammonium chloride (<10% ai) + 2-(2-butoxy) ethanol (<10% ai)	Farm Cleaner Detergent (Taipan Traders)
	Alkyl benzyl dimethyl ammonium chloride (30-60% ai) + phosphoric acid (<20% ai)	Banana Shed/Equipment Cleaner No. 2 (Taipan Traders)

Table 2 (continued)

Category	Active ingredient	Commercial examples
Miscellaneous disinfectants	2,2',2''-(Hexahydro-1,3,5-triazine-1,3,5-triyl) triethanol (70-80% ai) + 2-aminoethanol (1-<3% ai)	Antisept E12 (Fuchs Lubricants)
	N,N'-methylenebismorpholine (>60% ai)	Bactofix P (Fuchs Lubricants)
Undefined (detergent based)	Pyridine-2-thiol 1-oxide, sodium salt (0-5% ai)	Bio-Cleanse (Queensland Cleaning Solutions) Bio-Cleanse Plus (Queensland Cleaning Solutions) Spore Attack (Queensland Cleaning Solutions)
	Alkali metal salts of alkylbenzene sulfonic acid (5-10% ai) + coconut diethanolamide (1-5% ai)+ pyridine-2-thiol 1-oxide, sodium salt (0.1-1% ai)	Farmcleanse (Castrol Australia)
	Sodium hydroxide (24g/kg) and butoxyethanol (10-30% ai)	H.D. Gensolve (Custom Chemicals International P/L)
	Sodium dodecylbenzenesulphonate (5-15% ai) + cocoampho disodium dipropionate (5-10% ai) + sodium tripoly phosphate (5-10% ai) + sodium xylene sulphonate (1-5% ai)	Fleetmaster Harvest Kleen (Minehan Agencies)
Bio-flavonoids	Bitter orange extract (8-12% ai) + octanoic acid (2-4% ai) + lactic acid (2-4% ai)	Citran 1 (Organic Farming Systems)
	Bioflavonoid mixture (1-5% ai) + glycerine (2-4% ai) + Citran 1 (2-10% ai)	Citran 2 (Organic Farming Systems)
	Bioflavonoid mixture (5-10% ai) + organic acid blend (15-30% ai) + glycerine (30-60% ai) + ethanol (5-10% ai) + essential oil blend (1-5% ai)	EvoTech 213 (Evolution Organics)
Oxidising agent (Bleach)	Sodium hypochlorite (100g/L)	Sanichlor (DP Cleaning)

Inoculum preparation

A mixture of the four soil broth isolates of *Foc* R1 (VCG 0124 and 0125) was used to obtain the inoculum. The mycelial mat was extracted from each bottle (4-6 in total) using a wire loop and placed in a 250 ml beaker to which sterile distilled water was added to a volume of 50 to 100 ml. The mycelia mat was macerated using a stick mixer until it was no longer visible. A haemocytometer was used to determine the concentration of chlamyospores in the suspension which was then adjusted to 10^5 per ml. The same concentration rate was used in each experiment.

Experiment setup

Petri plates of $\frac{1}{2}$ strength potato dextrose agar ($\frac{1}{2}$ PDA) plus the antibiotic (approx. 30 units streptomycin sulphate per ml) were prepared prior to each experiment. For treatments without soil, 900 μ l of the disinfectant solution was added to a microcentrifuge tube (1.75 ml) that contained 100 μ l of the prepared chlamyospore suspension. The solution was pipetted a few times to ensure the solution was well mixed, prior to taking a 100 μ l aliquot and dispensing onto the surface of a petri plate. A sterile glass spreader was then used to spread the droplet evenly around the plate. This process was repeated for all three replicates and the remaining treatments without soil. Digital timers were subsequently set for 5 and 30 minutes so that further samples could be taken from the same tubes at these timings.

The process for soil treatments was slightly different. One gram of soil was pre-weighed into 50ml flat bottomed centrifuge tubes. The diluted disinfectant was poured into the tube to the 20 ml mark (1 part soil to 20 parts solution), and vigorously shaken before an aliquot of 900 μ l was added to a microcentrifuge tube that contained 100 μ l of the prepared chlamyospore suspension. From this point the same process was used as stated above for treatments without soil. All petri plates were placed in an incubator set to 25°C. Samples were again taken from all the tubes after 24 h.

Colony counts were conducted at 48 and 72 h, recording the number of *Foc* colonies present on each plate. In some instances, where colony numbers were high, plates were halved or quartered, one section was counted and the number used to calculate the number of colonies per plate. In other cases where it was not possible to count the number of colonies due to the intensity, a maximum colony number of 800 was recorded. Where 'zero' colony counts were recorded at 72 hours, further assessments were conducted at either day 6 or 7 post inoculation.

On-farm testing

To confirm some of our laboratory results in relation to the quaternary ammonium products, two farms located on the Atherton Tablelands were selected to assess the effectiveness of their drive-through dips. The product Steri-Max® was used in the drive-through dip at farm A, whilst farm B used Path-X® at the recommended dilution rate of 1:100. Both products contained the active ingredient didecyl dimethyl ammonium chloride (DDAC) at a concentration of 120 g/L. Samples were collected from each property and the method outlined above used to determine survival of chlamyospores in each sample. Each sample was replicated three times.

Results

Experiment 1 – Soil broth and chlamydo spores production

Chlamydo spores were produced in broth made from Searles Superior Garden Soil Mix without added glucose. Chlamydo spores were not formed in broth produced from Kickalong® Organic Premium Potting Mix or the UC Mix II treatments with or without the addition of glucose (data not shown). Chlamydo spores were not produced from isolates 23970 and 24261, and these isolates were discarded and not used in any further experiments.

Experiment 2 – Disinfectant Treatment

The three experiments were statistically analysed for the mean final counts of *Foc* at the range of dilutions, contact times and in the presence and absence of soil. The full random model was applicable for this analysis and a \log_{10} transformation was required to comply with assumptions of normally distributed data. All main effects and interactions were highly significant ($p < 0.001$).

There are some generalisations we can conclude from the analysed data:

1. Soil decreases the efficacy of the treatment, especially at the weakest dilution
2. The longer the contact time, the greater the reduction in colony formation
3. Quaternary ammonium compounds (>12% a.i.) and EvoTech 213 (bioflavonoid) were effective at achieving a 'zero detectable' level at rates of 1% across the range of contact times and in the presence of soil
4. Detergent based products appeared to only suppress or delay colony development.
5. No *Foc* colonies were recorded in the un-inoculated water or soil control treatments.

The activity of some products (Castrol Farmcleanse®, Fleetmaster Harvest Kleen (1:10 dilution), Bio-Cleanse (1:10 dilution), Bio-Cleanse Plus (1:10 and 1:100 dilution) and H.D. Gensolve) appeared to only delay germination and subsequent growth of *Foc* with colonies not visible or unable to be counted until 6 or 7 days after treatment. It should also be noted that the suppressive nature of the product did not necessarily occur at all contact times.

On-farm testing

Results from the drive-through dip solutions tested at the 0 and 5 min contact times indicated that the products (Steri-Max® and Path-X® – 120 g/L DDAC) were effective at reducing *Foc* colonies to a 'zero detectable' level, even after a period of 3-4 weeks without being topped up.

Discussion

The methods used by Bennet and Davis (2013) were successfully adapted to produce viable quantities of chlamydo spores in our experiments. The isolates and the soil broth solutions that did not produce chlamydo spores may have required a longer timeframe for this to occur, however, time did not permit this to happen as other isolates could produce chlamydo spores more efficiently.

The contact times of 0 and 5 min were designed to simulate the length of time to walk through a footbath or drive a vehicle or machinery through a drive-through dip containing a disinfectant. The longer contact times of 30 minutes and 24 hour were considered as a treatment for waste/runoff water in a holding tank or for soaking boots or equipment such as secateurs or cane knives.

It was imperative that the testing carried out was practical and closely simulated practices carried out in an on-farm situation. Given the destructive nature of *Foc*, and the implications the disease has on the banana industry, products were not perceived as applicable for use unless a 'zero detectable' level was achieved in the laboratory testing.

Of the five different categories of disinfectants assessed, the quaternary ammonium compounds were the most effective at reducing colony numbers to a 'zero detectable' level, particularly at the 1:100 dilution rate. However, there were differences between products that contained the same active ingredient, which could be due to the rate of the active ingredient or an effect of the other components that were present in the product.

In the bioflavonoid group, the product EvoTech 213 was equally as effective as the quaternary ammonium products at the rate of 1:100. Based on the rates tested (1:10 and 1:100), we are unsure if EvoTech 213 could be effective at the 1:1000 dilution as it was expected that the product would lose efficacy at 1:100 dilution.

The detergent based products were not effective at the 1:100 dilution, while most achieved a 'zero detectable' level this was only at the contact time of 24 hours. These products would be best placed to be used as a general clean down product to assist in the removing all soil and organic matter, prior to the use of disinfectant product that has efficacy at a shorter contact time.

The miscellaneous disinfectants and bleach were also not as effective as the quaternary ammonium products at the dilution of 1:100 until at least 30 minute contact or more was achieved. Due to bleach being inexpensive, it could be a viable option for treating run-off waste water in a holding tank, again providing the contact time was at least 30 minutes.

The results from the on-farm testing of the drive-through dips demonstrated that the products tested still remained efficacious after a timeframe of 3-4 weeks. This is in contrary to the literature which suggests that the effectiveness of the products is greatly reduced when in contact with soil and organic matter.

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Supplement to the report titled: Effect of Commercial Disinfectants on Survival of Propagules of *Fusarium oxysporum* f.sp. *cubense* (Race 1)

1. Additional chemical screening against Foc R1.

In three further experiments, several additional disinfectants Agriquat, Anosan, Atmosphere, EvoCrop 204, EvoTech-Modified, Path-X and X-OUT were tested for their efficacy against chlamydospores of Foc R1. For some products (Steri-Max, Atmosphere, EvoCrop 204, EvoTech modified and EvoTech 213) additional dilutions were added, at the manufacturers request. All experiments were conducted using methods as outlined in the previous milestone report. The additional products were quaternary ammonium products (Agriquat, Path-X, Atmosphere), oxidising agents (Anosan), bioflavonoids (EvoCrop 204 and EvoTech Modified), and a biological drain cleaner (X-OUT).

Results

The products Anosan and Atmosphere, not previously tested failed to eliminate colony development of Foc R1. Dilutions greater than 1:100 for Atmosphere and Steri-Max also failed to eliminate Foc R1, compared to the label rate (1:100) which is effective. EvoTech 213 was retested, however at the 1:100 dilution rate, the same results were not achieved as per the original testing as colonies were evident at the '0' contact time. Fresh product was supplied by the manufacturer (EvoTech 213) for further testing. EvoTech 213 and EvoTech (modified) reduced the colony count to a zero detectable level in the presence of soil. The other bioflavonoid product EvoCrop 204 did not reduce colony numbers to the same degree as the previous two mentioned. Agriquat performed similar to other quaternary ammonium products and would be an effective substitute for those products at a 1:100 dilution. The drain cleaner (X-OUT) had no effect on reducing Foc colonies. The control treatments were not included in the analysis of the third experiment as they performed as expected.

2. Soil type and dilution effects on disinfectant activity

Background

An experiment to determine the effect of soil type and the ratio amount of soil required before the activity of the disinfectants is compromised was conducted. Products tested included EvoTech 213 along with two DDAC products Path-X and Steri-Max used at their recommend rates (1%). Two field soil types (red ferrosol as used in previous experiments) and sandy soil collected from a previous banana cropping site and used at four rates (1, 5, 10 and 15 grams) were added to 20 ml of disinfectant solution. Aliquots (100ul) were taken at 3 contact times: '0' instant, 30 min and 24 hrs and plated onto PDA, all following procedures were carried out according to the 'Experimental setup' in the previous disinfection experiments.

Data analysis

Two different analyses were performed on the data, the first being a generalised linear model (GLM), assuming a Negative Binomial distribution and log link function. A split-plot-in-time treatment structure was used to account for the repeated plate counts at 48 and 72 hours (data for 72 hours presented in black). The second analysis excludes colony counts where a '+' was used, indicated *Foc* was present but colony numbers couldn't be physically counted due to the presence

of other fungal organisms. Where this occurred (predominantly in the high soil rates), a '+' was replaced by a missing value.

Results and Discussion

We can report that:

- Red soil rates above 1 gram reduced the activity of all the DDAC disinfectants tested and across all contact times
- At 24 hours contact, EvoTech was effective at reducing colony counts to zero detectable level. This was also achieved when the highest rate of soil (15g) was used. This indicates that the ratio of soil to water can be greater with this product compared to the DDAC compounds, providing 24 hour contact is achieved.
- In sandy soil, results were similar to that of red soil except that only a 30 minute contact was sufficient to reduce colony counts to a zero detectable level.
- The DDAC compounds only reduced colony counts to a zero detectable level after 24 contact with 5 grams of soil added.
- We suggest the active ingredient of the DDAC products is bound to the clay component of the soil, therefore reducing activity.

3. Disinfectants to decontaminate tools

Three disinfectants were tested for their ability to eliminate chlamydo spores added to soil and present on field sampling equipment such as cane knives. Products included: Diggers Violet Methylated Spirits at four rates (neat product, 90%, 80% and 70%), 1% Bleach (100g/L active) and 1% Steri-Max. Chlamydo spores of FocR1 were applied to PDA plates and allowed to dry for different lengths of time '0' (instant), 5 minutes or 1 hour, prior to being sprayed with the disinfectant treatments listed above. This technique was designed to simulate as close as possible field sampling activities and decontamination procedures

Results

The final colony counts indicate that both Bleach and Steri-Max at 1% were effective at eliminating colony development across all drying times. For the methylated spirits treatments, colony numbers reduced as the product rate increased, however complete elimination was not observed. It needs to be noted that this and other experiments are carried out on a 'worse case' scenario.

4. Evaluation of the recommended practices for decontaminating against *Foc*

Background

Biosecurity QLD questioned the efficacy of the recommended QA based products, either mixed with or following application of Farmcleanse. We utilised the previously developed bioassay to assess a range of product combinations and treatments. The standard practice of spraying with Farmcleanse, followed by a water rinse then a 10 minute contact with a QA (Steri-Max) and again followed by a water rinse. Our method mixed Farmcleanse and a quaternary ammonium product (Agriquat and Steri-Max) or Farmcleanse was allowed to drain from the vessel before adding the QA compounds. The plating technique and contact times was as previously described.

Results

Farmcleanse (drained) followed by a QA treatment was as effective as a QA treatment alone. Combining Farmcleanse with either of the QA products compromised the performance with colonies present at all contact times except at 24 hours.

Appendix 4 – Effect of Commercial Disinfectants on Survival of Propagules of *Fusarium oxysporum* f.sp. *cubense* (Tropical Race 4)

Methods

Preparation of cultures

A total of four *Foc* TR4 isolates were used in this study. All the TR4 cultures were preserved at -80°C on Microbank™ porous beads (Pro-Lab Diagnostics, Toronto, Canada). Working cultures were prepared by resuscitation of TR4 from -80°C storage on Spezieller Nährstoffarmer Agar followed by incubation at 25°C for 7 days. These cultures were then subcultured on carnation leaf agar (CLA) under black light to allow them to produce conidia, which were subsequently used to inoculate soil broth to produce chlamydospores.

Disinfectant products

A total of 31 disinfectant products received from chemical companies, resellers and local banana producers were used for testing (Table 1). They included 19 quaternary ammonium compounds, three bioflavonoids products, one oxidising agent, six detergent-based and two miscellaneous products. All products were diluted with sterile distilled water to the rates of 1:10, 1:100 and 1:1000 for treatments and dilutions were made up fresh prior to experiments. The tested dilution rates varied depending on the performance of individual product.

Preparation of soil broth for chlamydospores production

The Searles Superior Garden Soil Mix (Searles® Pty Ltd, Queensland, Australia) was used to prepare soil broth following the soil broth technique described by Bennett and Davis (2013) with some modifications. Briefly, 125 g of air-dried soil mix was mixed with 500 ml of reverse osmosis water in 2L flasks and agitated on an orbital shaker at 90 rpm for 1 hr. Following agitation, the soil-water mixture was poured through a 1.7 mesh sieve to remove large particles and then filtered through 8 layers of cheesecloth. The flow-through was filtered again through filter paper disks to achieve clear broth. The broth was autoclaved at 121°C for 20 min and then placed on the bench overnight to allow sediments to settle completely. The top clear soil broth was decanted into 250 ml-bottles (50 ml of soil broth per bottle) and autoclaved again in the following day.

Chlamydospores production

To stimulate *Foc* to produce chlamydospores, every 50 ml of the clear sterile soil broth was inoculated with 400 µl of 2.5×10^6 conidia suspension (to achieve a total of 10^6 conidia) prepared from 8-day *Foc* cultures grown on CLA under black light. The inoculated soil broth was agitated at 90 rpm on an orbital shaker at room temperature for at least 5 days to allow *Foc* to produce chlamydospores. To harvest chlamydospores, the soil broth cultures were passed through a layer of sterile cheesecloth to trap mycelia clumps, which contain the spores. The mycelia clumps were rinsed with sterile distilled water (SDW) over the cheesecloth to reduce the number of conidia and then transferred to a sterile small beaker using a volume of approximately 10 ml of SDW. Mycelium clumps were stirred using a sterile stirring bar to release chlamydospores. Stirring was continued until the mycelia clumps were no longer visible. The spore suspension was passed through a layer of sterile cheesecloth to remove remaining clumps and mycelia. The concentration of spore suspensions was determined using an improved Neubauer Haemocytometer and then adjusted to 10^5 spores per ml before use for disinfectant experiments.

Disinfectant experiments

In this study, *Foc* propagules were treated with disinfectants for ≤ 30 sec, 5 min, 30 min and 24 hr in the absence and presence of soil. The exposure times of ≤ 30 sec and 5 min were designed to simulate the length of time to walk through a footbath or drive a vehicle or machinery through a drive-through dip containing a disinfectant. The longer contact times of 30 min and 24 hr were designed to simulate the length of time required for treatment of runoff water in a holding tank or for soaking boots or equipment such as secateurs or cane knives. Treatments without soil were made by adding 100 μ l of the chlamyospore suspension prepared as described above into 2-ml tubes containing 900 μ l of disinfectants diluted to desired rates and the tubes were then briefly vortexed or pipetted a few times to ensure the solution was well mixed. After desired exposure times, a 100 μ l aliquot was taken and plated on half-strength potato dextrose agar plates supplemented with 30 units/ml of streptomycin sulfate (Sigma, MO, USA). All the plates were then incubated at 25°C for 48-72 hrs, after which the number of *Foc* colonies developed on each plate was recorded. Where “zero” colony counts were recorded at 72 hrs, further assessments were conducted at either day 6 or 7 post incubation to confirm whether the disinfectant products effectively eliminated *Foc* or just delayed fungal colony development. Treatments resulting in no colony detected after 6 or 7 days of incubation were considered to be effective against *Foc*.

Treatments with soil were performed in the same process as stated above for treatments without soil, except that one part of soil was added and mixed with 20 parts of disinfectant solutions before treatments occur. The soil used for the trials on *Foc* TR4 was collected from Coastal Plains Research Station in Middle Point, Northern Territory, Australia. The soil was air-dried, sieved (2 mm) to remove any large rocks or clods, placed into glass petri dishes, moistened, mixed and autoclaved twice before use.

Due to a substantial number of treatments, testings were carried out in multiple experiments. For every experiment, the controls consisting of water alone, water plus inoculum with and without soil and Castrol Farmcleanse (1:10 dilution) were included. This was done to allow all treatments to be combined in a single multi-experiment analysis. Three replicates of each treatment was performed in every experiment.

Results

The effects of disinfectant treatments on the survival of *Foc* TR4 propagules including chlamyospores in aqueous suspensions without and with soil added, determined based on the number of *Foc* colonies formed following treatment for ≤ 30 sec, 5 min, 30 min and 24 h, were assessed. In general, effects achieved from all disinfectant treatments were consistent between R1 and TR4. Of the five categories of disinfectants assessed, the quaternary ammonium (QA) compounds were the most effective against the survival of propagules of both *Foc* R1 and TR4 with no colony detected in treatments at 1:100 dilution both with and without soil added across all the four contact times for 16 out of 19 QA products tested. For the other three products that include F10SC Disinfectant, Farm Cleaner Detergent and Atmosphere Forte Blue Concentrate, although treatments at 1:100 dilution significantly ($p < 0.05$) reduced *Foc* colony numbers as compared to positive controls, zero colony counts were only achieved at 5 min of contact in treatments without soil or longer in treatments with soil added. Despite being completely effective at 1:100 dilution, all QA products became less effective when used at 1:1000 dilution. In particular, zero colony counts across all the four exposure times were only observed in treatments without soil for 10 out of 19 QA products when tested on R1 and 12 out of 18 QA products when tested on TR4. In the presence of

soil, none of the QA products resulted in zero colony counts of either R1 or TR4 across all the four contact times.

As compared to QA compounds, the bio-flavonoids, oxidising, miscellaneous and detergent-based products were less effective against propagules of both *Foc* races with none of them showing zero colony counts across all the four contact times in treatments at 1:100 dilution both with and without soil added. Of the three bio-flavonoids tested, zero colony counts across the four contact times were only observed for EvoTech 213 at 1:10 dilution. At this dilution, Citran 1 resulted in zero colony counts after 5 min of contact regardless the absence or presence of soil. However, this was not case for Citran 2, where both R1 and TR4 colonies were detected at all the four contact times in all treatments at 1:10 dilution both with and without soil present. For the oxidising agent, bleach containing 10% sodium hypochloride diluted to a 1:100 rate only completely inhibited *Foc* colony formation after 30 min of contact with R1 propagules and after 5 min of contact with TR4 propagules. At this dilution rate, the concentration of sodium hypochloride in bleach is equivalent to 1000 ppm. Diluting bleach to 100 ppm of sodium hypochloride prolonged the contact times to achieve zero colony count to 24 hr for R1 and 30 min for TR4. The two miscellaneous products, Antisept E12 and Bactofix P used at 1:10 and 1:100 dilutions were also not effective against R1 until 30 min and 24 hrs of contact, respectively, were achieved and therefore testing of their efficacy against TR4 was not proceeded.

For the six detergent-based products, their activity were first assessed on R1 and none of them resulted in zero colony count across all the four contact times in treatments at 1:100 dilution both with and without soil. Zero colony count of R1 was mostly achieved at 24-hr treatments at 1:10 dilution but this was only observed for Bio-Cleanse, Bio-Cleanse Plus and Fleetmaster Harvest Kleen. Because of their poor performance against R1, only Castrol Farmcleanse and Fleetmaster Harvest Kleen were tested on TR4 and it was found that both of them did not inhibit TR4 colony formation across all the four contact times in all treatments at 1:10 dilution both with and without soil present. Although detergent-based products did not effectively suppress the colony formation of both *Foc* R1 and TR4 propagules, they were found to delay the development of colonies of both *Foc* races up to 6 or 7 days post incubation as opposed to 2 to 3 days as observed in treatments with other disinfectants.

With respect to interactions between disinfectant treatments and exposure times, the results of statistical analysis showed that the colony count significantly ($p < 0.05$) reduced with increasing exposure times but this was only observed for the QA products in treatments at 1:1000 dilution with soil added. The colony counts were also significantly ($p < 0.05$) lower at 24 hr of contact than at early contact times in most of treatments with the bio-flavonoids, bleach, miscellaneous and detergent-based products. For interactions between disinfectant treatments and soil, significantly ($p < 0.05$) higher colony counts were recorded in treatments with soil added than without soil for QA products at 1:1000 dilution and Citran 1 at 1:100 dilution. These results suggest that both exposure times and the presence of soil affect the activity of certain disinfectant treatments.

Conclusions/Discussion

A total of 31 commercial disinfectants belonging to five different categories at different dilution rates were trialled against the survival of propagules of *Foc* R1 and TR4 in the absence and presence of soil at the four contact times of ≤ 30 sec, 5 min, 30 min and 24 h. Of all the disinfectants tested, the QA compounds were found to be the most effective against the survival of propagules of both *Foc* R1 and TR4 as compared to bio-flavonoids, oxidising, miscellaneous, and detergent-based products. This result is consistent with those of previous studies, which reported that a QA product

Sporekill was more effective in inhibiting germination or mycelial growth of *Foc* TR4 and STR4 isolates than other products including sodium hypochlorite and a detergent-based product Farmcleanse (Meldrum et al., 2013; Nel et al., 2007). In our study, 16 out of 19 QA products including Sporekill completely inhibited the colony formation of all propagules of both *Foc* R1 and TR4 across all the four exposure times regardless the absence or presence of soil when used at 1:100 dilution. However, this is not the case for F10SC Disinfectant, Farm Cleaner Detergent and Atmosphere Forte Blue Concentrate, which did not kill all *Foc* propagules at ≤ 30 sec of contact or even at longer contact times in the presence of soil. This could be attributed to the relatively low concentrations of active ingredients in these three products as compared to other QA products. More specifically, these three products contain less than 10% of active ingredients of benzalkonium chloride (BC) or alkyl benzyl dimethyl ammonium chloride (ABDAC) while other effective QA products at least 10% of active ingredients. For examples, Bactex CF contains 10% of BC, Banana Shed/Equipment Cleaner No. 1 has at least 30% of ABDAC and 12% of didecyl dimethyl ammonium chloride (DDAC) is found in Sporekill. Despite showing complete effectiveness against both *Foc* races at 1:100 dilution, all the QA products seemed to lose their efficacy when diluted to 1:1000 rate. Based on these findings, our study suggests that the QA products containing $\geq 10\%$ active ingredients at 1:100 dilution could be potentially implemented in on-farm biosecurity procedures including footbaths, drive-through dips, decontamination of farm machinery and equipment. For the products containing less than 10% active ingredients such as F10SC Disinfectant, Farm Cleaner Detergent and Atmosphere Forte Blue Concentrate, they can be used for long-term treatments such as soaking farm equipment but should not be applied for instant decontamination procedures such as footbaths or drive through dips. This principle may also apply for the bio-flavonoids, bleach and miscellaneous products at 1:100 dilution as these treatments generally required longer contact times to be effective against the survival of propagules of both *Foc* R1 and TR4.

Castrol Farmcleanse, a detergent-based product, was found to be effective at killing spores of *Fov* (Moore et al., 2001). However, the results from the present study showed that all the six detergent-based products, including Castrol Farmcleanse did not eliminate the propagules of both R1 and TR4 although they can delay the fungal colony development. This suggest that these products should not be applied for decontamination procedures against Panama disease. Castrol Farmcleanse was also found to deactivate the activity of QA products against R1 propagules (data not shown) and therefore, it is recommended that this product should not also be incorporated with QA products in decontamination procedures against Panama disease.

In addition, our study also showed that an increase in treatment times enhanced the efficacy of most disinfectants treatments against both *Foc* R1 and TR4. However, the presence of soil may decrease the efficacy of disinfectants against both *Foc* races. Our result supports the finding of Bentley et al. (2011) who reported that soil or the matter in soil decrease the efficacy of anti-fungal cleansers against conidia and chlamydo spores of *Fov*. Based on this result, we would suggest that soil attaching to contaminated items should be removed before application with disinfectants in order to maintain the efficacy of treatments.

For selecting a suitable disinfectant for on-farm decontamination procedures, in addition to the product efficacy, other factors such as product cost, corrosiveness and longevity should be considered. For example, the use of bleach as a disinfectant against *Foc* can be a cost-effective option because it is inexpensive. However, bleach is known to be highly corrosive and its active ingredient, which is sodium hypochlorite, quickly degrade. In the present study, bleach products were measured for sodium hypochlorite concentration prior to being subject for testings and it was found that all the bleach products contained only approximately 4.5% sodium hypochlorite, which is

less than half of the value stated on the label of their containers, which is 12.5%. This suggests that sodium hypochlorite in bleach can degrade even when they are stored in closed containers and therefore its degradation is expected to occur faster when used in the field. For QA products, it is unknown whether they products are corrosive to materials used in farm equipment and machinery and whether they are degradable over the duration of storage. Also, the effects of field conditions of their activity have not been extensively investigated except for Sporekill, which has been reported to remain its efficacy against microconidia germination of a TR4 isolate after 6 months of exposure to sunlight and diurnal temperature variation in the field (Meldrum et al., 2013). Detailed investigations on the corrosiveness, longevity of QA products and the effects of field conditions on their activity against *Foc* are therefore required.

In conclusion, this study has evaluated the efficacy of commercial disinfectants against propagules including chlamydospores of *Foc* R1 and TR4 for the first time and has determined that the QA products were the most effective disinfectants against the survival of propagules of both *Foc* R1 and TR4. These products can be used for all on-farm decontamination procedures for Panama diseases including footbaths, drive-through dips, decontamination of farm machinery and equipment provided that they contain at least 10% active ingredients and are not diluted more than a 1:100 rate before use. The presence of soil or organic matter in soil may decrease the efficacy of disinfectants and hence, attached soil on footwear or farm equipment should be completely washed off before treatment with disinfectants. However, further investigations on the corrosiveness of QA products on materials used in farm equipment and machinery, their longevity, and studies on the effects of field conditions such as sunlight and temperature on their activity against *Foc* are required before they can be applied in practice.

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Table 1. Disinfectants assessed in this study

Category	Disinfectant trade name	Active ingredient (%)
Quaternary ammonium	Agriquat	BC (25%) ^a
	Algacide 1200/9/2015	DDAC (12%) ^b
	Anti-Fungal SS No. 1 Cleaner	DDAC (<40%), ABDAC (<40%) ^c
	Atmosphere Forte Blue Concentrate	BC (<10%)

	Bactex CF	BC (10%)
	Banana Basher Cleaner	DDAC (10-30%), ABDAC (10-30%), ethyl alcohol (<10%), 2-butoxyethanol (<10%)
	Banana Buster Cleaner	DDAC (10-30%), ABDAC (10-30%), ethyl alcohol (10-30%)
	Banana Disease Blockbuster	DDAC (10-30%), ABDAC (10-30%), BC (10-30%), terpene hydrocarbons (<10%)
	Banana Disease Buster	DDAC (10-30%), ABDAC (10-30%), BC (10-30%)
	Banana Shed/Equipment Cleaner No. 1	ABDAC (30-60%)
	Banana Shed/Equipment Cleaner No. 2	ABDAC (30-60%), phosphoric acid (<20%)
	Banana Vehicle/Truck wash Cleaner	DDAC (<20%)
	F10SC Disinfectant	BC (5.4%), polyhexamethylene biguanide hydrochloride (0.4%)
	Farm Cleaner Detergent	ABDAC (<10%), 2-(2-butoxy) ethanol (<10%)
	Organic Food Fungal Cleaner	DDAC (<20%), ABDAC (<20%)
	Path-X	DDAC (12%)
	Sporekill	DDAC (12%)
	Steri-Max	DDAC (12%)
	Super Cleaner Anti-Fungal	ABDAC (<60%)
Bio-flavonoids	Citran 1	Bitter orange extract (8-12%), octanoic acid (2-4%), lactic acid (2-4%)
	Citran 2	Bioflavonoid mixture (1-5%), glycerine (2-4%) Citran 1 (2-10%)
	EvoTech 213	Bioflavonoid mixture (5-10%), organic acid blend (15-30%), glycerine (30-60%), ethanol (5-10%), essential oil blend (1-5%)

Oxidising agent	Bleach	Sodium hypochlorite (10%)
Miscellaneous	Antisept E12	2,2',2''-(Hexadydro-1,3,5-triazine-1,3,5-triyl) triethanol (70-80%), 2-aminoethanol (1-<3%)
	Bactofix P	N,N'-methylenebismorpholine (>60%)
Detergent-based	Bio-Cleanse	Pyridine-2-thiol 1-oxide, sodium salt (0-5%)
	Bio-Cleanse Plus	Pyridine-2-thiol 1-oxide, sodium salt (0-5%)
	Castrol Farmcleanse	Alkali metal salts of alkylbenzene sulfonic acid (5-10%), coconut diethanolamide (1-5%), pyridine-2-thiol 1-oxide, sodium salt (0.1-1%)
	Fleetmaster Harvest Kleen	Sodium dodecylbenzenesulphonate (5-15%), cocoampho disodium dipropionate (5-10%), sodium tripoly phosphate (5-10%), sodium xylene sulphonate (1-5%)
	H. D. Gensolve	Sodium hydroxide (2.4%), butoxyethanol (10-30%)
	Spore Attack	Pyridine-2-thiol 1-oxide, sodium salt (0-5%)

Appendix 5 – Quaternary ammonium disinfectants – monitoring methods, longevity and corrosion potential

Summary

The use of disinfectants to decontaminate vehicles, machinery, tools and equipment is a critical on-farm biosecurity practice, as it reduces the risk of spreading pests and diseases. Quaternary ammonium (QA) compound based disinfectants such as didecyl dimethyl ammonium chloride (120 g/L DDAC) applied at a 1% solution (1200ppm), the recommended label rate have shown to kill fungal spores that cause Panama disease Race 1 and Tropical race 4. Prior to this research growers had no means of monitoring and managing effective concentrations of QA compounds.

Various QA compound test kits which measure the concentration of active ingredient in a sample were trialled in this research. The test kits were trialled against three QA products containing 120 g/L DDAC – Sporekill[®], Steri-max[®] and Path-X[™]. Although a number of different QA compound test kits were trialled, the Precision Laboratories High Level 0-1500ppm test strips (0-1500ppm) provided consistent and accurate results, and are proving to be an effective tool for routine monitoring of QA compounds. A sample QA compound test kit was developed for growers to test their QA's and evaluate the Precision Laboratories High Level 0-1500ppm test strips in a real situation.

Approximately 90 test kits have been supplied to banana growers and industry stakeholders through industry events including; the Panama R&D Open Day, Australian Banana Industry Congress, Panama Tropical Race 4 update meetings and farm visits.

The longevity of QA's - Sporekill[®], Steri-max[®] and Path-X[™] made at 1% (1200ppm) solution with and without the addition of soil, and exposed to field conditions was investigated. After 8 months exposure to field conditions, the inoculation results had shown that all three QA products without the addition of soil maintained complete efficacy, with zero colony growth of Foc Race 1 detected. At the 8 month mark, the Precision Laboratories High Level QAC 0-1500ppm test strips started to show a reduction in concentration for some of the QA products. However, despite the reduction in concentration, the QA products without the addition of soil remained at an effective concentration after 8 months exposure to field conditions. The addition of 5% soil gave contrasting results early on in the experiment. Using the Precision Laboratories High Level 0-1500ppm test strips as indicators, it appeared that the concentration of active ingredient was reducing over time. Inoculation results of the QA products with the addition of soil at the 4 month mark had shown that colony growth was detected at an instant contact time for all QA's. A contact time of up to 30 minutes was required to reach zero colony growth for the QA's with the presence of soil. Sporekill[®] appeared to be the most effective product, having the least amount of colony growth detected, followed by Steri-max[®], and Path-X[™] which had the greatest number of colonies detected overall.

The interaction of Sporekill[®] (120 g/L DDAC) with the addition of soil over time was investigated, (two different soil types, a sandy soil and clay soil). Using the test strips as indicators, the results of this experiment suggest that there may potentially be a soil and time interaction with QA products such as Sporekill[®]. It is suggested that further investigation of QA products with the addition of different soil types is required to determine if the presence of soil and type of soil reduces the effectiveness of the QA product over time.

An investigation was conducted to determine if sunlight, temperature and evaporation have an effect on QA's – Sporekill[®], Steri-max[®] and Path-X[™]. Following exposure to field conditions for two and a half weeks, all three QA products (and replicates) appeared to remain at a 1% (1200ppm) solution, which indicates that exposure to field conditions may not reduce the concentration of active ingredient in QA products such as Sporekill[®].

Two experiments were conducted to investigate the corrosive abilities of various cleaning and disinfecting products on different types of metals when submerged in solution or dipped frequently. The products used included; Farmcleanse (10% solution), Sporekill®, Steri-max® and Path-X™ (1% solution) and water. The metals used were; unpainted steel, painted steel, galvanised, aluminium and stainless steel (grade 304). In the first experiment, when submerged in 3 different QA compound disinfectants (1% DDAC) and a detergent (10% Farmcleanse) for 2 months, the products did not corrode the metals significantly more than water alone, and water appeared to have similar (or potentially greater) corrosive abilities as the other products tested in this experiment. Painted and unpainted steel were the most susceptible metals to rust development and would be the least suitable metals for constructing long term on-farm biosecurity infrastructure. Oxidation of the aluminium and galvanised plates was observed for the majority of products, including water. The stainless steel appeared to remain in good condition throughout the experiment, with minor rust development present on the exposed cut edges.

The second experiment involved frequent dipping (2-3 times per week) of the metals in the cleaning and disinfecting products, followed by exposure to field conditions. The results had shown that unpainted steel was the most susceptible metal to rust development, and the three QA products – Sporekill®, Steri-max® and Path-X™ appeared to be more corrosive on unpainted steel than the water, Farmcleanse and control treatments. All three QA's were more corrosive on unpainted steel with the frequent dipping application method as opposed to the submerged in solution method, where all treatments including water had similar corrosive abilities. The painted steel, stainless steel, aluminium and galvanised plates remained in good condition throughout the experiment with the frequent dipping application method.

Quaternary ammonium (QA) compound based disinfectants for effective on-farm biosecurity management

The use of disinfectants to decontaminate vehicles, machinery, tools and equipment is a critical on-farm biosecurity practice, as it reduces the risk of spreading pests and diseases such as Panama disease. Quaternary ammonium (QA) compound based disinfectants such as didecyl dimethyl ammonium chloride (120 g/L DDAC) applied at a 1% solution (1200ppm) have shown to kill fungal spores that cause Panama disease (Race 1 and Tropical race 4). Prior to this research growers had no means of monitoring and managing effective concentrations of QA compounds.

QA compound test kits

A number of different QA compound test kits which measure the concentration of active ingredient in a sample were trialled. The test kits were trialled against three QA products which contain the active ingredient didecyl dimethyl ammonium chloride (120 g/L DDAC) – Sporekill®, Steri-max® and Path-X™. The QA test kits trialled in this research include:

Sporekill® test kit

The Sporekill® test kit is a titration style test kit that was developed specifically for Sporekill®. The test kit works by undertaking a titration to get a colour change. The number of drops required to get a colour change determines the concentration of product in the sample. When tested against Sporekill® and two other QA products, Steri-max® and Path-X™, initial tests had shown that the test kit underestimated the concentration of the solution. As the Sporekill® test kit had underestimated the concentration of the solution and it was not a practical test for growers, it was not explored any further and other test kits were trialled.

Hydrion® QT-10 test papers 0-400ppm

The Hydrion® QT-10 test papers can be used for measuring the amount of active ingredient of QA products between 0-400ppm. The test papers are used by dipping the paper into the solution for 10 seconds and instantly comparing the resulting colour to the colour chart. As a 1% solution is equivalent to 1200ppm, a dilution of the sample is required for readability in the colour range of the test paper.

Precision Laboratories QAC QR® test strips 0-400ppm

The Precision Laboratories QAC QR® test strips also measure the concentration of active ingredient of QA compound products between 0-400ppm. They are used by dipping the test strip into the test solution for 1-2 seconds and comparing the strip to the colour chart within 10 seconds. Similar to the Hydrion test papers, a dilution of the sample is required for readability in the colour range of the test strip.

Quantofix® Quat test strips 0-1000ppm

The Quantofix® Quat test strips are used to measure the concentration of QA compounds between 0-1000ppm. The test strip works by dipping the strip into the test solution for 5 seconds, waiting 15 seconds and comparing test strip colour to the colour chart. The Quantofix® test strip can be used to measure QA compounds in general, however, the reference compound for the colour scale is benzalkonium chloride. A dilution of the QA sample is required for readability in the colour range of the test strip.

Precision Laboratories High Level QAC test strips 0-1500ppm

The Precision Laboratories High Level QAC test strip measures the concentration of active ingredient between 0-1500ppm. This test strip works by dipping the test strip into the test solution for 1-2 seconds and comparing the test strip colour to the colour chart within 5 seconds. As a 1% solution is equivalent to 1200ppm, a dilution of the sample is not required for this test strip.

La Motte QAC QR® test strips 0-1500ppm

The La Motte QAC QR test strip measures the concentration of active ingredient between 0-1500ppm. The test strip is used by dipping the strip into the test solution for 5 seconds, shaking off excess liquid and comparing test strip colour to the colour chart. Similar to the Precision Laboratories high level 0-1500ppm test strip, a dilution of the sample is not required for La Motte QAC QR® test strip.

Research activities conducted on QA compound test kits

Various research activities were conducted on the QA compound test strips to determine if any would be suitable for growers to conduct routine testing of their QA disinfectants. Below is an outline of the research activities conducted on the different test strips.

Activity 1

Aim: The efficacy of the Hydrion® 0-400ppm QT-10 test papers, Precision Laboratories QAC QR® 0-400ppm test strips and the Quantofix® Quat 0-1000ppm test strips were tested against QA's – Sporekill®, Steri-max® and Path-X™.

Method: A 1% (1200ppm) stock solution of each QA product was made. As each of the test strips do not measure the exact concentration of a 1% solution, a dilution was undertaken to get to the desired concentration for readability within the colour range of the test strips. Each product was diluted to 100 and 400ppm, and each combination was replicated 3 times. To determine the accuracy of the test strips, each combination and replicate were tested with each of the different test strips.

Results: When compared to the colour chart, the Hydrion® QT-10 test papers showed a consistent colour when tested at 400ppm, (Table 1, Figure 1), however when tested at the lower concentration of 100ppm, the test paper colour appeared difficult to read. Furthermore, the Hydrion® test paper become saturated quickly which resulted in changes to colour development, (Table 1, Figure 2).

The Quantofix® test strips appeared to have overestimated the concentration of active ingredient for both concentrations, which may be an effect of the reference ingredient being benzalkonium chloride. As a result, the Quantofix® test strip would not be suitable for on-farm testing of disinfectants containing 120 g/L DDAC.

The results of this experiment had shown that the Precision Laboratories QAC QR® 0-400ppm test strip was the most effective test strip for both 100ppm and 400ppm dilutions, (Table 1, Figure 1 and 2).

Table 1: Results from test strips dipped in Sporekill®, Steri-max® and Path-X™ diluted to 100 and 400ppm

	Sporekill® 100ppm	Steri- max® 100ppm	Path- X™ 100ppm	Sporekill® 400ppm	Steri- max® 400ppm	Path- X™ 400ppm
Hydrion® QT-10	✗	✗	✗	✓	✓	✓
Precision Laboratories QAC QR®	✓	✓	✓	✓	✓	✓
Quantofix®	✗	✗	✗	✗	✗	✗

Note: as the Quantofix® test strip is calibrated to benzalkonium chloride, a conversion calculation is required where a 400ppm solution of product should appear as 500ppm on the Quantofix® colour chart. A 100ppm solution of product should remain the same and appear as 100ppm on Quantofix® colour chart (follow the colour chart directions).



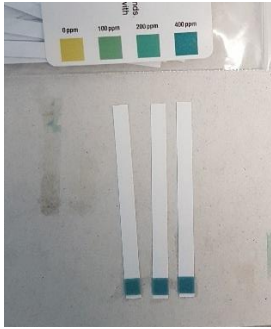
Hydrion®
Path-X™ 400ppm



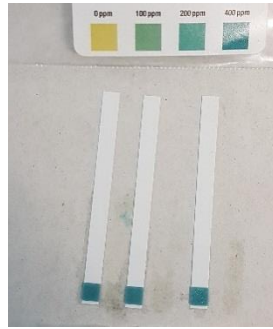
Hydrion®
Steri-max® 400ppm



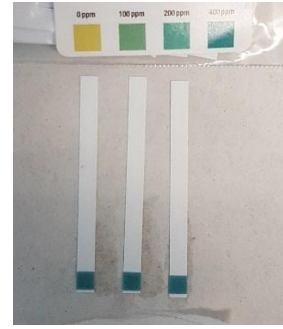
Hydrion®
Sporekill® 400ppm



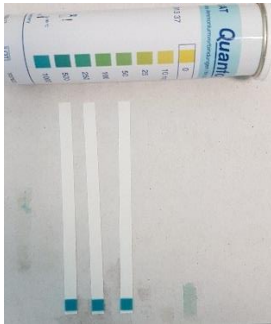
Precision Laboratories®
Path-X™ 400ppm



Precision Laboratories®
Steri-max® 400ppm



Precision Laboratories®
Sporekill® 400ppm



Quantofix
Path-X™ 400ppm



Quantofix
Steri-max® 400ppm



Quantofix
Sporekill® 400ppm

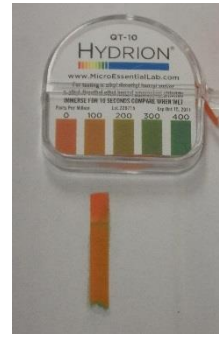
Figure 1: The Hydrion®, Precision Laboratories QAC QR® and Quantofix® test strips tested at 400ppm



Hydrion®
Path-X™ 100ppm



Hydrion®
Steri-max® 100ppm



Hydrion®
Sporekill® 100ppm

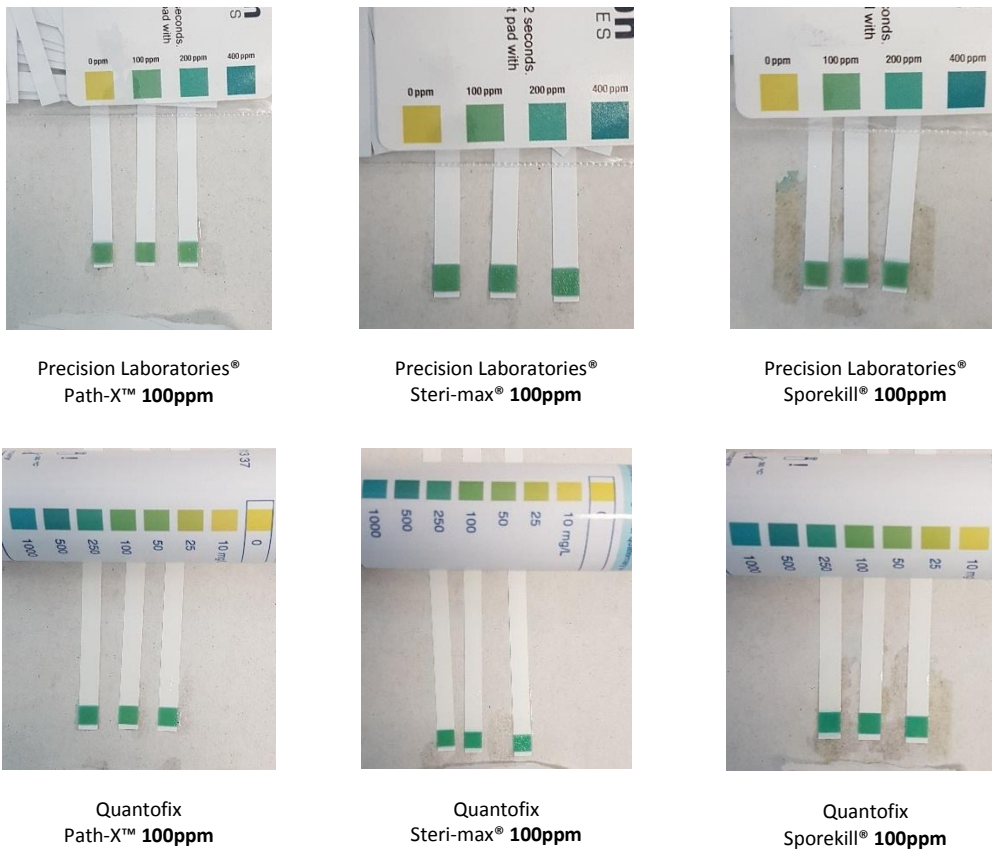


Figure 1: The Hydriion®, Precision Laboratories QAC QR® and Quantofix® test strips tested at 100ppm

Activity 2

Aim: As the Precision Laboratories QAC QR® 0-400ppm test strip provided consistent and accurate results for both 100 and 400ppm concentrations and each QA product, the next experiment undertaken was a calibration of the colour chart for each concentration indicated on the colour chart – 0, 100, 200 and 400ppm.

Method: A 1% (1200ppm) stock solution of the QA's – Sporekill®, Steri-max® and Path-X™ was made. Each product was diluted to 100, 200 and 400ppm with water as a control (0ppm). No replications were undertaken in this experiment. The test strips were dipped into the test solution (1-2 seconds) and the resulting colour was compared to the colour chart within 10 seconds.

Results: When dipped in the test solution for each concentration, the test strip colour positively matched the colour chart for all three QA products, (Figure 1). There were only slight differences in colour development between the 200 and 400ppm concentrations, therefore the optimum dilution concentration for readability is 200ppm.

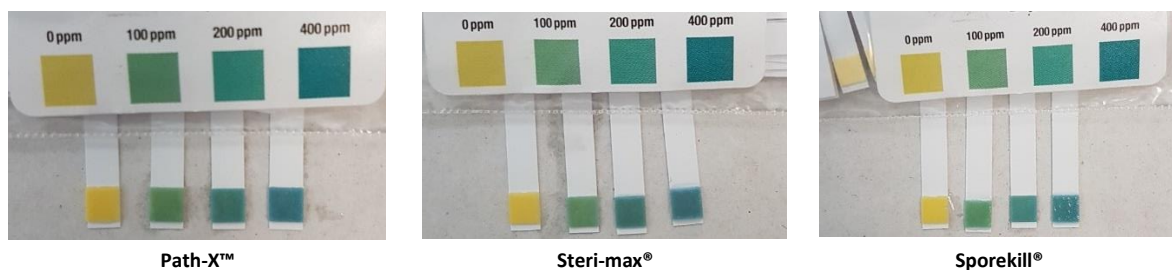


Figure 1: Calibration of the Precision Laboratories QAC QR® 0-400ppm test strips for QA's – Path-X™, Steri-max® and Sporekill®

Activity 3

Research shows that when 5% soil is added to a 1% solution of QA compounds such as Sporekill®, Steri-max® and Path-X™ (e.g. 1g soil to 20ml 1% solution), the solution is still effective at managing spores that cause Panama disease. Whereas when 25% soil is added to a 1% solution (e.g. 5g soil to 20mL 1% solution) it is no longer effective against managing spores that cause Panama disease.

Aim: Although the Precision Laboratories QAC QR® 0-400ppm test strip is not designed to test QA's with the presence of soil or organic matter, this experiment investigated the accuracy of the test strip against Sporekill®, Steri-max® and Path-X™ when different quantities of soil (5% and 25% soil) were added to the system.

Method: A 1% (1200ppm) stock solution of each product was made. 1g of soil was added to test tubes (x9), followed by the addition of 20ml of the 1% stock solutions of each QA product. 5g soil was added to another 9 test tubes followed by 20ml of the 1% stock solution of each QA product. Each of the solutions were then diluted to 200ppm (optimum concentration for readability). The Precision Laboratories QAC QR® 0-400ppm test strips were dipped in the test solutions for 1-2 seconds and compared to the colour chart within 10 seconds.

Results: With the addition of 5% soil, the test strip colour appeared to positively match the 200ppm colour chart for all three QA products, (Figure 1). However, the addition of 25% soil appeared to reduce the concentration of active ingredient in the sample, where all three QA products indicated a concentration between 0-100ppm, (Figure 2).



Figure 1: Addition of 5% soil



Figure 2: Addition 25% soil

Overall, efficacy testing conducted on the Precision laboratories QAC QR® 0-400ppm test strips had shown that this test strip would be suitable for growers to undertake on-farm testing of QA products in biosecurity infrastructure. However, as the test strip measures concentration between 0-400ppm and requires a dilution of the sample, it is not practical and easy to use for growers. For this reason, alternative QA compound test strips with a higher concentration range that do not require a dilution were explored.

Activity 4

Aim: The Precision Laboratories High Level 0-1500ppm test strip and La Motte 0-1500ppm test strip were trialled in this experiment. Using QA's – Sporekill®, Steri-max® and Path-X™, this experiment calibrated the test strips for each concentration indicated on the colour chart.

Method: A 2% (2400ppm) stock solution was made for each QA product. Each QA product was diluted to 200, 400, 700, 1000, 1200 and 1500ppm, with water as a control (0ppm). No replications

were undertaken in this experiment. The Precision Laboratories High Level 0-1500ppm test strips were dipped into the test solution for 1-2 seconds and the resulting colour was compared to the colour chart within 5 seconds. The La Motte 0-1500ppm test strips were dipped into the test solution for 5 seconds and compared to the colour chart within 15 seconds.

Note: The sensitivity range for each brand of test strip differentiates. The 700 and 1200ppm concentrations are not included on the colour chart, however, for consistency both test strips were tested at these concentrations.

Results: The test strip colour for both brands of test strip appeared to positively match the colour chart for each concentration (excluding 700ppm and 1200ppm which are not included on the colour chart). However, when tested at the higher concentrations (1000, 1200 and 1500ppm), colour development was similar and made it difficult to determine for both test strips. Moreover, colour development of the Precision Laboratories test strips appeared to be a darker teal than the La Motte test strips, (Figure 1 and 2). As colour development was a darker teal and appeared to be easier to compare to the colour chart, the Precision Laboratories High Level 0-1500ppm test strips were then selected for on-farm testing of QA's.

Precision Laboratories High Level QAC test strips



Figure 1: Calibration of Precision Laboratories High Level QAC test strips

La Motte QAC QR test strips

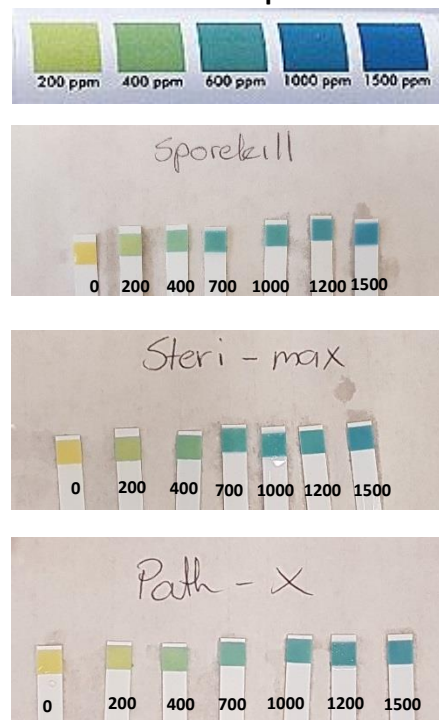


Figure 2: Calibration of La Motte QAC QR test strips with

Activity 5

Aim: Evaluate the Precision Laboratories High Level 0-1500ppm test strips in a real situation by testing on-farm biosecurity infrastructure containing QA disinfectants (e.g. Sporekill®).

Method: A 1% (1200ppm) standard solution of Sporekill® was made which was used as a control to compare with QA disinfectants that were collected from north Queensland banana farms. The

Precision Laboratories High Level 0-1500ppm test strip were dipped into the 1% standard solution and test solution simultaneously, for 1-2 seconds, (Figure 1). Both test strips were compared to the colour chart within 5 seconds, (Figure 2).



Figure 1: Test strips dipped into 1% standard solution and footbath sample simultaneously

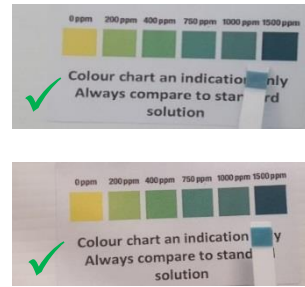


Figure 2: 1% standard solution (top), footbath sample (bottom)

Results: Overall, 26 disinfectant samples were collected from north Queensland banana farms. The samples were collected from 18 footbaths, 5 spray shuttles, 1 spray pack, 1 automatic dosing system and the waste water from 1 spray grid. Of the 26 samples tested, 18 samples appeared to be a 1% solution (1200ppm) or greater, while 8 samples appeared to be less than a 1% solution. The results of the on-farm testing were communicated back to growers, and growers topped up or replaced disinfecting solutions accordingly.



Figure 1: On-farm testing of QA disinfectants using the Precision Laboratories High Level 0-1500ppm test strips

Activity 6

The final activity consisted of developing a sample QA test kit for growers to test their QA's and evaluate the Precision Laboratories High Level 0-1500ppm test strips in a real situation. The test kits consisted of instructions, 10 test strips, a colour chart, a 1% standard solution and product safety data sheet, (Figure 1).

Overall, approximately 90 test kits have been supplied to banana growers and industry stakeholders through industry events including the Panama R&D Open Day (May 2017), Australian Banana Industry Congress (June 2017), Panama Tropical Race 4 update meetings in Tully, Innisfail and the Tablelands (August 2017) as well as farm visits.

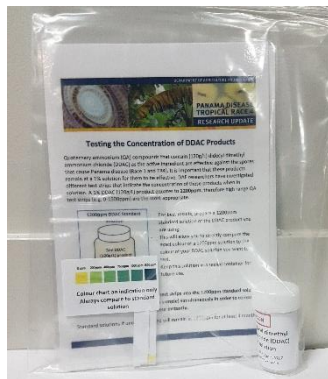


Figure 1: QA Test kit developed for banana growers



Figure 2: Test kits supplied to growers at the Panama R&D Open Day

Longevity of Quaternary Ammonium Compounds

Aim: The longevity of Sporekill®, Steri-max® and Path-X™ made at 1% (1200ppm) solution, with and without the addition of soil, and exposed to field conditions was investigated.

Method: In plastic storage containers, each QA product was made at 1% solution (1200ppm) with and without the addition of 5% soil, replicated 3 times (total of 18 test solutions). In plastic storage containers, deionised water with and without the presence of 5% soil was added to the containers, and replicated 3 times (total of 6 test solutions). The solutions were placed on an outside bench with the lids on, and exposed to field conditions for 8 months. The Precision Laboratories High Level 0-1500ppm test strips were used as indicators to test the disinfectants on a fortnightly basis for the first 6 weeks, and then monthly assessments were conducted thereafter. A fresh 1% standard solution was made at each assessment as a control to compare against test solutions. Sub samples of the 1% solutions without the addition of soil were collected at the 4 and 8 month mark and inoculated with *Fusarium oxysporum* f.sp *cabense* (*Foc*) Race 1. Sub samples of the 1% solutions with the addition of soil (both the supernatant and stirred solution) were collected at the 4 month mark only, and inoculated with *Foc* Race 1. To determine the effectiveness of the QA products, the number of Race 1 colonies detected after an instant, 5 minute and 30 minute contact time were recorded.



Figure 1: QA's exposed to field conditions at the South Johnstone DAF research station

Results

1% solutions without the addition of soil

After being exposed to field conditions including sunlight, humidity and temperature for 4 months, the inoculation results show that all three QA products were able to inhibit microconidia germination of *Foc* Race 1 across all contact times, (Figure 2). Using the Precision Laboratories High Level 0-1500ppm test strips as indicators, when tested at the 4 month mark, the test strip colour appeared to be a 1% solution for all QA's when compared to a fresh 1% standard solution, (Figure 3).

When the QA products were tested again at the 8 month mark, the inoculation results had shown that all three QA's maintained complete efficacy, with zero colony growth of *Foc* Race 1 detected, (Figure 4). However, at the 8 month mark the Precision Laboratories High Level 0-1500ppm test strips started to show a reduction in concentration for some of the QA products, particularly Path-X™ and Steri-max® which indicated concentrations between 750-1000ppm, (Figure 5). This suggests that although there may be a reduction in concentration, the QA products have remained at an effective concentration as no colony growth was detected at 8 months exposure to field conditions.

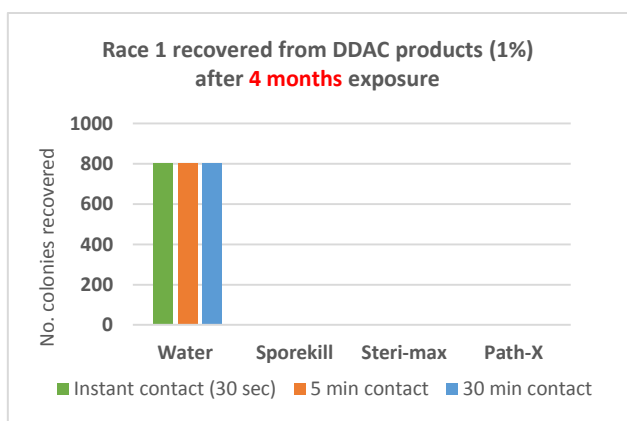


Figure 2: Race 1 colonies recovered from QA products at 4 months

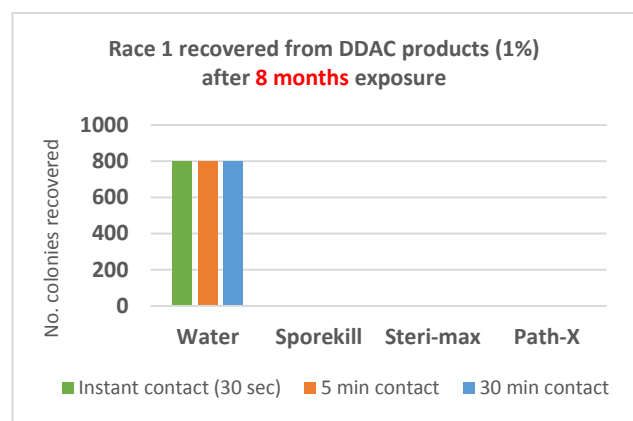


Figure 4: Race 1 colonies recovered from QA products at 8 months

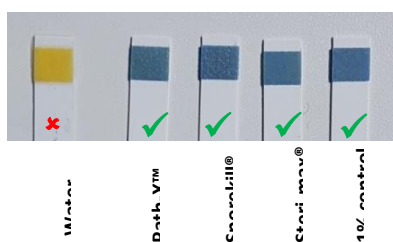


Figure 3: QA products tested at 4 months

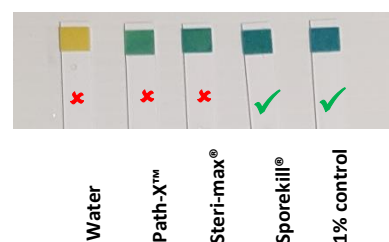


Figure 5: QA products tested at 8 months

1% solutions with the addition of soil

The addition of 5% soil gave contrasting results early on in the experiment, and it appeared that the concentration of active ingredient was reducing over time. Using the Precision Laboratories High Level 0-1500ppm test strips as indicators, when tested at the day of mixing, all three QA products appeared to be a 1% solution. However, when tested at 2 weeks, the same disinfectant solutions appeared to be less than a 1% solution. By week 4 the test strips indicated a further reduction and were showing close to 0ppm for all QA's and replicates, (Figure 6). As the test strips were indicating a reduced concentration early on in the experiment, assessments using the test strips were no longer taken.

At the 4 month mark, samples of the solutions (both supernatant and stirred) were collected and inoculated with *Foc* Race 1. The inoculation results show that colony growth was detected at an instant contact time for all QA's with the addition of soil. The Sporekill® solutions had the least number of colonies detected for both the supernatant (152 colonies) and stirred (163 colonies) samples. Whereas the Path-X™ solutions had the greatest number of colonies detected at an instant contact time, with greater than 800 colonies detected for both the supernatant and stirred solutions. After a contact time of 5 minutes, zero colonies were detected with the Sporekill® solutions. The Steri-max® solutions had some colony growth – 5 colonies detected in the supernatant and 3 colonies detected in the stirred samples. Again, the Path-X™ solutions had the greatest number of colonies detected at a 5 minute contact time, with 214 colonies detected in the supernatant and 116 colonies detected in the stirred samples. At the 30 minute contact time, no colony growth was detected for any of the QA's, which shows that some disinfecting is taking place, (Figure 7 and 8).

Overall, when exposed to field conditions for 4 months, a contact time of up to 30 minutes was required to reach zero colony growth for QA's with the presence of soil and organic matter. Sporekill® appeared to be the most effective product, having the least amount of colony growth detected, followed by Steri-max®, and Path-X™ which had the greatest number of colonies detected overall.

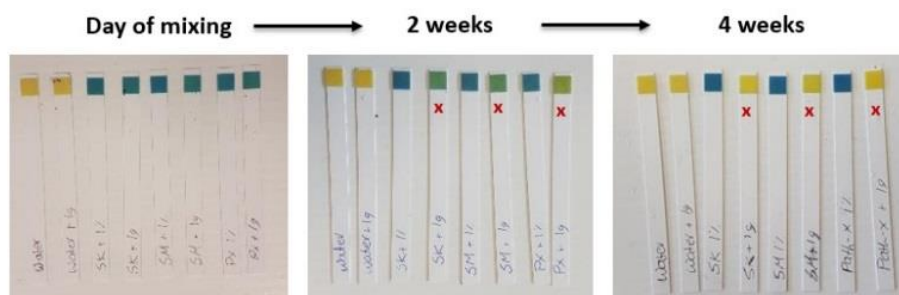


Figure 6: QA products with the addition of soil tested at day of mixing, 2 weeks and 4 weeks

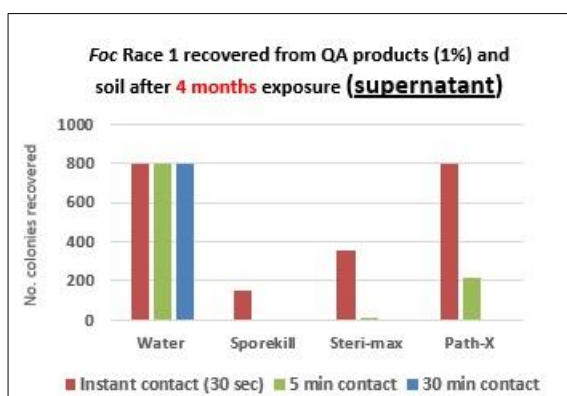


Figure 7: Race 1 colonies recovered from QA products and soil (supernatant)

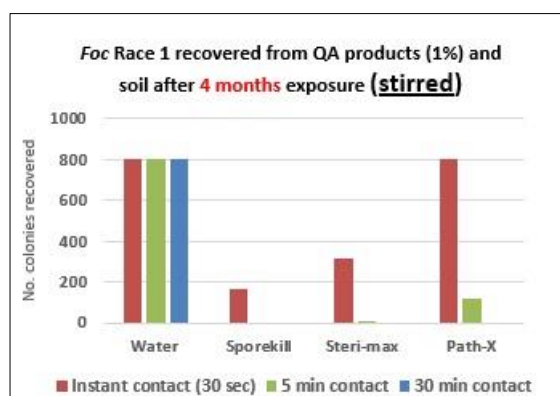


Figure 8: Race 1 colonies recovered from QA products and soil (stirred)

Interaction of soil and DDAC products over time

Aim: The aim of this experiment was to investigate the interaction of Sporekill® with the addition of soil over time.

Method: In this experiment two different soil types were used (clay and sand) and various amounts of soil were added to the Sporekill® solutions. A 1% standard stock solution of Sporekill® was made. 20ml of the 1% Sporekill® solution was added to clean test tubes (total of 10 test tubes). Various amounts of each soil type were weighed and added to the test tubes, (no soil, 0.25g, 0.50g, 0.75g and 1g). No replications were undertaken in this experiment. The Precision Laboratories High Level 0-1500ppm test strips were used as indicators to test the concentration of active ingredient in each sample. The samples were tested with the test strips at day of mixing, day 9 and day 28.

Results

The Sporekill® solutions without the addition of soil appeared to remain at a 1% (1200ppm) solution throughout the duration of the experiment, (Figure 1, 2 & 3). When tested approximately 30 minutes after adding soil to the system, all solutions appeared to remain at a 1% (1200ppm) solution for both soil types and all soil amounts, (Figure 1).

However, when the same solutions were tested 9 days later, the results indicate that there was a reduction in the concentration of active ingredient as more soil is added to the system. For both soil types with the addition of 1g soil, the solutions appeared to be approximately 875ppm, (Figure 2). Whereas, with the addition of a small amount of soil (0.25g), the solutions appeared to remain at 1% (1200ppm) for both soil types 9 days after mixing.

The same Sporekill® solutions were tested 28 days after mixing and the results had shown a further reduction in the concentration of active ingredient for both soil types and all soil amounts. There appeared to be more of a reduction in concentration of active ingredient for the clay soil as opposed to the sandy soil, (Figure 3). With the addition of 1g soil, the clay soil appeared to be approximately 300ppm, while the sandy soil appeared to be 400ppm. With the addition of 0.75g soil, the clay soil was approximately 750ppm, while the sandy soil appeared to be 1000ppm. When 0.5g soil was added to the system, the clay soil appeared to be 875ppm with the sandy soil being 1000ppm. They addition of 0.25g clay soil appeared to be 875ppm and the sandy soil appeared to be 1100ppm.

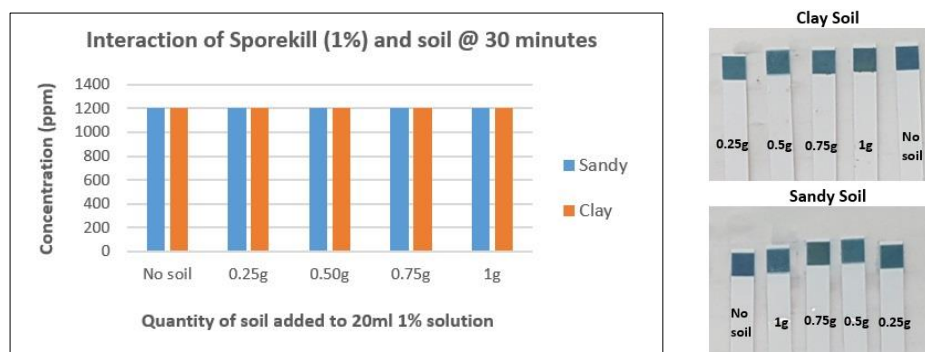


Figure 1: Sporekill and the addition of soil after 30 minutes

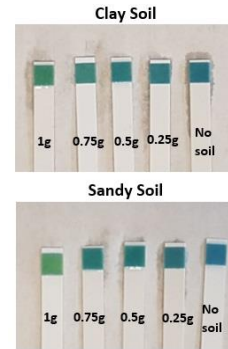
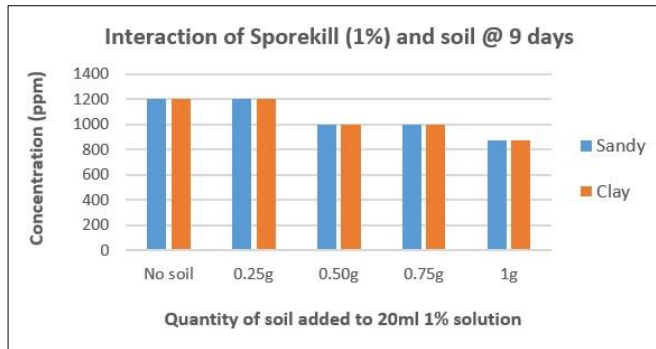


Figure 2: Sporekill and the addition of soil 9 days later



Figure 3: Sporekill with the addition of soil 28 days later

Overall, the results suggest that there may be a soil and time interaction with QA products such as Sporekill®. It is suggested that further investigation of QA products with the addition of soil and different types of soil, inoculated with Race 1 is required to determine if the presence of soil over time reduces the disinfecting capacity of the QA. This will also assist in gaining more information on whether the presence of soil over time has an effect on test strip colour and its effectiveness to accurately measure the concentration of active ingredient in a sample.

Does evaporation reduce the efficacy of QA disinfectants?

Aim: The aim of this experiment was to investigate whether sunlight, temperature and evaporation have an effect on QA's – Sporekill®, Steri-max® and Path-X™.

Method: In plastic storage containers, each QA product was made at 1% solution (500mL volume) and replicated 3 times, (9 test solutions). 500mL of deionised water was added to clean plastic containers, and replicated 3 times (3 test solutions). The solutions were placed on an outside bench with the lids off. To avoid dilution during a rainfall event, large storage containers were placed over the top of each replicate and secured to the bench with ricky straps. The storage containers were placed on timber chocks to allow for airflow, (Figure 1). The Precision Laboratories High Level 0-1500ppm test strips were used as indicators to test the disinfectants at the day of mixing, week 1, week 2 and week 2.5. The QA products and water controls had completely



Figure 1: QA products exposed to field conditions

evaporated by week 3. When the disinfectants were tested at week 2.5, there appeared to be approximately 50mL of solution left in each the containers.

Results: Following exposure to field conditions for two and a half weeks, all three QA products appeared to remain at a 1% (1200ppm) solution, (Figure 1). This indicates that exposure to field conditions such as sunlight, temperature and evaporation may not reduce the concentration of active ingredient in QA compound based disinfectants such as Sporekill®, Steri-max® and Path-X™. These results are particularly important for growers who have biosecurity infrastructure such as vehicle dips that are not under cover and exposed to field conditions on a daily basis. As it will provide confidence that their QA disinfecting products will remain at an effective concentration. It is important to remember that the presence of soil, organic matter and dilution from rainfall can reduce the efficacy of QA compound based disinfectants.

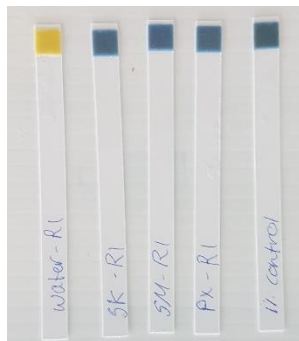


Figure 2: QA's at day of mixing

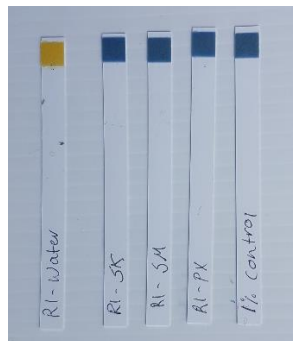


Figure 3: QA's at week 1

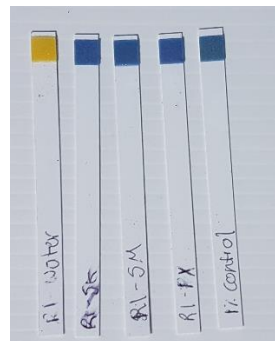


Figure 4: QA's at week 2

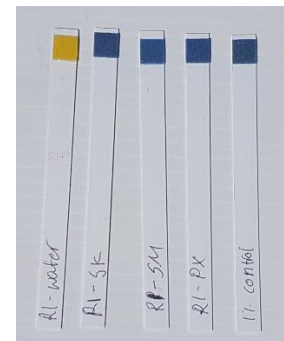


Figure 5: QA's at week 2.5

Are cleaning and disinfecting products corrosive?

Infrastructure such as footbaths and wash-down facilities are vital components of effective on-farm biosecurity. Two experiments were conducted to investigate the corrosive abilities of various cleaning and disinfecting products on different types of metals. This research is important as it will allow banana growers to make more informed decisions about the types of materials used for constructing on-farm biosecurity infrastructure that will withstand the test of time.

- **Cleaning and disinfecting products** - Farmcleanse (10% solution), Sporekill®, Steri-max® and Path-X™ (1% solution) and water.
- **Metal types** - Unpainted steel, painted steel, galvanised, aluminium and stainless steel (grade 304).



Figure 1: Cleaning and disinfecting products used in experiments

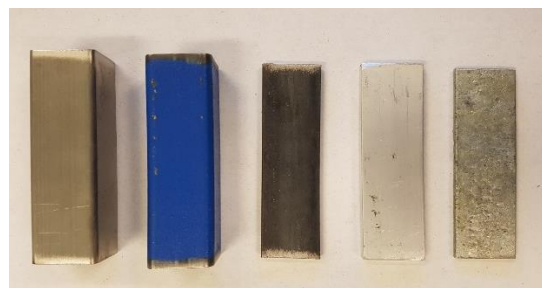


Figure 2: Metal types used in experiment

Experiment 1 – Submerged in solution

Aim: This experiment investigated the corrosive ability of the various cleaning and disinfecting products on different types of metal surfaces when submerged in solution for 2 months.

Method: The experiment was replicated three times and a Graeco Latin Squares design was used. A 1% solution was made for each QA product and replicate (3 QA's, 5 metals, 3 reps = 45 solutions), and a 10% solution of Farmcleanse was made for each replicate (1 product, 5 metals, 3 reps = 15 solutions). Water was used as a control treatment in this experiment (1 product, 5 metals, 3 reps = 15 solutions). Visual assessments were undertaken weekly and ratings of rust development were conducted on the cut edges and top and bottom surfaces. Rust development ratings were; 0 = 0%, 1 = cut edges only, 2 = 1 - 25%, 3 = 26 - 50%, 4 = 51 - 75%, 5 = 76 - 100%



Figure 3: Metals submerged in solution

Results

When various types of metals were submerged in 3 different QA compound disinfectants (1% DDAC) and a detergent (10% Farmcleanse) for 2 months, they did not corrode the metals significantly more than water alone. When submerged in solution, water may have similar (or potentially greater) corrosive abilities as the other products tested in this experiment.

Painted and unpainted steel were the most susceptible metals to rust development, (Figure 6 and 7). Pitting corrosion was observed for both painted and unpainted steel for all products and would be the least suitable metals for constructing long-term on-farm biosecurity infrastructure. The paint on the painted steel had bubbled and peeled for the majority of products, with the water treatments having the least amount of paint that had bubbled and peeled, (Figure 7).

Rust development was present on the cut edges of the stainless steel for the majority of products, (Figure 8). Rust development may be a result of a number of factors including; sensitisation from heat treatment and high temperatures, the stainless steel surface (cut edges) being covered with steel particles, or the chromium oxide layer failing to protect the stainless steel from oxygen. Although rust development was present on the cut edges, the standard grade stainless steel would be a suitable metal for constructing on-farm biosecurity infrastructure.

Aluminium had oxidised for the majority of products except Steri-max[®], where Steri-max[®] appeared to have no oxidising effect on the aluminium, (Figure 9). The aluminium plates had a chalky texture and a white powder had developed over the surface when scraped. Further investigation is required to determine if the structural integrity of the aluminium plates was compromised.

For the majority of products (except Farmcleanse) a white precipitate had developed on the surface of the galvanised metal plates, (Figure 10). Rust had developed on the cut edges for galvanised metal treated with water which may be a result of the exposed edges no longer having a protective galvanised coating, and potential microbial activity. As a white precipitate had developed for the majority of products when submerged in solution for 8 weeks, further research is required to determine if the structural integrity of the galvanised plates was compromised.

	Farmcleanse	Path-X™	Sporekill®	Steri-max®	Water
Aluminium	5.00	5.00	5.00	0.00	5.00
Galvanised	0.67	2.33	1.33	2.67	1.67
Painted	1.33	0.67	0.67	2.00	3.67
Stainless	0.00	0.67	1.00	1.00	1.00
Unpainted	3.67	2.67	2.00	2.33	2.67

Experiment 2 – Dipped frequently

Aim: The second experiment consisted of the same products and metals, however, the metals were dipped 2-3 times a week and exposed to field conditions for 3 months.

Method: This experiment was replicated three times. To avoid cross contamination between the different types of metals, the cleaning and disinfecting solutions were made for each metal type. A 1% solution was made for each QA product and metal type (3 QA's, 5 metals = 15 QA solutions) and a 10% solution of Farmcleanse was made for each metal type (5 metals = 5 Farmcleanse solutions). Plastic containers were filled with water which was used as a treatment for each metal type (5 metal types = 5 water solutions). Metals receiving the control treatment were not treated with any cleaning or disinfecting product. Each metal was dipped for 10 seconds in its allocated cleaning and disinfecting solution 2-3 times a week for 3 months. Two assessments of rust development were conducted in this experiment. The first assessment was conducted prior to commencement of the experiment, and the second assessment was conducted at the end of the experiment. In this experiment ratings were conducted on the top surface of the metal plates. No ratings were conducted on the exposed cut edges in this experiment. Rust development ratings were; 0 = 0%, 1 = 1 - 25%, 2 = 26 - 50%, 3 = 51 - 75%, 4 = 76 - 100%.



Figure 4: Metals placed on racks and exposed to field conditions



Figure 5: Metals dipped frequently in cleaning and disinfecting products

Results

Unpainted steel was the most susceptible metal to rust development. The three QA products – Sporekill®, Steri-max® and Path-X™ appeared to be more corrosive on unpainted steel than the water, Farmcleanse and control treatments, (Figure 6). Furthermore, all three QA's appeared to be more corrosive on unpainted steel with this particular application method as opposed to the submerged in solution method, where all treatments including water had similar corrosive abilities. Overall, if treated frequently with QA products such as Sporekill®, Steri-max® and Path-X™, unpainted steel would be the least suitable material for on-farm biosecurity infrastructure.

With the frequent dipping application, the painted steel appeared to remain in good condition for all products. Unlike the submerged in solution application method, the paint did not bubble and peel and remained intact for all products. Minor rust development was present where there were chips in the paint. Moreover, visual observations had shown rust development present on the exposed cut edges for all treatments, (Figure 7).

Despite a small amount of rust development present on the exposed cut edges, the stainless steel remained in good condition for all products with the frequent dipping application, (Figure 8). Similarly, with the frequent dipping application, the aluminium and galvanised plates remained in good condition with no oxidation or rust development observed, (Figure 9 and 10).

Table 2. Mean level of rust development with frequent dipping application after 12 weeks

	Farmcleanse	Path-x™	Sporekill®	Steri-max®	Water	No treatment
Aluminium	0.00	0.00	0.00	0.00	0.00	0.00
Galvanised	0.00	0.00	0.00	0.00	1.00	0.33
Painted	0.00	1.00	1.00	1.00	0.00	1.00
Stainless	0.00	0.00	0.00	0.00	0.00	0.00
Unpainted	1.00	4.00	4.00	4.00	2.00	2.00



Figure 6: Unpainted steel, submerged in solution (left), dipped frequently (right)



Figure 7: Painted steel, submerged in solution (left), dipped frequently (right)



Figure 8: Stainless steel, submerged in solution (left), dipped frequently (right)



Figure 9: Aluminium, submerged in solution (left), dipped frequently (right)



Figure 10: Galvanised steel, submerged in solution (left), dipped frequently (right)

Appendix 6 – Development and testing for specificity and sensitivity of a TaqMan MGB qPCR assay for *Foc* TR4 from soil

The aim of this work is to design a quantitative PCR (qPCR) assay for the sensitive and specific detection of *Fusarium oxysporum* f. sp. *ubense* Tropical race 4 (*Foc* TR4) in soil and water samples.

Part 1 – Development and testing of qPCR assay

Based on sequence information available in GenBank qPCR assays were tentatively designed for the detection of *Fusarium oxysporum* f. sp. *ubense* Tropical race 4. The Intergenic Spacer (IGS) of ribosomal DNA was chosen as target as it is in multiple copies in the genome, which improves assay sensitivity. Two forward primers, two reverse primers and one TaqMan MGB probe were designed. The TaqMan MGB assays are used routinely by SARDI Molecular Diagnostic Centre to quantify target organisms.

A collection of *Fusarium* isolates was organised by Lucy Tran-Nguyen (Department of Primary Industry and Fisheries, Darwin). It included several isolates of *F. oxysporum* f. sp. *ubense* TR4, Subtropical Race 4 and Race 1, and a range of *Fusarium* species/subspecies from Australia and overseas. This large collection of more than 100 isolates was used to assess the specificity and sensitivity of the assays designed. All DNA concentrations were adjusted to 200pg/ul so qPCR results were directly comparable between isolates.

The four possible primer combinations were first tested against a limited number of isolates to identify the best one (Table 1). All primer combinations allowed detection of the *Foc* TR4 DNA but Ct (Cycle threshold) values indicated that F1R1 was the most sensitive combination i.e. the one giving the lowest Ct value (17.4). This combination also detected one *Foc* subtropical race 4 isolate but with a much lower sensitivity (i.e. higher Ct value) and showed a slight cross reaction with *F. oxysporum* f. sp. *vasinfectum*.

Table 1. Preliminary assessment of 4 primer combinations (F1R1, F1R2, F2R1, F2R2) for *Fusarium oxysporum* f. sp. *ubense* detection.

Species	Ct Value			
	F1R1	F1R2	F2R1	F2R2
<i>F. oxysporum</i> f. sp. <i>ubense</i> tropical race 4	17.3	19.8	18.5	21.3
<i>F. oxysporum</i> f. sp. <i>ubense</i> subtropical race 4	31.2	33.3	32.6	34.9
<i>F. oxysporum</i> f. sp. <i>ubense</i> race 1	UD	UD	UD	UD
<i>F. oxysporum</i> f. sp. <i>vasinfectum</i>	37.4	UD	38.5	UD
<i>F. oxysporum zingiberi</i>	UD	UD	UD	UD
<i>F. oxysporum</i> f. sp. <i>tracheiphilum</i>	UD	UD	UD	UD
<i>F. oxysporum</i> f. sp. <i>niveum</i>	UD	UD	UD	UD
Negative control	UD	UD	UD	UD

The assay using F1R1 was selected for further assessment against the whole *Fusarium* collection (Table 2). The assay detected all but one (Taiwanese) *Foc* TR4 isolates with high sensitivity. Most SR4 and R1 isolates were also detected with high Ct values, although two R1 isolates (Sri Lanka and Malaysia) were detected with lower Ct values (29 and 19). All *Foc* genotypes from banana as well as other *F. oxysporum* subspecies were detected with Ct values ranging from approx. 26 to 38. One isolate of *F. oxysporum* f. sp. *lycopersici* was detected with a Ct value of 17. The assay did not detect *F. subglutinans*.

Table 2. Specificity assessment of the selected qPCR assay

CAV #	Origin	Identity	Host	Ct Value
604	Indonesia	1216 <i>Foc</i> tropical race 4	Banana	17.8
610	Malaysia	1213 <i>Foc</i> tropical race 4	Banana	17.5
789	Australia	01213/16 <i>Foc</i> tropical race 4	Banana	17.3
847	Indonesia	01213/16 <i>Foc</i> tropical race 4	Banana	17.1
1185	Philippines	01213/16 <i>Foc</i> tropical race 4	Banana	16.1
2307	Taiwan	1216 <i>Foc</i> tropical race 4	Banana	15.6
2346	Taiwan	1213 <i>Foc</i> tropical race 4	Banana	36.0
NT1415-404-1-2	Qld	<i>Foc</i> tropical race 4	Banana	20.1
SS NT1415-404-1-2	Qld	<i>Foc</i> tropical race 4	Banana	21.1
95	South Africa	0120 <i>Foc</i> subtropical race 4	Banana	36.3
191	Indonesia	0120/15 <i>Foc</i> subtropical race 4	Banana	35.1
289	Brazil	0120 <i>Foc</i> subtropical race 4	Banana	32.9
1889	India	1220 <i>Foc</i> subtropical race 4	Banana	35.3
2400	Bangladesh	1220 <i>Foc</i> subtropical race 4	Banana	34.2
1089	Australia	129 <i>Foc</i> subtropical race 4	Banana	34.5
Dc 43289	WA	<i>Foc</i> subtropical race 4	Banana	UD
BRIP 40376-2 VCG 0120/15	Qld	<i>Foc</i> subtropical race 4	Banana	UD
BRIP 40334-2 VCG 0129	Qld	<i>Foc</i> subtropical race 4	Banana	UD
BRIP 42331 VCG 0120	Qld	<i>Foc</i> subtropical race 4	Banana	UD
BRIP 44073-1 VCG 01211	Qld	<i>Foc</i> subtropical race 4	Banana	UD
941	Thailand	125 <i>Foc</i> race 1	Banana	35.3
967	Philippines	0124/5 <i>Foc</i> race 1	Banana	34.3
968	India	0124/5 <i>Foc</i> race 1	Banana	34.9
2123	Cambodia	124 <i>Foc</i> race 1	Banana	37.7

2260	Sri Lanka	124 <i>Foc race 1</i>	Banana	29.4
2282	Malaysia	125 <i>Foc race 1</i>	Banana	19.5
Dc 43281	WA	<i>Foc race 1</i>	Banana	36.1
Dc 43287	WA	<i>Foc race 1</i>	Banana	UD

600	Philippines	Gen 2	Banana	37.5
615	Philippines	Gen 8	Banana	27.1
616	Philippines	Gen 7	Banana	37.8
618	Philippines	122	Banana	26.4
620	Philippines	Gen 9	Banana	36.7
624	Philippines	Gen 10	Banana	38.6
626	Malawi	0124/5	Banana	37.4
632	USA	1210	Banana	30.8
785	USA	126	Banana	35.0
788	USA	1210	Banana	27.5
793	Indonesia	126	Banana	34.5
818	Indonesia	Gen 3	Banana	36.6
848	Indonesia	1219	Banana	35.7
853	Malaysia	Gen 4	Banana	38.7
871	Malaysia	1217	Banana	31.1
898	Philippines	Gen 11	Banana	35.8
909	Malaysia	Gen 5	Banana	35.0
939	Thailand	123	Banana	32.2
998	Uganda	1222	Banana	30.7
1003	Vietnam	Gen 13	Banana	37.4
1004	Vietnam	Gen 14	Banana	38.7
1011	Vietnam	Gen 12	Banana	35.6
1024	Mexico	Gen 6	Banana	37.1
1050	Honduras	126	Banana	31.0
1959	Sri Lanka	1217	Banana	33.0
1983	Philippines	123	Banana	31.0
2013	Vietnam	128	Banana	33.9
2037	Bangladesh	128	Banana	26.2
2119	Cambodia	1221	Banana	40.1
2226	Cambodia	1221	Banana	31.4

2234	Cambodia	123	Banana	37.8
2249	Cambodia	1222	Banana	30.5
2285	Malaysia	1218	Banana	31.2
2318	Taiwan	121	Banana	33.6
2365	Taiwan	121	Banana	32.8
DRC 54	DRC	1212	Banana	29.2
Phil 4	Philippines	122	Banana	25.2
Tz57	Tanzania	1212	Banana	29.5
Bur 57	Burundi	1212	Banana	38.7
201	South Africa	<i>F. oxysporum</i>	Non-pathogen	35.7
202	South Africa	<i>F. oxysporum</i>	Non-pathogen	36.2
528	South Africa	<i>F. oxysporum</i>	Non-pathogen	36.0
529	South Africa	<i>F. oxysporum</i>	Non-pathogen	36.7
531	South Africa	<i>F. oxysporum</i>	Non-pathogen	34.3
Dc 43278	WA	<i>F. oxysporum</i>	Cucumber	UD
Dc 43279	WA	<i>F. oxysporum</i>	Leek	40.2
Dc 43282	WA	<i>F. oxysporum</i>	Cotton	UD
Dc 43283	WA	<i>F. oxysporum</i>	Rice	UD
Dc 43285	WA	<i>F. oxysporum</i>	Tomato	UD
Dc 43286	WA	<i>F. oxysporum</i>	Basil	38.4
Dc 43288	WA	<i>F. oxysporum</i>	Pineapple	UD
Dc 43290	WA	<i>F. oxysporum</i>	Grapevine	UD
Dc 43291	WA	<i>F. oxysporum</i>	Pine tree	UD
Dc 43284	WA	<i>F. oxysporum redolens</i>	Bean	UD
315	PPRI 5456	<i>F. oxysporum lycopersici</i>	Tomato	17.0
316	PPRI 5457	<i>F. oxysporum lycopersici</i>	Tomato	33.4
330	CBS 413.90	<i>F. oxysporum lycopersici</i>	Tomato	32.0
317	PPRI 4923	<i>F. oxysporum melonis</i>	Melon	33.7
324	SP.257	<i>F. oxysporum niveum</i>	Watermelon	34.7
Dc 43280	WA	<i>F. oxysporum niveum</i>	watermelon	UD

327	CBS 491.97	<i>F. oxysporum dianthi</i>	Carnation	37.4
328	CBS 137.97	<i>F. oxysporum gladioli</i>	Gladiolus	35.4
329	CBS 259.51	<i>F. oxysporum lini</i>	Flax	35.2
331	CBS 195.65	<i>F. oxysporum tulipae</i>	Tulip	32.8
333	CBS 935.73	<i>F. oxysporum phaseoli</i>	Climbing bean	35.1
334	CBS 127.73	<i>F. oxysporum pisi</i>	Pea	31.3
336	CBS 488.76	<i>F. oxysporum raphanni</i>		35.5
337	CBS 411.90	<i>F. oxysporum vasinfectum</i>	Cotton	34.8
338	CBS 783.83	<i>F. oxysporum elaeidis</i>	Oil palm	37.9
339	CBS 179.32	<i>F. oxysporum nicotianae</i>	Tobacco	31.9
340	CBS 744.79	<i>F. oxysporum passiflorae</i>	Passion fruit	34.6
341	CBS 794.70	<i>F. oxysporum perniciosum</i>	Mimosa	33.4
343	CBS 424.90	<i>F. oxysporum melonis</i>	Melon	31.0
345	CBS 129.81	<i>F. oxysporum chrysanthemi</i>	Ornamentals	29.6
385	MRC 2301	<i>F. proliferatum</i>		32.2
Dc 43219	NSW	<i>F. subglutinans</i>	Banana	UD
Dc 43220	WA	<i>F. subglutinans</i>	Banana	UD
Dc 43221	WA	<i>F. subglutinans</i>	Banana	UD
Dc 43222	WA	<i>F. subglutinans</i>	Banana	UD
Negative control				UD

Conclusions and proposed actions

The assay designed detects most *Foc* TR4 with a high sensitivity. However, the detection of most other *Foc* races and many *F. oxysporum* subspecies, albeit with a lower sensitivity, limits the potential application of the assay.

The apparent lack of specificity of the assay could be due to insufficient sequence information as design was based on the only few sequences available in GenBank. Moreover, some of the results such as the poor detection of the Taiwanese *Foc* TR4 and the strong detection of two *Foc* R1 and one *F. oxysporum* f.sp. *lycopersici* isolates suggest that there could have been some misidentification or contamination. Additional work is proposed to sequence a targeted region in the IGS for all isolates in Table 2. This will help to clarify the identification of each isolate and identify a better location for a TaqMan MGB assay.

Part 2 - TaqMan MGB assay redesigned based on IGS sequence data for all isolates in Table 2.

Introduction

The aim of this work is to design a quantitative PCR (qPCR) assay for the sensitive and specific detection of *Fusarium oxysporum* f. sp. *ubense* Tropical race 4 (*Foc* TR4) in soil and water samples.

Based on sequence information available in GenBank, a TaqMan MGB qPCR assay, targeting the Intergenic Spacer (IGS) of ribosomal DNA was designed for the detection of *Foc* TR4 (Milestone 2). The assay was assessed for sensitivity and specificity against a collection of more than a 100 isolates including several *Foc* TR4, Subtropical Race 4 (SR4) and Race 1 (R1) isolates and a range of *Fusarium* species/subspecies from Australia and overseas. The assay detected most *Foc* TR4 with a high sensitivity but cross reacted with most other *Foc* races and many *F. oxysporum* subspecies, with a lower sensitivity. The apparent lack of specificity suggested that the assay may need redesigning and warranted further investigation.

Outcome

The IGS of a selection of 16 isolates from the collection was sequenced in both directions to generate sequence information and confirm identification (Figure 1). Sequences were successfully generated for 14 isolates. This clearly indicated that two isolates had been misidentified: one isolate of *Foc* TR4 from Taiwan that is detected with a Cycle Threshold (Ct) of 36.0 sequenced as *Foc* R1 while one isolate of *F. oxysporum* f. sp. *lycopersici* detected with a Ct value of 16.9 in fact sequenced as *Foc* TR4 (Figure 1). Moreover, it showed that, based on their sequence results some isolates cannot theoretically be detected by the primers and probe used in the assay, suggesting that some DNA may be contaminated (red grouping, in Figure 1). Further analysis of the qPCR results obtained with the collection of isolates highlighted that all Australian non-target isolates behaved as expected with no or very high Ct whereas all isolates from a South African collection showed some levels of cross reaction (Table 3).

Conclusions and future work

Overall, the results showed that the qPCR assay designed in the IGS is sensitive. They also provided strong evidence that some isolates may have been contaminated or misidentified.

A new collection, including pathogenic/non-pathogenic and Australian/overseas isolates will be assessed in order to verify the results and validate the assay design. In parallel, work will start to assess the sensitivity of the assay in soil which will involve testing soil naturally infected from Australia and overseas and artificially spiking non contaminated soil with known amount of *Foc* TR4 spores. This will allow checking the specificity of detection in soil and determining detection threshold.

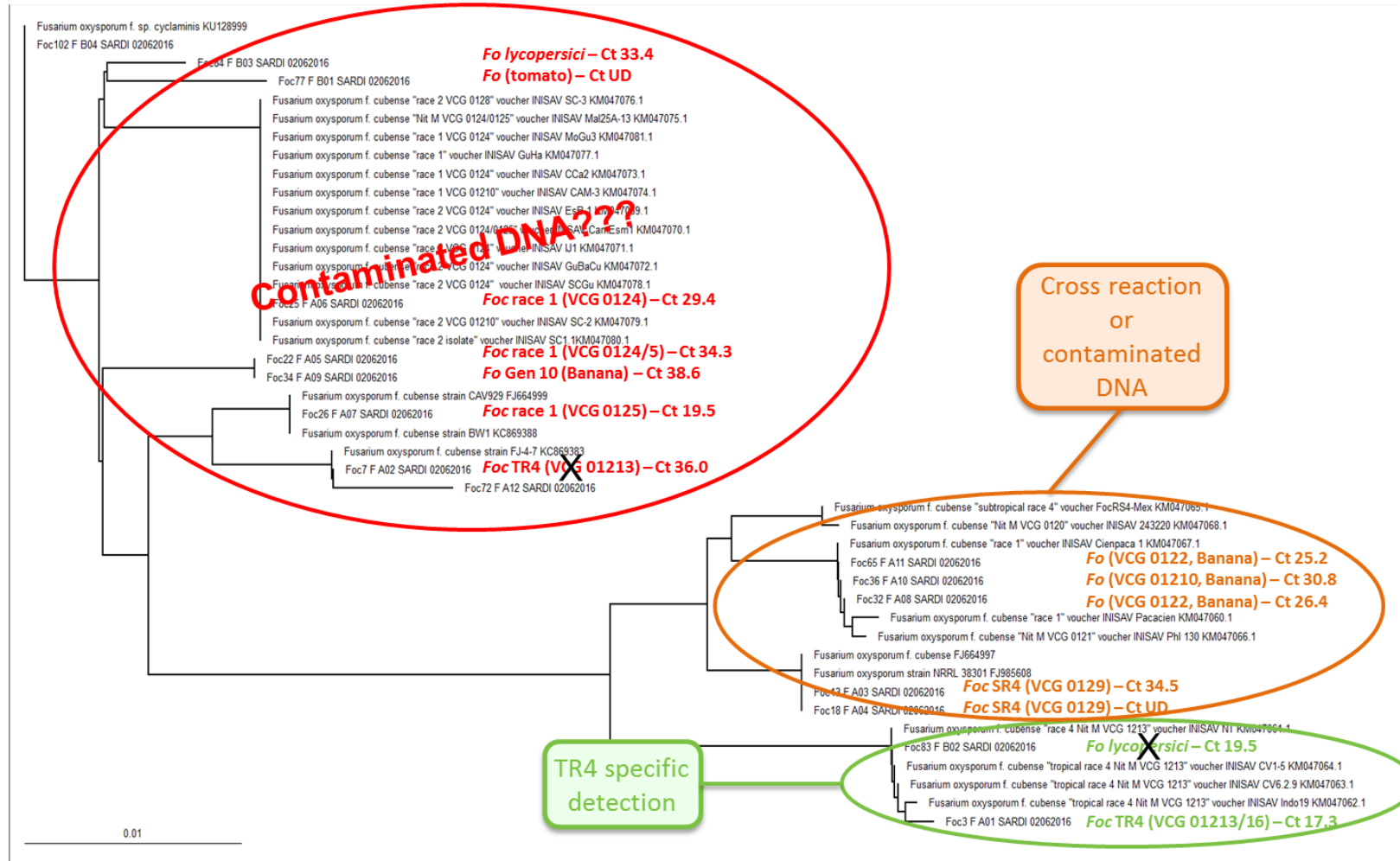


Figure 1. Phylogeny based on the IGS sequences of *Foc* isolates selected from public database. The test has been designed to detect isolates in the green section. Isolates in the orange section should not be detected while those in the red section cannot theoretically be detected by the primers and probe selected for the assay. Two isolates indicated with a black cross, were misidentified (*Foc* TR4 in the red section sequencing as *Foc* race 1 and *F. oxysporum* f. sp. *lycopersici* in the green section sequencing as *Foc* TR4). Sequences of the isolates in the red section strongly suggest that the DNA has been contaminated.

Table 3. Presentation of qPCR results (as Ct values) highlighting inconsistencies. The *Foc* TR4 isolate from Taiwan with a Ct of 36 sequenced as *Foc* race 1 while the *F. oxysporum* f. sp. *Lycopersici* with a Ct of 16.9 sequenced as *Foc* TR4. All non-target isolates from Australia (lighter grey section) generated very high or not Ct values, as expected whereas all non-target isolates from the South African collection (darker grey section) were detected non-specifically.

Identity	No of isolates	Origin	Ct values
<i>Foc</i> TR4	10	Australia, Indonesia, Malaysia, Philippines, Taiwan	16.1 - 21.2
	1	Taiwan	36.0
<i>Foc</i> SR4	4		UD
<i>Foc</i> Race 1	2		36.1 - UD
<i>F. oxysporum</i> (various f. sp.)	10		38.2 (1), 40.6 (1) and UD
<i>F. oxysporum niveum</i>	1	Australia	UD
<i>F. oxysporum redolens</i>	1		UD
<i>F. subglutinans</i>	4		UD
<i>F. solani</i>	1		UD
<i>Foc</i> SR4	6		32.9 - 36.3
<i>Foc</i> race1	6		19.5 - 35.3
<i>Foc</i>	39		25.2 - 40.1
<i>F. oxysporum</i>	5	South African collection	34.3 - 36.7
<i>F. oxysprum</i> (various f. sp., including one f. sp. <i>niveum</i>)	16		29.6 - 37.9
<i>F. oxysporum</i> f.sp. <i>lycopersici</i>	3		16.9 , 32.0, 33.4
<i>F. proliferatum</i>	1		32.2

Part 3 – Validate *Foc* TR4 DNA assay with DNA from international isolates and assess sensitivity against field samples

Outcome

Extended specificity assessment

A new collection including pathogenic/non-pathogenic and Australian/international isolates was received from DAF Queensland and used to further assess the specificity of the assay (Table 4). The results, together with those reported previously (Milestone 3) indicate that the qPCR assay preferentially detects *Foc* TR4, but some isolates are detected well (VCG 1213/1216), some less well (VCG 0122) and others are hardly detected (VCG 0121). The results also show that other *Foc* races may be detected infrequently and usually at Ct values >30 for 0.8ng DNA/well. The new results confirm that some isolates may have been contaminated or misidentified as shown by the difference in CT values between the two DNA collections for Genotypes 2-14 (Table 4).

Spiking experiment

Two experiments were conducted to assess the sensitivity of the *Foc* TR4 assay. Two hundred gram soil samples were spiked with 50,000, 5,000, 500, 50, 5 and 0 spores and with 500, 250, 100, 50, 5 and 0 *Foc* TR4 spores, in the first and second experiment respectively, with three replicate samples for each spiking level. The assay consistently detected 50,000, 5,000 and 500 spores/200g, with Ct values averaging 29.1, 32.1 and 37.9, respectively. Samples with 250, 100, 50 and even 5 spores/200g were detected erratically; no *Foc* TR4 was detected in the un-spiked samples. Overall, the results indicate that the limit of detection of the new assay is about 2.5 spores/g of soil.

Assessment of field samples

Soil samples were collected on a farm near Darwin, previously infected by *Foc* TR4. Following the outbreak the banana plants have been removed and the block kept host free. DNA was extracted from four composite samples and tested by qPCR. *Foc* TR4 DNA was detected in all samples with Ct values ranging from 31.2 to 34.9.

Additional soil samples were collected from several *Foc* TR4 infected and non-infected banana farms in Northern Territory, Queensland and New South Wales. In excess of 70 samples were analysed after DNA extraction (Table 5). *Foc* TR4 was only detected in soil samples originating from known infected farms with lower Ct values (i.e. higher *Foc* TR4 concentration) at sites with recent infection compared to sites with older infections; all other samples returned a below detection level result including those from farms with known *Foc* R1 and SR4 infections.

To investigate if the assay could detect non-target *Fusarium* species, next generation sequencing was performed on soil DNA of all samples in which the assay detected *Foc* TR4 and a selection of soils with below detection results. *Foc* TR4 sequences were found only in soil samples that had returned a positive results in qPCR, and not in the others, further supporting the specificity of the assay.

Conclusions

The results obtained throughout the project show that the assay can detect most *Foc* TR4 VCGs and is sensitive enough to detect as few as 2.5 spores per gram of soil. Assessment of a range of soil samples from Northern Territory, Queensland and New South Wales, show that the assay detects *Foc* TR4 in soil where it is expected with no background cross reaction with *Foc* R1 and SR4, nor with known non-infected soils. Next generation sequencing results further supports the specificity of the assay.

SARDI is currently following up on the poor detection of *Foc* TR4 isolates from VCG 0121 with Dr J. Henderson (University of Queensland, Centre for Plant Science), who has developed qPCR assays targeting other regions of the genome.

The assay is now commercially available through SARDI Molecular Diagnostics Centre. It has already being used to assess research samples for HIA Project BA14014 - Fusarium wilt Tropical Race Research program. The assay could potentially be used for surveillance programs. However, where a new incursion is detected, it is recommended that this be confirmed by sequencing to confirm identification.

Table 4. Specificity assessment of the *Foc* TR4 qPCR assay on a collection of Australian and international isolates. Results are given as Cycle threshold (Ct) values. All DNA were used at 200pg/ul. Low Ct values indicate sensitive detection, whereas Ct values close to 40 indicate poor detection. The column ‘Previous results’ indicate results obtained on another DNA extract from the same isolate (Milestone 3 report). ND: not detected.

Collection Number	BRIP/CAV \ Number*	Strain	ID	Host	Country	Ct value [^]	Previous results [#]
Endophytic <i>F. oxysporum</i>	BRIP 64639a			<i>Macaranga tanarius</i>	Australia	38.2	NA
	BRIP 62826b			Cavendish	Australia	36.8	
	BRIP 62833			Cavendish	Australia	ND	
	BRIP 62830a			Unknown grass	Australia	ND	
	BRIP 62834			Cavendish	Australia	39.2	
	BRIP 64626a			Cavendish	Australia	32.0	
	BRIP 64636f			Solanum sp.	Australia	39.1	
	BRIP 64642b			<i>Drymaria cordata</i>	Australia	ND	
	BRIP 64625a			<i>Axonopus compressus</i>	Australia	ND	
	BRIP 64636d			Solanum sp.	Australia	ND	
Indo 035	BRIP 58634	VCG 01219		Pisang Raja Serah (AAB)	Indonesia	ND	NA
Uganda 05	BRIP 59170	VCG 01222		Kainja (ABB)	Uganda	ND	
22994	BRIP 22887	VCG 0128		Bluggoe (ABB)	Australia	ND	

24248	BRIP 44611	VCG 0128		Bluggoe (ABB)	Australia	ND	
INDO016	BRIP 59040		Genotype 2	Pisang Ambon Putih (AAA)	Indonesia	ND	37.5
INDO54	BRIP 58653		Genotype 3	Pisang Susu (AAB)	Indonesia	ND	36.6
MAL04	BRIP 59144		Genotype 4	Pisang Awak (AAB)	Malaysia	ND	38.7
MAL67	BRIP 58731		Genotype 5	Musa acuminata subsp. malaccensis	Malaysia	ND	35.0
PHIL01	BRIP 62784		Genotype 7	Latundan (AAB)	Philippines	ND	37.8
PHIL18	BRIP 62789		Genotype 8	Latundan (AAB)	Philippines	28.2	27.1
PHIL24	BRIP 59159		Genotype 9	Latundan (AAB)	Philippines	ND	36.7
PHIL26	BRIP 62793		Genotype 10	Latundan (AAB)	Philippines	ND	38.6
PHIL22	BRIP 62791		Genotype 11	Katali (ABB)	Philippines	ND	35.8
VIET12	BRIP 62978		Genotype 12	Chuo xiem (ABB)	Viet Nam	39.1	35.6
VIET01	BRIP 62970		Genotype 13	Chuo ngop cao (?)	Viet Nam	ND	37.4
VIET07	BRIP 62973		Genotype 14	Chuo xiem (ABB)	Viet Nam	ND	38.7
	BRIP 5188		Race 1	Lycopersicon esculentum	Australia	ND	NA
	BRIP 16809		Race 3	Lycopersicon esculentum	Australia	ND	
Mal 010	BRIP 58683	VCG 01217	Race 1	Pisang Rastali (AAB)	Malaysia	32.0	
Indo 012	BRIP 58619	VCG 01218	Race 1	Pisang Siem (ABB)	Indonesia	33.4	
Thai 37	BRIP 59169	VCG 0123	Race 1	Kluai Namwa (ABB)	Thailand	ND	
Thai 14	BRIP 58794	VCG 0124	Race 1, 2	Kluai Namwa (ABB)	Thailand	35.7	
Brazil 06	BRIP 62953	VCG 0124	Race 1, 2	Bluggoe (ABB)	Brazil	ND	
Thai 07-1	BRIP 58788	VCG 0125	Race 1, 2	Kluai Namwa (ABB)	Thailand	30.9	
India 033	BRIP 62957	VCG 0125	Race 1, 2	Koribontha (ABB)	India	ND	
24426	BRIP 40309	VCG 0129	SR4	Lady finger (AAB)	Australia	ND	
N5331	BRIP 63589	VCG 0129	SR4	Williams (AAA)	Australia	ND	
Indo 014	BRIP 58620	VCG 0120	SR4	Pisang Ambon Putih (AAA)	Indonesia	34.2	
PNG 01	BRIP 59161	VCG 0126	SR4	Cooking banana	Papua New Guinea	ND	

23631	BRIP 44073	VCG 01211	SR4	SH 3142 (AA)	Australia	30.5
24218	BRIP 42102	VCG 01220	SR4	Williams (AAA)	Australia	ND
24219	BRIP 42173	VCG 01220	SR4	Williams (AAA)	Australia	ND
Taiwan 16	BRIP 62969	VCG 0121	TR4	Cavendish (AAA)	Taiwan	UD
09.02.02A.01	BRIP 63220	VCG 0121	TR4	Pisang Buai (AAA)	Indonesia	36.1
Phil 10	BRIP 59154	VCG 0122	TR4	Grande Naine (AAA)	Philippines	27.8
Indo 155	BRIP 62560	VCG 01213	TR4	Grande Naine (AAA)	Indonesia	16.9
001/PAPUA	BRIP 63198	VCG 01213/16	TR4	Pisang Raja (AAB)	Indonesia	18.4
Mal 072	BRIP 58736	VCG 01216	TR4	Grande Nain (AAA)	Malaysia	16.9

* DNA was tested at 200pg/ul. ^ Low Ct values indicate sensitive detection; the closer the Ct values is to 40, the poorer the detection. # Results obtained on another DNA extract from the same isolate (Milestone 3 report). ND: not detected.

Table 5. Assessment of soil samples using the *Foc* TR4 qPCR assay. Results are listed as Cycle threshold (Ct) values. The closer the Ct value is to 40, the lower the concentration of *Foc* TR4 DNA in the sample.

Sample identification	State	Known status	No samples tested	No samples with <i>Foc</i> TR4 detected	Ct Value
BRF		Not infected	6	0	BDL*
CPRS		Not infected	3	0	BDL
CPRS (R1 to R5)		Old <i>Foc</i> TR4 infection	5	1	38.1
CPRS (pit 1 to pit 3)	NT	Old <i>Foc</i> TR4 infection	3	3	30.1 to 37.4
DBF (pineapple 1 to 3)		Old <i>Foc</i> TR4 infection	3	2	35.1 to 36.1
DBF (old rotation site 1 to 3)		Old <i>Foc</i> TR4 infection	3	3	35.1 to 36.6

DBF "A" 1 to "A" 3		Old <i>Foc</i> TR4 infection	3	1	35.320
Pheeney 1 to 3		Old <i>Foc</i> TR4 infection	3	2	34.8 to 38.1
KRS 1 to 3		Not infected	3	0	BDL
Cormack site 1		Not infected	3	0	BDL
Cormack Site 2		Not infected	3	0	BDL
Down hill from infected sites			6	0	BDL
Up hill from infected sites		Recent <i>Foc</i> TR4 infection	5	0	BDL
Infected site. Plant destroyed			1	1	26.8
Grant Dwarf Ducasse <i>Foc</i> R1 Rep A to E	QLD	<i>Foc</i> R1 infection	5	0	BDL
Gaia banana site 1 to 7		Not infected	7	0	BDL^
SJRS		Not infected	6	0	BDL
NSW Property - SR4 patch - 1 to 3		<i>Foc</i> SR4 infection	3	0	BDL
NSW Property - R1 patch	NSW	<i>Foc</i> R1 infection	1	0	BDL

BRF: Berrimah Research Farm; CPRS: Coastal plains Research Station; SJRS: South Johnstone Research Station.

*BDL: Below Detection limit; ^ One sample out of 7 from the Gaia banana site produced a Ct value of 42.7

Appendix 7 – Managing inoculum load in pseudostems, soil and water

1. Determining the effect of urea and lime on *Fusarium oxysporum* f.sp. *cupense* Race 1

Aim: To measure the effect that high rates of urea, lime and the two combined have on the survival of *Fusarium oxysporum* f.sp. *cupense* VCG0124 (*Foc*) in field soil.

Method:

1. Field soil was collected from the Centre for Wet Tropics Agriculture research station, pH measured, dried, sieved to 2mm, and the water holding capacity determined.
2. 50g of dried, sieved soil was placed into a sterile 250ml vented jar.
3. Spore suspensions of three different concentrations were added. High 38,500 conidia/gram of soil, medium 385 conidia/gram of soil, and low 3.85 conidia/gram of soil.
4. Sterile H₂O was added to bring the soil moisture up to 50% water holding capacity.
5. Jars were then incubated for 7 days at 26°C in the dark before the treatments were added.
6. The four treatments were as follows:
 - a. Control x 3 replicates
 - b. Lime @ 5 Tonne/ha x 3 replicates
 - c. Urea @ 5 Tonne/ha x 3 replicates
7. Jars were then incubated in the same conditions for a further 21 days.
8. Pieces of *Foc* susceptible banana pseudostem were harvested, cut to length, surface sterilised in 10% ETOH: 3% NaOCl for 1 minute. Rinsed in sterile water and allowed to dry on sterile blotting paper in the lamina flow cabinet.
9. Each piece was then placed bottom cut side down onto the soil surface within the jar and firmed to ensure contact with the soil. The jar was then resealed and allowed to incubate for a further 7 days.
10. The pseudostem piece was then removed from the jar. Washed to remove any remaining soil, and surface sterilised in 70% ETOH for 1 minute.
11. Four 2mm slices were then taken from the end that had been in contact with the soil. The piece in direct contact was discarded. 35 samples were then cut from the remaining three pieces and plated out onto half strength PDA + Streptomycin sulphate. (5 plates, 7 pieces each)
12. These plates were then assessed for FOC growth at 72 and 96 hours.

Results:

Treatment	Total Positive to FOC	Average No. Positive to FOC
High Control	81	5.4
High Lime	70	4.7
High Urea	0	0.0
High Urea & Lime	0	0.0
Medium Control	51	3.4
Medium Lime	23	1.5
Medium Urea	0	0.0
Medium Urea & Lime	0	0.0
Low Control	77	5.1
Low Lime	65	4.3
Low Urea	0	0.0
Low Urea & Lime	0	0.0

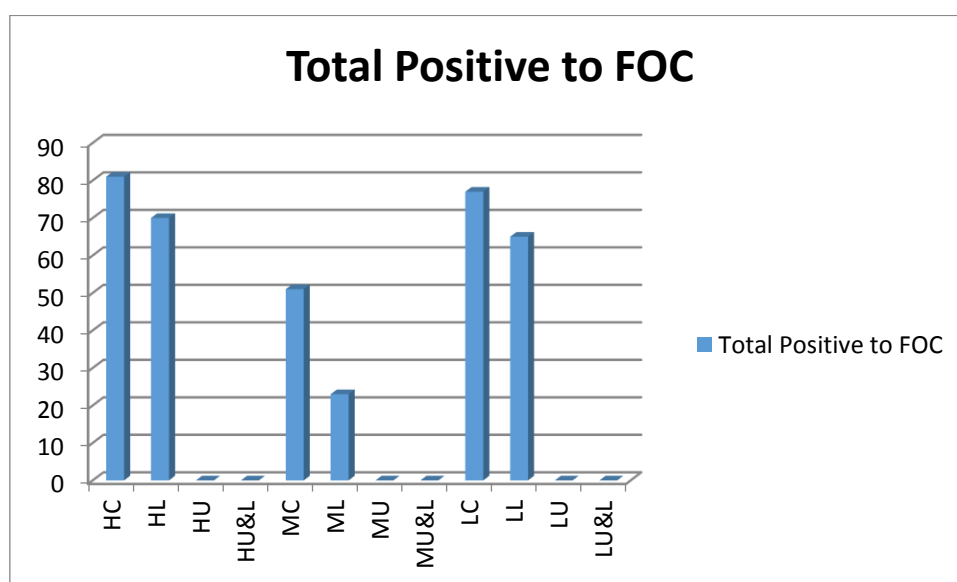


Figure 1. The results showed that regardless of inoculum pressure (high, medium, or low), once urea was added at a rate of 5T/ha *Foc* could not be recovered.

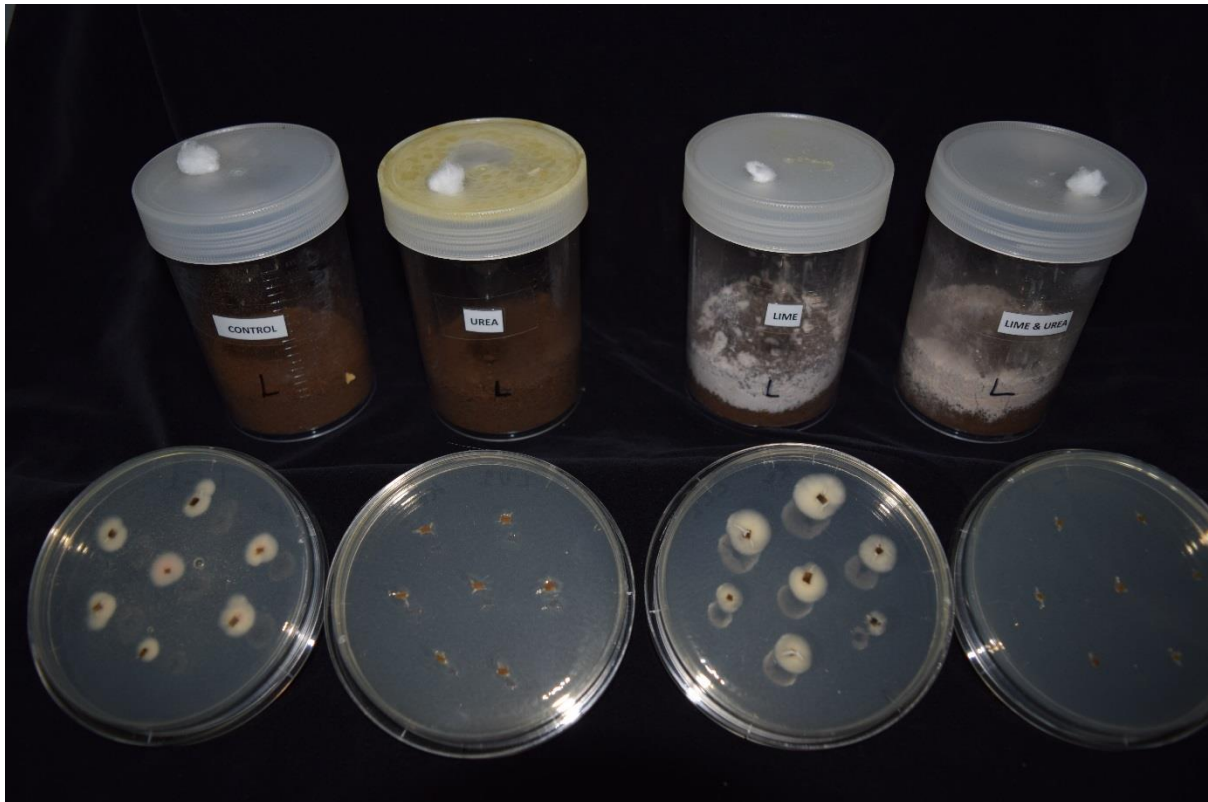


Figure 2. Treatment jars along with corresponding culture plates.

2. Altering nitrogen availability to reduce *Foc* populations in soil

Objective:

To explore the effectiveness of urea treatments at lower rates and investigate chemical alternatives which have been suggested as toxic (either directly or by interrupting the nitrogen metabolic pathway) to *Fusarium oxysporum* f.sp. *ubense* VCG0124 (*Foc*).

Method:

1. Field soil was collected from the Centre for Wet Tropics Agriculture research station, pH measured, dried, sieved to 2mm, and the water holding capacity determined.
2. 50g of dried, sieved soil was placed into a sterile 250ml vented jar.
3. The soil was inoculated using a FOC spore suspension equivalent to 80 conidia/g soil (40,000 conidia/50g soil).
4. Sterile H₂O was added to bring the soil moisture up to 50% water holding capacity.
5. Jars were then incubated for 7 days at 26°C in the dark before the treatments were added.
6. The treatments were as follows:
 - a. Potassium chlorate @ 4%, 8%, 16% (w/v),
 - b. Sodium nitrite @ 200ppm, 400ppm, 800ppm,
 - c. Nitrite @ 200ppm, 400ppm, &
 - d. Urea 0.5kg/m², 0.25kg/m², 0.125kg/m².
7. Jars were then incubated in the same conditions for a further 21 days.
8. Pieces of FOC susceptible banana pseudostem were harvested, cut to length, surface sterilised in 10% ETOH: 3% NaOCl for 1 minute. Rinsed in sterile water and allowed to dry on sterile blotting paper in the lamina flow cabinet.

9. Each piece was then placed bottom cut side down onto the soil surface within the jar and firmed to ensure contact with the soil. The jar was then resealed and allowed to incubate for a further 7 days.
10. The pseudostem piece was then removed from the jar. Washed to remove any remaining soil, and surface sterilised in 70% ETOH for 1 minute.
11. Four 2mm slices were then taken from the end that had been in contact with the soil. The piece in direct contact was discarded. 35 samples were then cut from the remaining three pieces and plated out onto half strength PDA + Streptomycin sulphate. (5 plates, 7 pieces each)
12. These plates were then assessed for FOC growth at 72 and 96 hours.

Results:

Table 1. Recovery of *Foc* from soil treated with different nitrogen forms and nitrogen metabolic inhibitors

Treatment	% FOC Recovery
Potassium Chlorate 16%	100
Potassium Chlorate 8%	100
Potassium Chlorate 4%	97
Sodium Nitrite 800ppm	95
Sodium Nitrite 400ppm	100
Sodium Nitrite 200ppm	96
Nitrite 400ppm	98
Nitrite 200ppm	100
Urea 5t/ha	0
Urea 2.5t/ha	0
Urea 1.25t/ha	0

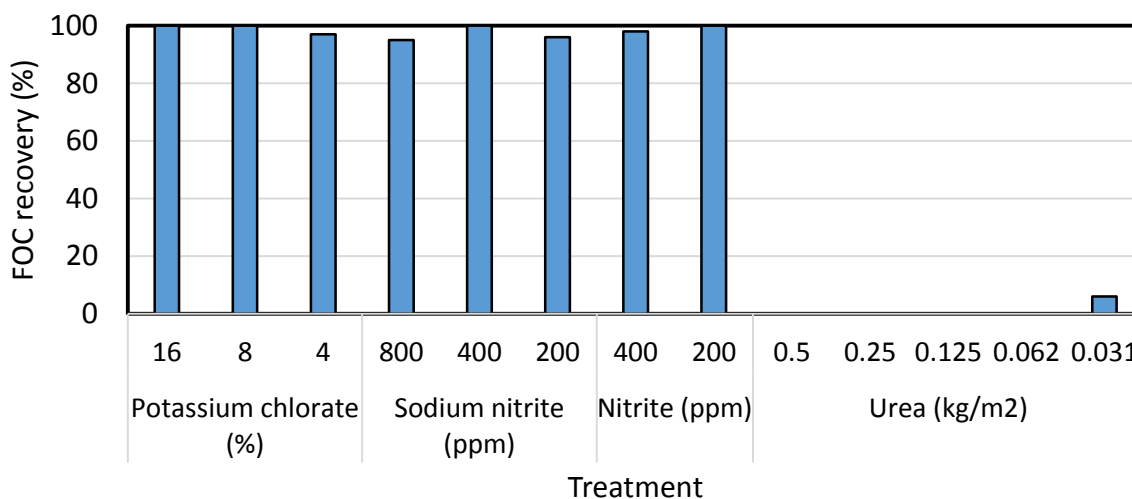


Figure 3. *Foc* recovery from soil treated with different nitrogen forms and nitrogen metabolic inhibitors

Outcome:

Urea at rates greater than 0.031 kg/m² were effective at preventing FOC from being recovered from the soil, whereas nitrite forms and potassium chlorate a nitrogen cycle disruptor were ineffective at reducing *Foc* recovery

3. Efficacy of ammonia against *Foc* R1**Objective:**

To determine the limit of the effectiveness of urea treatments between 0.0625kg/m² and 0.03125kg/m², whilst comparing them to alternative sources of ammonia at equivalent concentrations.

Method:

1. Field soil was collected from the Centre for Wet Tropics Agriculture research station, pH measured, dried, sieved to 2mm, and the water holding capacity determined.
2. 50g of dried, sieved soil was placed into a sterile 250ml vented jar.
3. The soil was inoculated using a *Foc* spore suspension equivalent to 80 conidia/g soil (40,000 conidia/50g soil).
4. Sterile H₂O was added to bring the soil moisture up to 50% water holding capacity.
5. Jars were then incubated for 7 days at 26°C in the dark before the treatments were added.
6. The treatments were as follows:
 - a. Urea
 - b. Ammonium Nitrate + Potassium hydroxide
 - c. Aqueous ammonia.

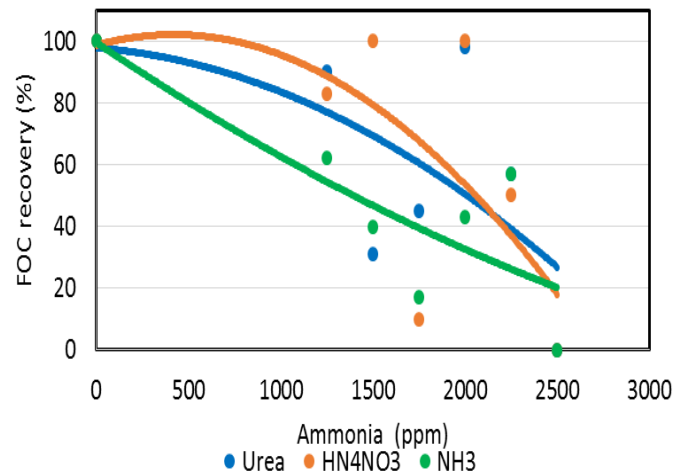
Each of the above treatments were applied at rates equivalent to 2500, 2250, 1750, 1500, 1250 ppm of NH₃

7. Jars were then incubated in the same conditions for a further 21 days.
8. Pieces of FOC susceptible banana pseudostem were harvested, cut to length, surface sterilised in 10% ETOH: 3% NaOCl for 1 minute. Rinsed in sterile water and allowed to dry on sterile blotting paper in the lamina flow cabinet.
9. Each piece was then placed bottom cut side down onto the soil surface within the jar and firmed to ensure contact with the soil. The jar was then resealed and allowed to incubate for a further 7 days.
10. The pseudostem piece was then removed from the jar. Washed to remove any remaining soil, and surface sterilised in 70% ETOH for 1 minute.
11. Four 2mm slices were then taken from the end that had been in contact with the soil. The piece in direct contact with the soil was discarded. 35 samples were then cut from the remaining three pieces and plated out onto half strength PDA + Streptomycin sulphate. (5 plates, 7 pieces each)
12. These plates were then assessed for FOC growth at 72 and 96 hours.

Results:

Table 2. Foc recovery from soil at various ammonium concentrations

Treatment	% Recovery
Urea = NH ₃ 2500ppm	0
Urea = NH ₃ 2250ppm	57
Urea = NH ₃ 2000ppm	98
Urea = NH ₃ 1750ppm	45
Urea = NH ₃ 1500ppm	31
Urea = NH ₃ 1250ppm	90
NH ₄ NO ₃ +KOH = NH ₃ 2500ppm	0
NH ₄ NO ₃ +KOH = NH ₃ 2250ppm	50
NH ₄ NO ₃ +KOH = NH ₃ 2000ppm	100
NH ₄ NO ₃ +KOH = NH ₃ 1750ppm	10
NH ₄ NO ₃ +KOH = NH ₃ 1500ppm	100
NH ₄ NO ₃ +KOH = NH ₃ 1250ppm	83
NH ₃ 2500ppm	0
NH ₃ 2250ppm	57
NH ₃ 2000ppm	43
NH ₃ 1750ppm	17
NH ₃ 1500ppm	40
NH ₃ 1250ppm	62



Outcome:

Ammonia (NH₃) from any source that produced a concentration equal to or above 2500 ppm was effective at preventing FOC from being recovered from the soil.

4. Urea application to reduce *Foc* population in soil

W On'Neill, J Pattemore, K Thompson, A Smyth, L Coates and K Pegg

1. Introduction

Urea, $(\text{NH}_2)_2\text{CO}$, is applied to confirmed Panama disease tropical race 4 (TR4) infestation sites as part of the Biosecurity Queensland destruction protocol. In soil, urea produces ammonia which is then oxidised to nitrite by bacterial metabolism. While ammonia has fungicidal effects, previous experimental work by Sequeira (1963) demonstrated that toxicity was correlated with nitrite accumulation and that nitrite levels of 200 ppm will almost eliminate *Foc* populations in soil under laboratory conditions. Löffler *et al* (1986) demonstrated that for *Fusarium oxysporum* f.sp. *dianthii* urea application prevented chlamydospore formation and enhanced their lysis, resulting in a significant population decline.

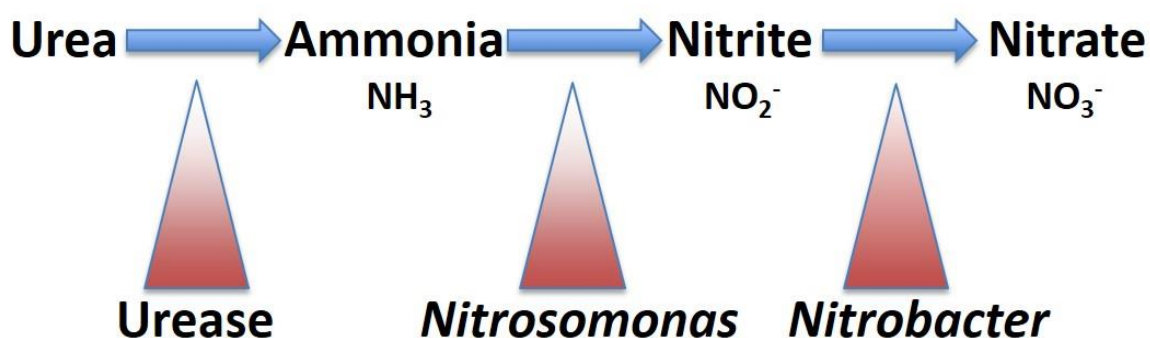


Figure 1 The hydrolysis of urea to ammonia is followed by nitrification

Urea is currently applied at a rate of 1 kg/m^2 to the soil surface, which is based on further work by Sequeira who found that 1.6 kg/m^2 was a very effective fungicide and also demonstrated that rates as low as 170 g/m^2 could have an effective fungicidal effect. This project aimed to assess whether or not appropriate levels of nitrite are attained in soil under field conditions after urea application, the effect of the application on the *Fusarium oxysporum* (F.o.) population in the soil and also to measure disease suppression through the use of bioassay plants. Results from this experiment will provide scientific justification for the current urea application treatment or provide information to modify the current strategy.

Urea in conjunction with a granular formulation of the soil fumigant Basamid (Dazomet, which releases methyl-isothiocyanate during decomposition) was utilised in subtropical race 4 infested soil in southern Queensland (Persley *et al.*, 1989). Basamid provides effective control of nematodes, soil inhabiting insects, soil fungi and germinating seeds of weeds. *Fusarium* species are named on the product label amongst the suite of fungi that Basamid can control. The project also tested the efficacy of Basamid in combination with urea against *Foc* to see if it provides an improved option for the reduction of soil inoculum.

As part of the destruction process, infected plant material is placed in plastic bags, urea is added at a rate of $1\text{kg}/\text{bag}$, and the bagged material is left on site in the destruction zone. An assessment was

also made of the effectiveness of urea for eliminating the pathogen in the bagged vegetative material.

2. Method

Soil treatment and sampling

A Panama disease (caused by *Fusarium oxysporum* f.sp. *cubense*, Foc) infested site was sourced at the race 1 screening block, Duranbah, NSW. The site has Red Krasnozem soil (ferrosol) with a 50% clay content and pH of 5.2. Twenty Dwarf Ducasse (*Musa*, ABB) stools were cut to ground level and assessed for internal symptoms of the disease. All growing points were removed with a gouging implement. A 1 metre X 1 metre plot was marked around each stool using a quadrat. Soil samples were taken from each plot at two depths (0 – 5cm and 5 – 15cm) for pre-treatment analysis. Each sample consisted of twenty bulked sub-samples taken at random locations within the plot which were thoroughly mixed by hand before bagging. Samples were placed in a cool esky in the field and were split for chemical and biological testing upon return to the laboratory. Samples were then frozen (chemical analysis) or refrigerated (biological analysis) until required.

The treatments for the experiment are as follows and were replicated 4 times and laid out in a randomised block design across 3 plant rows:

- 1 Urea 1 kg/m² covered with plastic
 - 2 Urea 1 kg/m², watered in, covered with plastic
 - 3 Urea 1 kg/m² + Basamid 50g/m², watered in, covered with plastic
 - 4 Plastic only
 - 5 Untreated
- } Plus 200g urea in gouged corm

For all of the urea treatments, 1kg of granular urea (Incitec Pivot, 46% nitrogen w/w) was spread evenly on the surface within each plot. As per TR4 destruction protocol, it was not incorporated in the soil. For treatment 3, 50g of the granular soil fumigant Basamid (Dazomet 940g/kg) was also evenly spread within the plots. In addition, 200 grams/plot of urea was distributed amongst the gouged holes in the banana corm for treatments 1, 2 and 3 in accordance with the current destruction strategy. Ten litres of water was then applied to treatments 2 and 3 plots using a watering can and all plots except for treatment 5 were rapidly covered with 1.5 X 1.5 metre squares of heavy duty plastic (200µm black builder's plastic). The plastic was secured at each corner using steel pins. The plastic was briefly removed when the trial was sampled at 6 and 15 days post-treatment, in the same manner as the original sampling (20 sub-samples bulked per plot, 2 depths sampled per plot).

Chemical analysis

Surface and sub-surface samples from each time point for each plot were forwarded to the Department of Science, Information Technology and Innovation Chemistry Centre at the EcoSciences Precinct, Brisbane. Mineral-N was extracted from air dry soil using 2 M KCl (1:10 soil to solution ratio for 1 h at 25°C), followed by automated colorimetric procedures to determine ammonium-nitrogen and nitrite-nitrogen on a SEAL QuAAtro39 Continuous Segmented Flow Analyzer. The ammonium-nitrogen procedure of the automated system is a modification of the method of Searle (1974), which uses the indophenol reaction with salicylate and hypochlorite. The nitrite-nitrogen procedure used was a modification of the nitrate-nitrogen automated colorimetric method of Best (1976) with the hydrazine sulfate replaced with water so that none of the nitrite is reduced to nitrate.

Determination of *Fusarium oxysporum* populations

Sub-soil (5-15cm sampling depth) samples were air dried for 24 hours before grinding to a fine consistency with a mortar and pestle. One gram of the ground soil was added to 9mL of sterile water and the soil suspension was thoroughly shaken before 0.5mL of the solution was evenly spread on a 90mm plate of Komada's medium (Komada, 1976). Distinctly pigmented *Fusarium oxysporum* colonies were counted after 7 days incubation at 25°C. Forty-eight *F. oxysporum* colonies from pre-treatment soil samples were subcultured and analysed to confirm their identity as F.o. (Edel *et al* 2000) and determine whether or not they were Foc (unpublished race 1 assay).

Infected plant material treatment

Pseudostem pieces of the Dwarf Ducasse plants with obvious signs of vascular discolouration were cut into approximately 15cm lengths and placed inside six heavy duty 240 litre plastic garbage bags. One kilogram of urea was added to three of the bags and three were left untreated. The bags were sealed by tying the opening in a knot and were then left in random order in the middle of the trial. Six weeks later, discoloured vascular strands were excised from pseudostem pieces within each bag and plated onto Potato Dextrose Agar amended with streptomycin and Nash-Snyder medium to assess survival of the pathogen.

Statistics

All statistical analysis was performed using Genstat 16 and statistical significance was determined using Fisher's unprotected least significant difference test.

3. Results

All banana plants removed prior to application of treatments had some indication of disease, ranging from discolouration in the corm only to more advanced symptoms including discolouration in the pseudostem and leaf yellowing.

Chemical analysis

Prior to the application of treatments the mean ammonium level across all plots was 53ppm in the surface soil layer (0 - 5cm) and 9ppm in the sub-soil (5 – 15cm). Ammonium levels at 6 and 15 days post-application are given in tables 1 and 2 below:

Table 1. Mean Soil NH₄ Levels (ppm) 6 Days Post Application

Treatment	Surface	Subsurface
Untreated	72 a	51 a
Plastic	77 a	59 a
Urea	4350 c	1334 b
Urea Watered	5565 d	3882 c
Urea+Basamid Watered	2065 b	2275 b

Means with the same subscript are not significantly different at 5% level. P<0.001

Table 2. Mean Soil NH₄ Levels (ppm) 15 Days Post Application

Treatment	Surface	Subsurface
Untreated	49 a	52 a
Plastic	51 a	66 a
Urea	5125 c	2370 b

Urea Watered	3340 b	3062 c
Urea+Basamid Watered	3085 b	2718 bc

Means with the same subscript are not significantly different at 5% level. P<0.001

At 6 days post-application, ammonium levels are highest for the Urea Watered treatment at both the surface and sub-surface depths suggesting that this treatment allowed more rapid conversion of urea to ammonia/ammonium and faster penetration through the soil profile. Ammonium levels for the Urea+Basamid Watered treatment were significantly lower than Urea Watered at both depths, suggesting that the fumigant may have inhibited some of the microorganisms involved in the breakdown of urea. In the Untreated and Plastic treatments, sub-surface ammonium levels had increased somewhat from the pre-treatment mean of 9ppm to 51 and 59ppm respectively, suggesting some sub-surface movement of the ammonia/ammonium from adjacent urea treated plots.

At 15 days post-application, ammonium levels at the surface were highest in the Urea treatment but had declined somewhat for the Urea Watered treatment. The ammonium level was two orders of magnitude higher for the urea treatment compared to the control. At the sub-surface depth, ammonium was increasing in the Urea treatment, but levels remained significantly higher in the Urea Watered treatment.

Nitrite nitrogen only reached detectable levels in a few of the urea treated plots and in general remained below the 2ppm detection threshold.

***Fusarium oxysporum* populations**

Counts of dilution plates determined the mean F.o. population to be 1372 colony forming units per gram of dry soil (cfu/g) prior to the application of treatments. There was minimal variation across the trial site with no significant difference between treatment plots. F.o. population counts at 6 and 15 days post-application are given in tables 1 and 2 below:

Table 3. Mean *Fusarium oxysporum* Colony Forming Units per Gram of Soil 6 Days Post Application

Treatment	Fo cfu/g
Untreated	2485 a
Plastic	1895 b
Urea	115 c
Urea Watered	15 c
Urea+Basamid Watered	55 c

Means with the same subscript are not significantly different at 5% level. P<0.001

Table 4. Mean *Fusarium oxysporum* Colony Forming Units per Gram of Soil 15 Days Post Application

Treatment	Fo cfu/g
Untreated	2485 a
Plastic	2375 a
Urea	35 b
Urea Watered	30 b
Urea+Basamid Watered	25 b

Means with the same subscript are not significantly different at 5% level. P<0.001

At 6 days post treatment the F.o. population in all of the urea treatments has declined markedly. Although the counts for the three urea treatments are not significantly different from each other, the higher ammonium level at this time point produced by watering in the urea may have resulted in

a more thorough reduction in the population. The Plastic (only) treatment resulted in a small but significant reduction in F.o. population compared with control

At 15 days post treatment, the F.o. population in all three urea treatments is at a similar level corresponding with the more even sub-surface ammonium levels at this time point (Table 2). The F.o. population in the Plastic treatment is not significantly different from control at 15 days.

Foc race 1 was determined to comprise approximately 4% of the overall F.o. soil population. This proportion is consistent with previous studies of Foc infected sites in northern New South Wales (O'Neill, unpublished data).

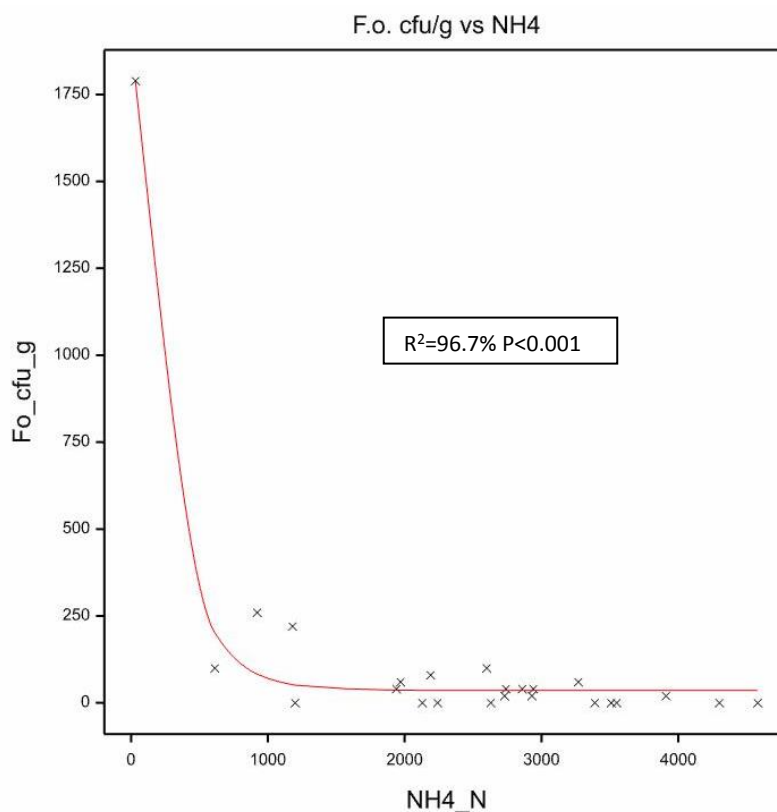
Pathogen recovery from Infected Plant Material Treatment

No *Fusarium oxysporum* was recovered from infected pseudostem tissue that had been bagged with urea. F.o. was recovered from two of the three untreated bags, however the pathogen was only confirmed as surviving in one of the bags.

4. Discussion

When all data points for ammonium concentration from all urea treatments are plotted against their corresponding F.o. cfu count (with mean NH₄ and F.o. value used for untreated plots) it can be seen that the F.o. population rapidly drops as the NH₄ concentration rises in the 0 – 1000 ppm range (Figure 1). By 2000ppm the F.o. population has been virtually eliminated.

Figure 2. Regression of F.o. Colony Forming Units vs NH₄ Concentration.



At 15 days post application the Urea treatment had the highest ammonium level in the surface layer, and all three treatments which included urea had similar levels at the sub-surface, although the Urea Watered treatment was significantly higher. The F.o. population at 15 days was not significantly different between the three urea treatments. The addition of Basamid provided no advantage in terms of reduction in F.o. population. Given the extra expense and WH&S issues associated with use

of such a fumigant product, there would appear to be no justification in adding it to the treatment regime when urea alone is providing equivalent efficacy. Urea is readily available, inexpensive and safe to handle.

The soil type at the Duranbah site is similar to Ferrosols which are commonly used for banana cultivation in north Queensland. Given that soil moisture in north Queensland would normally be higher than those at the commencement of this trial, it would be expected that the urea conversion to ammonium might proceed more rapidly than in this experiment (soil surface moisture around 12% at commencement) and may more closely mimic the Urea Watered treatment.

In this experiment nitrite was not found to have accumulated by the second post treatment sampling at 15 days. Sequira (1963) had recorded peak ammonia levels at 6 days followed by peak nitrite at 13 days after urea incorporation in a container study. The oxidation of ammonia to nitrite and nitrite to nitrate is largely performed by bacteria in the genera *Nitrosomonas* and *Nitrobacter* respectively. As *Nitrobacter* is more sensitive to ammonia than *Nitrosomonas*, nitrite tends to accumulate in the soil during the breakdown process until ammonia levels drop. In this experiment it is possible that the very high levels of ammonia/ammonium achieved were high enough to inhibit *Nitrosomonas* as well as *Nitrobacter*, resulting in minimal conversion to nitrite. Because nitrite is considered useful for longer term suppression of the pathogen, further experimentation would be desirable to see if nitrite accumulates with time using this treatment regime. It would also be desirable to determine the levels of the nitrogen compounds (and pathogen survival) at greater depths than those assessed in this study.

Due to the extremely high rates of urea used for this technique, the strategy is only useful for treating small "hotspots" which are detected early. Nutrient leaching may result in large amounts of nitrogen compounds leaving the treated site and potentially causing environmental issues. The treatment is also only likely to be effective if the pathogen is confined to a small disease focus. If the pathogen is more widespread, it will likely recolonise a treated area from adjacent untreated soil.

The brief investigation of Foc survival in bagged pseudostem pieces showed that the addition of urea inside the bags may be effective at killing the pathogen, although there appeared to be some natural attrition of the fungus in the untreated bags. A more thorough investigation is required to assess the effectiveness of the procedure and document the levels of nitrogen compounds achieved inside the bags.

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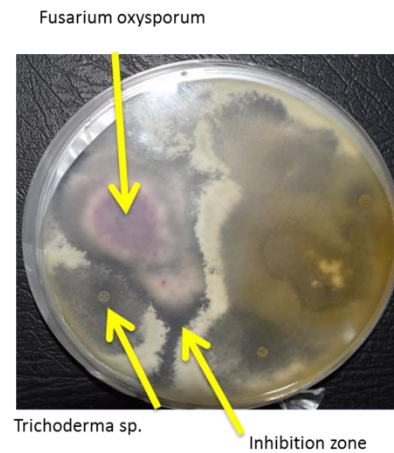
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Dr Angus McElnea and staff at the DSITI Chemistry Centre provided technical advice on chemical analysis of soil samples.



5. Pseudostem Disk Assay for Suppression Testing of *Trichoderma* Isolate 2055 Against *Fusarium oxysporum* f.sp. *ubense* Race 1

Aim: Whilst testing soil treatments for disease suppressive qualities from a fertiliser and groundcover trial on a site previously infected with *Fusarium oxysporum* f.sp. *ubense* Race 1 (*Foc*), a number of *Trichoderma* isolates were recovered. With *Trichoderma*'s success as a bio control agent for various fungal pathogens it was decided to complete a quick dual culture test with one of these isolates to see if there were any signs of FOC suppression. The result of this test can be seen in Figure 1. It was then decided to test this culture further in a bioassay designed to highlight any suppressive potential.

Method:

Figure 1 Dual culture test

1. Single spore cultures of the *Trichoderma* isolate 2055 and *Foc* VCG0124 were initiated on ½ strength Potato Dextrose Agar + Streptomycin sulphate (PDA+S) in 90mm petri plates, and allowed to grow until the surface was filled with sporulating culture.
2. Spore suspensions were then prepared using 10ml of sterile H₂O poured onto the surface of the plate. The conidia were then loosened by gently scraping the surface of the agar using a sterile glass rod. The resulting spore suspension was then collected and quantified using a haemocytometer.
3. Lengths of 40mm diameter pseudostem from *Foc* Race 1 susceptible plants was then collected, surface sterilised by dipping in 10% ETOH: 3% NaOCl for 1 minute. Rinsed in sterile water, allowed to dry on sterile blotting paper in the lamina flow cabinet, and then sliced into lengths of 20mm.
4. Field soil was collected from the Centre for Wet Tropics Agriculture research station, twice sterilised, air dried, sieved to 2mm, and the water holding capacity determined.
5. 50g of soil was then placed into a sterile 250ml vented screw top jar.
6. Sterile H₂O was added to bring the soil moisture up to 50% water holding capacity.
7. Pseudostem disks were then inoculated with the following treatments at a rate of 4×10^4 conidia per treatment per disk;

- i. Control
 - ii. *Foc* VCG0124
 - iii. *Trichoderma* 2055
 - iv. *Foc* VCG0124 + *Trichoderma* 2055
8. Treated pseudostem disks were then placed bottom cut side down onto the soil surface within the sterile jars and incubated in the dark at 26°C for 28 days.
 9. Inoculated disks were then removed and fresh 65mm lengths of surface sterilised (10% ETOH: 3% NaOCl for 1 minute), un-inoculated 40mm diameter pseudostem from an *Foc* Race 1 plant were added. Each piece was placed bottom cut side down onto the soil surface within the jar and firmed to ensure contact with the soil.
 10. The jar was then resealed and allowed to incubate for a further 7 days.
 11. The pseudostem piece was then removed from the jar. Washed to remove any remaining soil, and surface sterilised in 70% ETOH for 1 minute. The pseudostem pieces were then allowed to dry on sterile blotting paper in the lamina flow cabinet
 12. Four 2mm slices were then taken from the end that had been in contact with the soil. The piece in direct contact with the soil was discarded. 35 samples were then cut from the remaining three pieces and plated out onto modified Komada medium. (5 plates, 7 pieces each)
 13. These plates were then assessed for *Foc* growth at 72 and 96 hours.

Results:

Table 1 *Foc* recovery from pseudostem treated with *Trichoderma sp*

Treatment	Number Positive from 84	% Positive
Control	0	0.0
<i>Foc</i> VCG0124	45	53.6
Trich 2055	0	0.0
<i>Foc</i> VCG0124 + Trich 2055	9	10.7

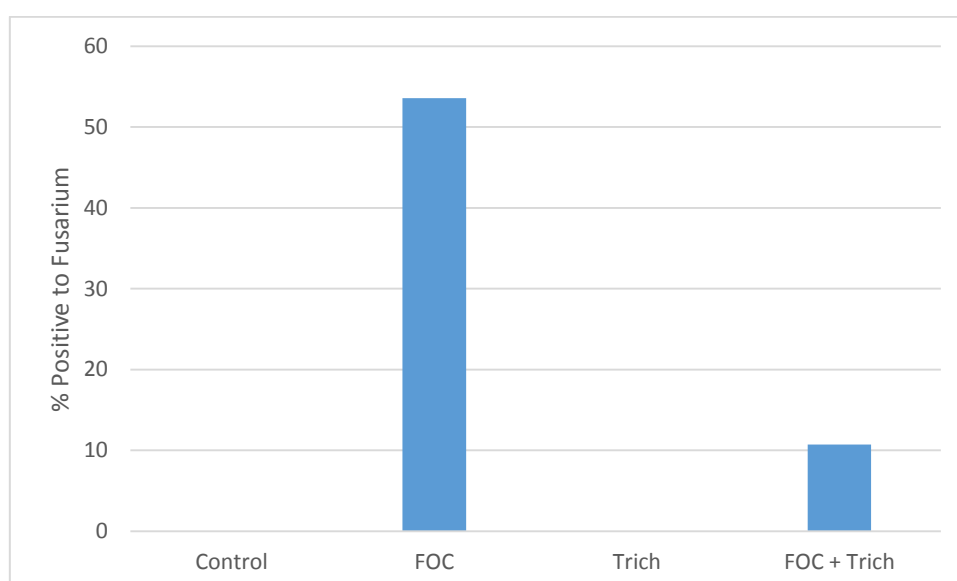


Figure 1. *Foc* recovery from pseudostem treated with *Trichoderma sp*

The amount of *Foc* recovered from the *Foc* + *Trichoderma* treatment was reduced by 42.9% when compared to the recovery percentage of the FOC alone treatment. This result suggests that the *Trichoderma* is having a suppressive effect on the FOC when paired together.

6. *Trichoderma* v's *Foc* Dual Culture Growth Plates

Aim: Whilst testing soil treatments for disease suppressive qualities from a fertiliser and groundcover trial on a site previously infected with *Fusarium oxysporum* f.sp. *cubense* Race 1 (*Foc*), a number of *Trichoderma* isolates were recovered. With *Trichoderma*'s success as a bio control agent for various fungal pathogens it was decided to put these isolates into a dual culture experiment with *Foc* and rate their suppressive/competitive qualities.

Method:

1. Single spore cultures of the 20 *Trichoderma* isolates (antagonist) and *Foc* 0124 (pathogen) were initiated on PDA + Streptomycin sulphate (PDA+S) in 90mm plates, and allowed to grow until the surface was filled with sporulating culture.
2. 6mm discs of each were cut from the cultures and the antagonist and the pathogen were placed 45mm apart on 90mm PDS+S plates in triplicate.
3. The dual culture plates were then incubated at 26°C, under 12 dark - 12 light (near infrared).
4. Growth measurements were then taken twice daily (8:00am and 3:00pm) until the cultures had met and overgrowth was evident. Measurements in mm were taken as follows:
 - i. Growth of pathogen towards the antagonist.
 - ii. Growth of pathogen away from the antagonist.
 - iii. Growth of antagonist towards the pathogen.
 - iv. Growth of antagonist away from the pathogen.
 - v. Pathogen control radius.
 - vi. Antagonist control radius.
 - vii. Width of zone of inhibition if present.
5. Analysis of the results were done using the following equations:
 - i. Percentage inhibition of Pathogen by Antagonist = $(\text{Pathogen control} - \text{Pathogen towards antagonist}) * 10 / \text{Pathogen control}$.
 - ii. Percentage inhibition of Antagonist by Pathogen = $(\text{Antagonist control} - \text{Antagonist towards Pathogen}) * 10 / \text{Antagonist control}$.
 - iii. Difference in Pathogen growth towards and away from Antagonist on Dual Culture
 - iv. Difference in Pathogen growth on Dual Culture and Control
6. Once the growth of the cultures had met and started to interact they were rated using the modified Bell scale from Bell et. al. (1982) as follows:
 - i. Class 1 – Antagonist completely overgrown pathogen. (100% Overgrowth)
 - ii. Class 2 – Antagonist overgrown $\frac{3}{4}$ of pathogen. (75% Overgrowth)
 - iii. Class 3 – Antagonist colonised on half of the growth of the pathogen. (50% Overgrowth)
 - iv. Class 4 – Pathogen and the antagonist locked at the point of contact.
 - v. Class 5 – Pathogen overgrown the antagonist.

Results:

Table 1. *Foc* inhibition by different *Trichoderma sp* isolates in dual culture

Antagonist Isolate	Average Percentage inhibition of FOC by Trich	Average Percentage inhibition of Trich by FOC	Average difference in FOC growth towards and away from Trich on Dual Culture	Average difference in FOC growth on Dual Culture and Control	Total Bell Scale
Trich-001	3.1	5.1	3.8	4.0	6.0
Trich-002	3.5	4.5	4.2	4.5	8.0
Trich-003	3.3	3.0	3.0	4.3	12.0
Trich-004	3.2	4.2	3.0	4.2	11.0
Trich-005	4.9	4.3	5.3	6.3	3.0
Trich-006	3.3	3.7	3.3	4.3	3.0
Trich-007	3.6	3.7	4.7	4.7	4.0
Trich-008	3.6	3.6	4.0	4.7	4.0
Trich-009	3.1	3.2	2.8	4.0	3.0
Trich-010	3.6	4.5	4.3	4.7	9.0
Trich-011	3.1	3.2	3.0	4.0	6.0
Trich-012	4.0	2.8	3.8	5.2	5.0
Trich-013	3.3	2.5	3.0	4.3	5.0
Trich-014	4.2	3.0	4.2	5.5	5.0
Trich-015	4.1	4.2	4.7	5.3	12.0
Trich-2051	4.2	3.5	4.5	5.5	4.0
Trich-2052	4.0	4.3	4.5	5.2	12.0
Trich-2053	2.8	3.8	4.0	3.7	10.0
Trich-2054	4.4	3.2	4.7	5.7	7.0
Trich-2055	3.6	4.5	4.0	4.7	11.0

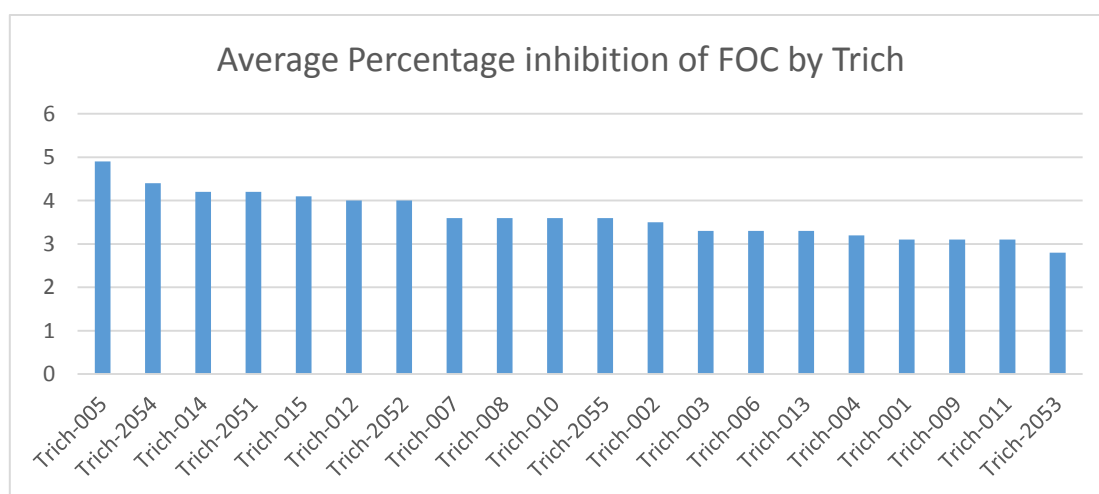


Figure 1. *Foc* inhibition by different *Trichoderma sp* isolates in dual culture

Trich-005 consistently outperformed all other isolates when averaged over all of the measurements. It demonstrated the highest percentage inhibition of the pathogen, the most rapid growth in relation to the pathogen, and was one of only three *Trichoderma* isolates to completely and consistently overgrow the pathogen.

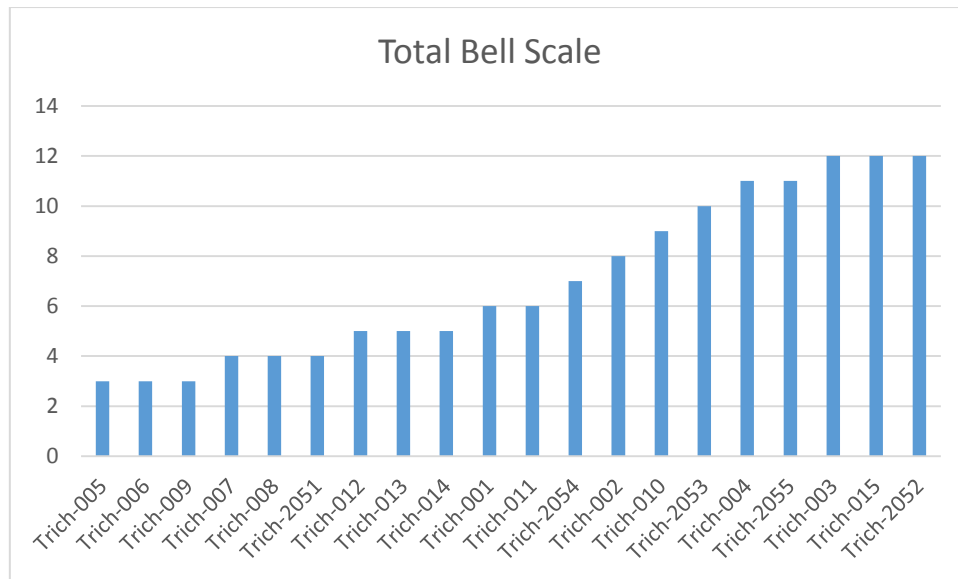


Figure 2. *Foc* inhibition using the Bell Scale by different *Trichoderma sp* isolates in dual culture

From the results shown above it was concluded that further pathogen suppression and control testing should concentrate on the following isolates:

1. Trich-005
2. Trich-007
3. Trich-014
4. Trich-015
5. Trich-2051
6. Trich-2054

7. Pseudostem Disk Assay for Suppression Testing of the Six Best Performing *Trichoderma* Isolates against *Fusarium oxysporum f.sp. cubense Race 1*

Aim: To repeat the previous pseudostem disk assay to test *Trichoderma* for *Fusarium oxysporum f.sp. cubense Race 1 (Foc)* suppression qualities, but include the six best performing isolates from the dual culture screening. The purpose of this is to highlight any outstanding individual isolates.

Method:

1. Single spore cultures of the *Trichoderma* isolate 2055 and *Foc* VCG0124 were initiated on ½ strength Potato Dextrose Agar + Streptomycin sulphate (PDA+S) in 90mm petri plates, and allowed to grow until the surface was filled with sporulating culture.
2. Spore suspensions were then prepared using 10ml of sterile H₂O poured onto the surface of the plate. The conidia were then loosened by gently scraping the surface of the agar using a sterile glass rod. The resulting spore suspension was then collected and quantified using a haemocytometer.
3. Lengths of 40mm diameter pseudostem from *Foc* Race 1 susceptible plants was then collected, surface sterilised by dipping in 10% ETOH: 3% NaOCl for 1 minute. Rinsed in sterile water, allowed to dry on sterile blotting paper in the lamina flow cabinet, and then sliced into lengths of 20mm.
4. Field soil was collected from the Centre for Wet Tropics Agriculture research station, twice sterilised, air dried, sieved to 2mm, and the water holding capacity determined.

5. 50g of soil was then placed into a sterile 250ml vented screw top jar.
6. Sterile H₂O was added to bring the soil moisture up to 50% water holding capacity.
7. Pseudostem disks were then inoculated with the following treatments at a rate of 4×10^4 conidia per treatment per disk;
 - i. Control
 - ii. *Foc* VCG0124
 - iii. Tri-005
 - iv. *Foc* VCG0124 + Tri-005
 - v. Tri-007
 - vi. *Foc* VCG0124 + Tri-007
 - vii. Tri-014
 - viii. *Foc* VCG0124 + Tri-014
 - ix. Tri-015
 - x. *Foc* VCG0124 + Tri-015
 - xi. 2051
 - xii. *Foc* VCG0124 + 2051
 - xiii. 2054
 - xiv. *Foc* VCG0124 + 2054
8. Treated pseudostem disks were then placed bottom cut side down onto the soil surface within the sterile jars and incubated in the dark at 26°C for 28 days.
9. Inoculated disks were then removed and fresh 65mm lengths of surface sterilised (10% ETOH: 3% NaOCl for 1 minute), un-inoculated 40mm diameter pseudostem from an *Foc* Race 1 plant were added. Each piece was placed bottom cut side down onto the soil surface within the jar and firmed to ensure contact with the soil.
10. The jar was then resealed and allowed to incubate for a further 7 days.
11. The pseudostem piece was then removed from the jar. Washed to remove any remaining soil, and surface sterilised in 70% ETOH for 1 minute. The pseudostem pieces were then allowed to dry on sterile blotting paper in the lamina flow cabinet
12. Four 2mm slices were then taken from the end that had been in contact with the soil. The piece in direct contact with the soil was discarded. 35 samples were then cut from the remaining three pieces and plated out onto modified Komada medium. (5 plates, 7 pieces each)
13. These plates were then assessed for *Foc* growth at 72 and 96 hours.

Results:

Table 1. *Foc* recovery from pseudostem discs treated with 6 *Trichoderma sp* isolates

Treatment	Total out of 84	% Positive
Control	0	0
Tri-005	0	0
Tri-005 + <i>Foc</i>	0	0
Tri-007	0	0
Tri-007 + <i>Foc</i>	0	0
Tri-014	0	0
Tri-014 + <i>Foc</i>	0	0
Tri-015	0	0
Tri-015 + <i>Foc</i>	26	31.0
2051	0	0
2051 + <i>Foc</i>	37	44.0
2054	0	0
2054 + <i>Foc</i>	33	39.3

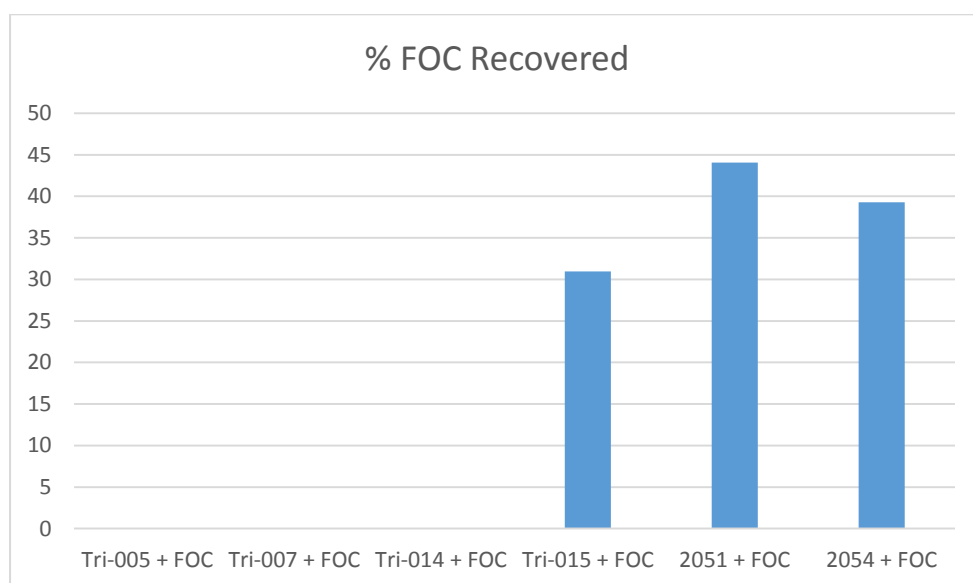


Figure 1. *Foc* recovery from pseudostem discs treated with 6 *Trichoderma sp* isolates

The treatments Tri-005 + *Foc*, Tri-007 + *Foc*, and Tri-014 + *Foc* had zero recovery of *Foc*, whilst the remaining three treatments between 31% and 44%. From these results it is clear that Tri-005, Tri-007, and Tri-014 are the isolates that should be included in future trials.

8. Effects of *Trichoderma virens* (Tri-005) on *Fusarium oxysporum f.sp cubense* Race 1 Conidia and Chlamydospore Production within the Pseudostem in-vitro

Aim: To determine the effect of *Trichoderma virens* (Tri-005) on *Fusarium oxysporum f.sp cubense* Race 1 (*Foc*) total conidia and chlamydospore production when grown on susceptible pseudostem under controlled conditions.

Method:

1. Pieces of *Foc* susceptible banana pseudostem were harvested, cut to length, sliced lengthways, and then surface sterilised in 10% ETOH: 3% NaOCl for 1 minute. Pieces were then removed, rinsed in sterile water and allowed to dry on sterile blotting paper in the lamina flow cabinet.
2. Once dry pseudostem lengths were placed into sterile plastic containers with a clip lock lid cut side up.
3. Single spore cultures of the *Trichoderma virens* (Tri-005) and *Foc*VCG0124 were initiated on ½ strength Potato Dextrose Agar + Streptomycin sulphate (PDA+S) in 90mm petri plates, and allowed to grow until the surface was filled with sporulating culture.
4. The following treatments were applied by placing 6mm agar plugs cut from the sporulating cultures onto the cut surface of the pseudostem;
 - I. Uninoculated control
 - II. *Foc*VCG0124
 - III. *Trichoderma virens* (Tri-005)
 - IV. *Foc*VCG0124 + *Trichoderma virens* (Tri-005) (plugs placed at opposing ends)
5. The containers were then sealed, and incubated in the dark at 26°C for 8 weeks.
6. Pseudostem pieces were then removed from the container and homogenised.
7. The homogenised pseudostem was then strained through eight layers of cheesecloth.
8. Counts were then conducted for total conidia, and total chlamydospores using a haemocytometer.

Results:

Table 1. Chlamydospore production by *Foc* R1 grow alone or in combination with *Trichoderma virens* (Tri-005)

Treatment	Total Conidia/mL	Chlamydospores/mL
Control	174800	2000
Tri-005	330800	0
<i>Foc</i> -R1	561600	34800
<i>Foc</i> -R1 v's Tri-005	369200	10200

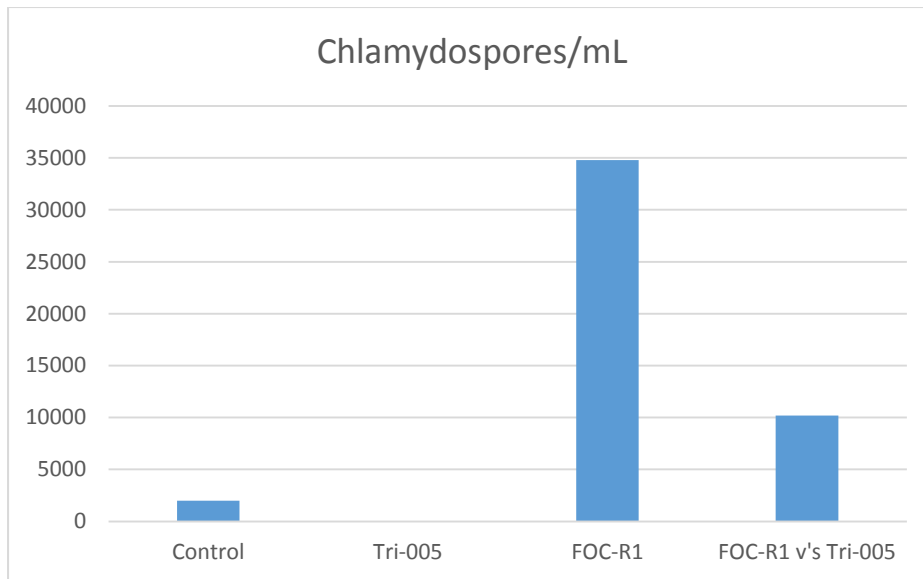


Figure 1. Chlamyospore production by *Foc* R1 grow alone or in combination with *Trichoderma virens* (Tri-005)

The results from this experiment show that when *Foc* is grown together with *Trichoderma virens* (Tri-005) on pseudostem under controlled laboratory conditions, it produces only one third of the quantity of chlamyospores than if it were grown alone. This suggests that Tri-005 has a potential use for reducing the proliferation of *Foc* within the pseudostem of an infected banana plant.

Appendix 8 - Identification of weed and ground cover species that play a role in hosting Panama disease

Introduction

Panama disease caused by the soil-borne fungus *Fusarium oxysporum* f.sp. *cabense* (*Foc*), is considered to be one of the most devastating and destructive plant diseases in the world, (Hennessey, Walduck, Daly, & Padovan, 2005). It affects almost all banana cultivars and the pathogen is highly complex in terms of races and strains (Hermanto et al, 2009). A particularly virulent strain of *Foc* known as Tropical Race 4 (TR4) has been identified in many banana growing regions throughout the world, including Australia. With more than 90% of banana production taking place in North Queensland and Cavendish (cv. Williams) being the most common variety, Panama disease TR4 poses a serious threat to the future of the Australian banana industry. Tropical Race 4 was detected on a commercial banana farm in the Northern Territory in 1997. Since then, the disease had spread to other banana farms throughout the Northern Territory, and in March 2015 TR4 was detected on a commercial banana farm in Tully, North Queensland. Following the first detection in North Queensland, the disease has been detected on a second commercial banana farm in the Tully Valley, (July 2017).

The soil-borne fungus is known to infect various species other than banana, and it can survive and persist for years until new host plants become available, (Beckman & Roberts, 1995). Waite and Dunlap (1953) first reported *Foc* infection of four non-banana hosts growing in an infected banana farm in Central America. Pittaway, Nasir and Pegg (1999), also reported the infection of *Foc* STR4 in two different weed species in Australia. Furthermore, Hennessey et al. (2005) identified four weed species that were infected with TR4 in the Northern Territory.

The identification of weed and ground cover species that allow *Foc* to survive and persist in banana farms is important for; inoculum control, identifying species that may assist in suppressing the pathogen, reducing the spread of the pathogen, and long-term management of Panama disease.

1. On-farm survey of common weed and groundcover species

Aim: Surveys of North Queensland banana farms were conducted to determine the most common weed and groundcover species co-habiting banana farms.

Method: Five commercial banana farms and the South Johnstone DAF Research Facility were surveyed, (Figure 1). Discussions were held with each grower to establish the most common species co-habiting their banana farm. Transect surveys were conducted on each farm in blocks that had not been recently slashed or treated with herbicide. The bed and interrow were surveyed for dominant weed and ground cover species. Photos were taken and species that were unable to be identified in the field were uprooted, cleaned of loose soil, sealed in two plastic bags and placed in a styrofoam box for further identification in the laboratory. Unidentifiable species were sent to the Queensland Herbarium for formal identification.



Figure 1. On-farm survey locations of north Queensland banana farms

Results

The small representative surveys determined the five most common weed and groundcover species co-habiting each farm, (Table 1). The surveys indicate that there are a number of species commonly found growing in North Queensland banana farms. Table one provides a complete list of the five most common weed and ground cover species found on each property, and below is a list of the six most common species found across all properties.

- Sourgrass (*Paspalum conjugatum*) - 5/6 farms
- Crowsfoot Grass (*Eleusine indica*) - 5/6 farms
- Mullumbimby couch (*Cyperus brevifolius*) - 4/6 farms
- Cinderella Weed (*Synedrella nodiflora*) – 4/6 farms
- Broadleaf Carpet Grass (*Axonopus compressus*) – 3/6 farms
- Pennywort (*Centella asiatica*) – 3/6 farms

Table 1. Common weed and ground cover species identified on North Queensland banana farms

Sample site	Common name	Scientific name
Farm 1 (South Johnstone)	Mullumbimby Couch	<i>Cyperus brevifolius</i>
	Sourgrass	<i>Paspalum conjugatum</i>
	Crowsfoot Grass	<i>Eleusine indica</i>
	Pennywort	<i>Centella asiatica</i>
	Broadleaf Carpet Grass	<i>Axonopus compressus</i>
Farm 2 (Tully)	Crowsfoot Grass	<i>Eleusine indica</i>
	Cinderella Weed	<i>Synedrella nodiflora</i>
	Praxelis	<i>Praxelis clematidea</i>
	Blackberry Nightshade	<i>Solanum americanum</i>
	Sedges	<i>Cyperus spp.</i>
Farm 3 (South Johnstone)	Sourgrass	<i>Paspalum conjugatum</i>
	Crowsfoot Grass	<i>Eleusine indica</i>
	Nut Grass	<i>Cyperus rotundus</i>
	Cinderella Weed	<i>Synedrella nodiflora</i>
	Summer Grass	<i>Digitaria ciliaris</i>
Farm 4 (Boogan)	Mullumbimby Couch	<i>Cyperus breuifolius</i>
	Sourgrass	<i>Paspalum conjugatum</i>
	Broadleaf Carpet Grass	<i>Axonopus compressus</i>
	Cinderella Weed	<i>Synedrella nodiflora</i>
	Green Panic	<i>Panicum maximum var. trichoglume</i>

Farm 5 (Tully)	Mullumbimby couch	<i>Cyperus brevifolius</i>
	Sourgrass	<i>Paspalum conjugatum</i>
	Crowsfoot Grass	<i>Eleusine indica</i>
	Cinderella Weed	<i>Synedrella nodiflora</i>
	Pennywort	<i>Centella asiatica</i>
DAF South Johnstone Research Station	Sourgrass	<i>Paspalum conjugatum</i>
	Crowsfoot Grass	<i>Eleusine indica</i>
	Pennywort	<i>Centella asiatica</i>
	Broadleaf Carpet Grass	<i>Axonopus compressus</i>
	Mullumbimby Couch	<i>Cyperus brevifolius</i>
	Common Sensitive Plant	<i>Mimosa pudica</i>
	Pinto Peanut	<i>Arachis pintoii</i>
	Nut Grass	<i>Cyperus rotundus</i>

2. Identification of weed and ground cover species as alternative hosts for Race 1

Aim: Field surveys of North Queensland banana farms were conducted to identify alternative hosts of *Foc* Race 1. Race 1 was used as a surrogate for TR4, as access onto TR4 infected properties in Queensland is restricted.

Method: Weed and ground cover species were collected from three banana farms growing susceptible varieties and known to be infected with Race 1, (Figure 2). Initial discussion was held with the property owner to gain information about the most suitable blocks which were likely to contain the highest relative inoculum load. Blocks that had been recently slashed or treated with herbicide were not selected for sampling. Initially, weed and groundcover species were collected from the interrow, bed and in close proximity to the pseudostem. However, due to little or no recovery of *Foc* Race 1, it was decided that samples would only be collected from the bed and growing in close proximity to banana plants that appeared to be showing external symptoms of Race 1 infection. For each species approximately three replicates were collected from different locations within each block, however, numbers of species collected varied depending on their abundance at the time of sampling. Each sample was uprooted, cleaned of loose soil, sealed in two plastic bags and placed in a Styrofoam box before being transported to the South Johnstone DAF laboratory.

In the laboratory, the roots were washed under running water to dislodge any excess soil for 30 minutes. Eight root pieces from each plant were then surface sterilised in 1% sodium hypochlorite solution for 1 minute and then rinsed twice in sterile distilled water for 30 seconds. Root pieces were then dried in sterile blotting paper and cut into smaller 1 cm segments. Root segments were placed into Petri plates containing a *Fusarium* selective media, and incubated at 25°C for 3-5 days to allow for fungal growth. Positive *Fusarium* recoveries were developed as pure cultures. The recovered populations were not able to be characterised at the South Johnstone DAF laboratory, therefore single spore cultures were sent to the Biosecurity Queensland *Foc* diagnostics team at the Eco-Sciences Precinct in Brisbane for formal identification. Cultures were identified by undertaking microscopic examination, and then polymerase chain reaction (PCR) tests were conducted to

determine if isolates were *Fusarium oxysporum*. Positive *Fusarium oxysporum* isolates underwent VCG testing to determine if the isolate is *Foc* Race 1 (VCG 0124). Elongation Factor sequencing was conducted on all of the isolates to allow characterisation to a species complex level.



Figure 2. Weed, grass and ground cover sample locations on north Queensland banana farms

Results: The first batch of samples sent to ESP consisted of 22 single spore isolates collected from farm 1. Nine isolates were identified as *Fusarium oxysporum*, however, no isolates tested positive to Race 1.

The second batch of samples collected from farm 2 were sent as whole plants (11 different species plus replicates). Twenty-one isolates were identified as *Fusarium oxysporum*, with 1 isolate testing positive to Race 1, (VCG 0124, BRIP 64652A).

The third batch of samples consisted of 68 single spore isolates collected from farm 3 and 22 isolates from farms 1 and 2. Of the 68 isolates from farm 3, 17 isolates tested positive to *Fusarium oxysporum*, and 4 isolates tested positive to Race 1, (VCG 0124, BRIP 64850, 64851, 64852, 64853). Of the 22 isolates from farms 1 and 2, 4 isolates tested positive to *Fusarium oxysporum* and no isolates tested positive to *Foc* Race 1.

The roots from 20 different species (115 samples in total) collected from three banana farms were analysed for the presence of the pathogen as an endophyte, (Table 2). Although these species were actively growing in commercial banana farms infected with *Foc* Race 1, none of these species had shown external symptoms similar to those apparent in banana plants infected with Panama disease. Race 1 was isolated from four different species commonly found co-habiting North Queensland banana farms. These species include, *Cleome aculeata* (2 isolates), *Youngia japonica* (1 isolate), *Eleusine indica* (1 isolate), and *Digitaria ciliaris* (1 isolate), (Table 2 and Figure 3). A study undertaken by Hennessey et al. (2005) shows that the chances of isolating *Foc* is reduced when a small number of samples per species is collected. This suggests that further investigation is required as a higher number of species and replicates sampled will increase the chance of isolating *Foc*. However, a range of factors may have an impact on recovering the pathogen and need to be taken into consideration, for example; inoculum load, root mass, proximity of weeds to infected host plants, whether the farm has an active weed management regime put in place and so on.

Table 2. Recovery of *Fusarium oxysporum f.sp cubense* Race 1 from plants collected from Race 1 infested banana farms

	Plant species	Number plants collected	Number isolates positive <i>Fo</i>	Numbers positive <i>Foc</i> R1	Percent positive	VCG Group
Farm 1	Crowsfoot Grass	4	0	0		
	Sourgrass	2	0	0		
	Mullumbimby Couch	3	0	0		
	<i>Phyllanthus sulcatus</i>	6	1	0		
	Pinto peanut	3	0	0		
	Awnless Barnyard Grass	3	5	0		
	Pennywort	3	1	0		
	Broadleaf Carpet Grass	4	2	0		
	Nut Grass	3	0	0		
	Spiny Spider Flower	3	0	0		
	Summer Grass	2	0	0		
	Green Amaranth	2	0	0		
	Cinderella Weed	3	0	0		
	Blue top (<i>Praxelis</i> sp)	6	0	0		
Farm 2	Cobbler's Pegs	4	1	0		
	Emilia	3	2	0		
	<i>Phyllanthus sulcatus</i>	4	3	0		
	Pennywort	4	3	0		
	Summer Grass	6	3	0		
	Green Amaranth	2	4	0		
	Spiny Spider Flower	3	2	1	33%	VCG 0124
	Broadleaf Carpet Grass	3	1	0		
	Blue top (<i>Praxelis</i> sp)	3	1	0		
	Cinderella Weed	3	1	0		
Farm 3	Nutgrass	3	1	0		
	Cinderella weed	3	0	0		
	Blue top (<i>Ageratum</i> sp)	3	1	0		
	Guinea grass	3	0	0		
	Cobbler's pegs	3	1	0		

Vernonia	3	2	0		
<i>Youngia japonica</i>	3	0	1	33%	VCG 0124
Summer grass	3	2	1	33%	VCG 0124
<i>Phyllanthus sulcatus</i>	3	1	0		
Crowsfoot grass	3	10	1	33%	VCG 0124
Spiny Spider Flower	3	0	1	33%	VCG 0124
Total	20 different species	115	48	5	

Table 3. Diagnostic summary of weed and groundcover species collected from Race 1 infected banana farms in North Queensland

Species	<i>Fusarium oxysporum f.sp cubense Race 1</i>	<i>Fusarium oxysporum species complex</i>	Other <i>Fusarium species complex</i>
Spiny Spider Flower <i>Cleome aculeate</i>	✓	✓	x
<i>Youngia japonica</i>	✓	✓	x
Summer Grass <i>Digitaria ciliaris</i>	✓	✓	✓
Crowsfoot Grass <i>Eleusine indica</i>	✓	✓	✓
<i>Phyllanthus sulcatus</i>	x	✓	x
Pinto Peanut <i>Arachis pintoi</i>	x	x	✓
Awnless Barnyard Grass <i>Echinochloa colona</i>	x	✓	✓
Pennywort <i>Centella asiatica</i>	x	✓	✓
Cobbler's Pegs <i>Bidens pilosa</i>	x	✓	x
Emilia <i>Emilia sonchifolia</i>	x	✓	x
Green Amaranth <i>Amaranthus viridis</i>	x	✓	x
Broadleaf Carpet Grass <i>Axonopus compressus</i>	x	✓	✓
Praxelis <i>Praxelis clematidea</i>	x	✓	x
Cinderella Weed <i>Synedrella nodiflora</i>	x	✓	x
Nutgrass <i>Cyperus rotundus</i>	x	✓	x
Sourgrass	x	x	✓

<i>Paspalum conjugatum</i>			
Vernonia <i>Cyanthillium cinereum</i>	x	✓	x
Blue Top <i>Ageratum conyzoides</i>	x	x	x
Guinea Grass <i>Panicum maximum</i>	x	x	x
Mullumbimby Couch <i>Cyperus brevifolius</i>	x	x	x



Figure 3. Species identified as potential hosts of *Fusarium oxysporum f.sp. cubense* Race 1 (VCG 0124). *Cleome aculeata* 'Spiny Spider Flower' (top left), *Eleusine indica* 'Crowsfoot Grass' (top right), *Youngia japonica* (bottom left), *Digitaria*

3. Glasshouse inoculation trial using *Foc* Subtropical Race 4

Aim: A glasshouse inoculation trial was conducted to determine survival of *Foc* Subtropical Race 4. STR4 (VCG 0120) was used as a surrogate for TR4, as experimentation on TR4 is prohibited in Queensland outside of a QC3 laboratory.

Method: Surveys of North Queensland banana farms were conducted to determine the most common weed and ground cover species co-habiting banana farms. Eighteen species were identified as being the most common species, or regarded as high risk due to their presence on TR4 infested farms in the Northern Territory and North Queensland. The species were collected from sites in North Queensland with no known history of banana production or Panama disease. Each species was potted in potting mix and raised in the South Johnstone DAF glasshouse. Plants were removed from pots (soil removed), wrapped in moist paper towel, placed in sealed plastic bags and transported bare root to the Eco-Sciences Precinct (ESP) in Brisbane. The species were re-potted and raised in glasshouses at ESP, and later transported to Redlands Research Station for inoculation.

Ten replicates of each species were inoculated with STR4 colonised millet, and another two replicates were treated with uncolonised millet and used as uninoculated controls. Ten Cavendish (cv. Williams) banana plants were inoculated with colonised millet and treated as positive disease controls. Another ten plants were treated with uncolonised millet and used as negative controls. The inoculation trial was set up using a randomised block design. To avoid contamination of the pathogen from inoculated plants through water splash, the uninoculated plants were randomised in a separate block.

Root isolations were conducted 3 months post-inoculation when the inoculated banana controls displayed early external symptoms of STR4 infection. Plants were removed from pots and excess soil on the roots was dislodged by washing them under running water for 30 minutes. Ten root pieces from each plant were surface sterilised in 1% sodium hypochlorite solution for 1 minute and then rinsed twice in sterile distilled water for 30 seconds. Root pieces were dried in sterile blotting paper and cut into smaller 1cm segments, which were placed onto 90-mm Petri plates (5 pieces per plate) containing Nash-Snyder medium (NS), and incubated at 25°C for 3-5 days to allow fungal growth.

After inoculation, *Fusarium* cultures growing from the root pieces were examined and putative *Fusarium oxysporum* isolates were subcultured onto PDA for microscopic identification. *Fusarium oxysporum* isolates recovered from alternative host plants were tested using SIX8b Polymerase chain reaction (PCR) to determine if they were positive or negative for STR4. Following molecular testing, confirmatory testing was conducted using vegetative compatibility group (VCG) testing.

Results: The positive Cavendish (cv. Williams) banana control plants all had internal discolouration typical of early stage Panama disease symptoms. Subtropical Race 4 was consistently isolated from the discoloured tissue of the control plants. The negative Cavendish (cv. Williams) banana controls displayed no disease symptoms and the pathogen was not recovered from any of the negative controls.

Subtropical Race 4 was isolated from more than one replicate for all of the species included in the experiment, except for Pinto Peanut where there was only 6 surviving replicates by the end of the experiment, and STR4 was only recovered from 1 replicate, (Table 4). Recovery of the pathogen was found to be more consistent from some species than others. For example, the pathogen was recovered from 80% of Mullumbimby Couch replicates, whereas the pathogen was only recovered from 20% Green Amaranth replicates, (Table 4). Although the experiment showed that all of the species collected from North Queensland had the potential to host STR4, the differences in the frequency of isolation may suggest that some species more readily host the pathogen than others. However, factors such as differences in root mass between species (e.g. a dense root mass) as well as maturity of species may affect the recovery rate of the pathogen.

	1	2	3	4	5	6	7	8	9	10	Percent isolated (%)
Pinto Peanut <i>Arachis pintoii</i>											16.7
Green Amaranth <i>Amaranthus viridis</i>											20.0
Pennywort <i>Centella asiatica</i>											20.0
Praxelis <i>Praxelis clematidea</i>											20.0

Signal Grass <i>Brachiaria decumbens</i>											22.2
Cinderella Weed <i>Synedrella nodiflora</i>											33.3
Blue Top <i>Ageratum conyzoides</i>											37.5
Crowsfoot Grass <i>Eleusine indica</i>											40.0
Rhodes Grass <i>Chloris gayana var. Callide</i>											40.0
Spiny Spider Flower <i>Cleome aculeata</i>											40.0
Tridax Daisy <i>Tridax procumbens</i>											40.0
Broadleaf Carpet Grass <i>Axonopus compressus</i>											50.0
Guinea Grass <i>Panicum maximum</i>											50.0
Nut Grass <i>Cyperus rotundus</i>											50.0
Sourgrass <i>Paspalum conjugatum</i>											50.0
Summer Grass <i>Digitaria ciliaris</i>											50.0
Humidicola Grass <i>Brachiaria humidicola</i>											60.0
Mullumbimby Couch <i>Cyperus brevifolius</i>											80.0

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Appendix 9 – Review of global banana breeding programs and recommendations for future directions

Banana Plant Improvement for the Australian Banana Industry – The Way Forward

Jeff Daniells

1. The Issues

The potential for new banana varieties with desirable pest and disease resistance traits has generated much interest among banana growers and marketers with the outbreak of Panama disease TR4 in the midst of the north Queensland industry in March 2015 (Figure 1). While resistant genetics hold the promise of effective control of Panama disease there are many other exotic and endemic pests and diseases, as well as production and marketing attributes that need to be considered in the evaluation and selection of new varieties.

The Australian banana industry has invested in the importation and screening of international selections and hybrids in the pursuit of improved pest and disease and production characteristics. The rigorous quarantine procedures required to protect the Australian industry from exotic pests and diseases means that the importation and screening process is relatively slow and expensive. As such it is important that a clear strategy for the selection and importation of new varieties exists to ensure the most efficient use of the R&D investment.

The development of new banana varieties is a slow and costly process and issues of infertility have made it difficult to breed from some commercially important varieties like Cavendish. This has resulted in the application of unconventional breeding techniques such as somaclonal selection and mutagenesis. The need to recoup some of the investment in breeding these varieties will almost certainly mean that future use of some varieties will be restricted and licenced with royalties due to the variety owner.



Figure 1. TR4 affected Cavendish in Tully

But Panama disease TR4 is not the only major problem facing the banana industry. As serious as Panama disease TR4 might be, in reality it is just one of many issues faced by the industry. The current impact and implications of the other issues may actually outweigh Panama disease TR4. In particular the impacts of overproduction on profitability have been enormous in recent times. So too, having to throw out so much (15-30%) oversized fruit does little to enhance profitability. In some cases varieties may also be the solutions to these problems – varieties with a greater proportion of the bunch meeting market specifications and those with IP allowing supply control would be greatly advantageous. Add to these problems the threats of exotic pest and diseases, potential bans on pesticides and aerial spraying, fruit imports and increasing demands and competition in the marketplace all call for industry innovation. It will be advantageous if solutions provided to Panama disease TR4 are resilient when confronted with new challenges. We must look

beyond Panama disease TR4. Furthermore varieties are only part of the solution for any of these problems. In this discussion paper international banana plant improvement programs are reviewed in the context of the applicability and availability of progeny for evaluation in Australia and prospects for partnering with them.

2. The Australian Scene

The Australian banana industry produces about 372,000 tonnes of fruit/year (ABGC, 2016) with a farm gate value of A\$600 Million, with almost all production going to the domestic market. Cavendish bananas (Williams, Mons Mari, Grande Naine) represent about 95% of this production. A further 4% is Lady Finger whilst the remainder is mostly made up of Ducasse, Sucrier, Goldfinger and Pacific Plantain. Bananas are sold as a commodity product with relatively limited product differentiation in the marketplace. Despite efforts to diversify the varieties grown, the market situation has changed very little in the last 25 years. This is for various reasons detailed in the final report by Daniells et al. 2011 ('New and alternative banana varieties designed to increase market growth' HAL Project BA09041), not least of them being the simplicity of having just the one variety to manage in what is a relatively complex supply chain which makes possible very successful mass marketing and the maintenance of such a status quo by various components of the supply chain.

About 95% of Australia's banana production is in north Queensland (wet tropical coast, Atherton Tablelands and Lakeland). The remainder comes mostly from northern NSW plus southern Queensland (Bundaberg), Carnarvon (WA) and the NT. There are no fresh banana imports to Australia because of quarantine issues.

3. The potential role of varieties in managing the Panama disease TR4 incursion

Initially the primary response is the application of biosecurity measures to minimise/prevent the spread of disease inoculum. Early disease detection combined with plant destruction and soil treatment that minimises disease spores that remain and improved soil erosion control in the industry are crucial components. If the disease should spread and affect the whole industry 'Williams' Cavendish would no longer be able to be grown. With currently available disease tolerant Cavendish selections grown for fewer ratoons we could expect yield (t/ha/yr) reductions of the order of 50% with massive impacts on costs of production and profitability. Experience with Panama disease generally has been that resistant varieties are only adopted as a last resort when the industry runs out of clean ground to move to. When that point is reached integrated crop management (ICM) needs to be ready to roll out. The ICM package requires a genetic resistance component with at least the resistance level of 'Formosana' (GCTCV 218) required (Figure 2). The improvement program needs to combine this resistance with plant, yield and fruit characteristics superior to what we currently have available amongst resistant Cavendish selections.

Whilst we focus on seeking resistant varieties and their potential to allow a relatively smooth transition to a new, relatively unchanged production system we must not allow ourselves as an industry to become complacent about the ongoing importance of biosecurity. The biosecurity measures employed as a result of Panama disease TR4 have potential benefits apart from its exclusion (<http://www.promusa.org/blogpost468-FocTR4-as-a-driver-of-agroecological-approaches-in-banana-production>).



Figure 2. Formosana (GCTCV 218) – A TR4 tolerant Cavendish selection from Taiwan

Regarding the subtropical industry, despite the ‘tropical’ label given to Panama disease TR4 this will not grant immunity for the subtropics based on current world distribution of the pathogen (Figure 3). Panama disease TR4 has yet to be detected in our subtropical industries so biosecurity is a must. However, the bigger problem remains of competition in the marketplace with north Queensland fruit. Product differentiation can be a solution to this problem and alternative varieties are seen as one means of doing this. Successful sustainable production of a new variety in the subtropics will require either supply control (e.g. exclusive variety like Little Gem or owning a brand such as ‘Eco-Banana’) and/or the ability to take maximum advantage of proximity to market (e.g. food miles minimised, freshness of product, full flavour development with more mature fruit etc.). The more vertically integrated a business is the better. Geographical diversification has been seen by the industry to be advantageous in the context of continued market supply following major cyclone damage in the wet tropics. So continuing to foster the sustainability of the subtropical industries, by the industry as a whole, is desirable.

The successful containment of Panama disease TR4 in Australia may open up export opportunities (primarily niche) if we have a supply capability for specialty varieties that may be in short supply elsewhere due to Panama disease TR4. Any new varieties (Panama disease TR4 resistant or otherwise) for which we can access IP would further improve our export and domestic prospects. Thus we should not be immediately dismissive of the prospects of such varieties found to be susceptible to Panama disease TR4 in our screening trials. Indeed IP is a very desirable feature to promote the prospects of a new variety but is of value and limited to only those licenced to do so. This would be a new world of sorts to the banana industry but is a scenario that is likely to play out.

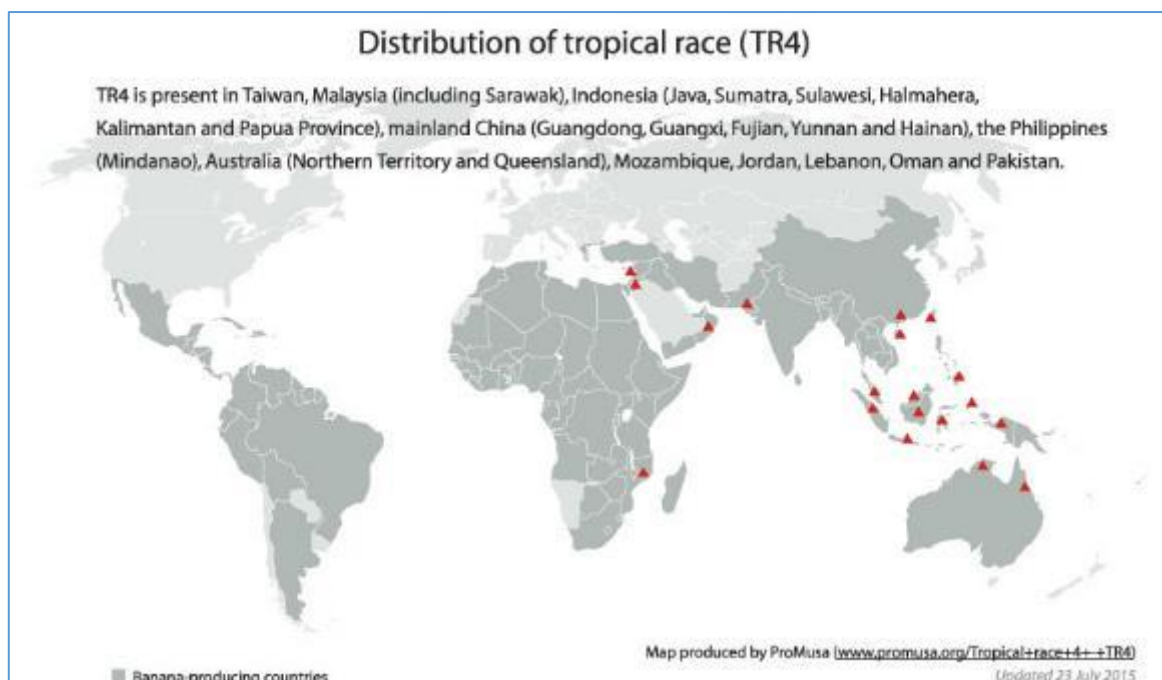


Figure 3. World distribution of TR4 in July 2015. It's called tropical race 4 but is not restricted to the tropics

3.1 Issues of commercialisation and roll out of new varieties

Unlike some other horticultural industries the banana industry has little experience with the commercialisation of new varieties. Currently the banana market in Australia is almost exclusively supplied with fruit of publicly available varieties over which no licencing or intellectual property is held. This will not necessarily be the case for future variety development and commercialisation. The long term investment required in developing new varieties means that new varieties will most likely be licenced and managed to maximise the return on the investment in breeding as has occurred in other crops like apples and mangoes. This will present a new scenario for most Australian banana producers and envisages a future where access to new varieties with desirable pest and disease resistance traits will not necessarily occur for all growers who want them.

An important component for the commercialisation of any new varieties that are controlled by DAF or the banana industry is to develop a strategy for the commercialisation of a new variety. The absence of such a strategy has contributed to a lack of action for some promising varieties like High Noon which has some Panama disease R1/SR4 resistance and has rated highly in consumer testing (Figure 4).



Figure 4. High Noon from FHIA was identified in HAL Project BA09041 as a variety with possible prospects to expand the banana market. It may be susceptible to TR4 but it's IP is available for managing supply

4. The Plant Improvement Programs

Current banana breeding methodologies fall into 3 broad categories:

- Conventional cross-breeding techniques – fertile male and female lines are crossed to produce seedling progeny which are often tetraploid in nature but manipulation can also produce triploid offspring.
- Somaclonal selection – uses natural or induced mutation in existing cultivars to produce progeny with desirable attributes such as pest or disease resistance, improved plant stature and increased yield.
- Genetic modification – involves the manipulation of the banana DNA by inserting identified genes for desirable characteristics from bananas or other organisms. Newer techniques include the manipulation of the plant's own genome without the introduction of any external genetic material.

According to Bioversity International (<http://www.promusa.org/blogpost363-Who-s-breeding-bananas>) in 2013 there were 14 active banana cross-breeding programs globally: in Brazil, Cameroon, China (2), Cote d'Ivoire, Guadeloupe, Honduras, India (3), Indonesia, Nigeria, Tanzania, Uganda and the USA. Of these there are really only 3 programs (Brazil, Guadeloupe and Honduras) with direct relevance to Australia in terms of products and their availability for importation to Australia. The African programs are focussing on cooking bananas - plantains and East African Highland Bananas, whilst India is not about to make available their germplasm and Indonesia and USA are quite insignificant.

Ortiz and Swennen (2014) indicate there are 6 banana genetic modification endeavours; Australia, Belgium, India, Kenya, Malaysia and Uganda. However, others would appear to exist including China.

There are more than 10 active somaclonal and/or mutagenesis programs globally (Stephan Nielen, pers. comm. 2017). These include Taiwan, China, Brazil (EMBRAPA), Austria (IAEA), South Africa (Stellenbosch Univ.), UK (Univ. of Leicester), Mauritius (FAREI) and our own Queensland DAF program.

Table 1. Summary of breeding methodologies used by the main global banana breeding programs

Country	Conventional cross pollination	Somaclonal selection	Genetic modification
		Natural/Induced	
Honduras – FHIA	X		
Guadeloupe – CIRAD	X		
Brazil – EMBRAPA	X	X	
Taiwan – TBRI		X	
China	X	X	X
Philippines		X	
Malaysia		X	X
Indonesia	X		
Australia		X	X
India	X		X
Belgium			X
Austria - IAEA		X	
South Africa - Stellenbosch		X	
UK (Univ. Leicester)		X	

4.1 Honduras - FHIA

Inception and breeding methodologies

The United Fruit Company, which became Chiquita, had a brief breeding program in Panama in the 1920s but this program was cancelled with the onset of the Great Depression and the germplasm was transferred to Tela in Honduras in 1930. The breeding program was resurrected at La Lima in Honduras in the late 1950s. Due to the long time frames involved and the lack of any commercially suitable hybrids produced after 25 years the program was donated to FHIA (Honduran Foundation for Agricultural Research) in 1984. In an effort to make the required progress major emphasis had been placed on the development of diploid parents which combined multiple disease resistance, dwarfism, yield and fruit quality. This greatly enhanced the attributes of tetraploid progeny derived from crosses of these diploids with triploid female parents such as Highgate, Prata (dwarf Lady Finger), plantains and so forth. Goldfinger (FHIA-01/SH-3481) is one such Prata hybrid developed about 30 years ago (Figure 5).



Figure 5. FHIA hybrid Goldfinger is resistant to TR4

Program objectives

At inception the primary focus of the program was to breed Panama disease Race 1 resistant replacements for Gros Michel. With the replacement of Gros Michel by Cavendish, and the incursion of black Sigatoka into Central America, the focus of the program moved more to breed for improved leaf disease and nematode resistance.

Typically the hybrids have resistance to Sigatoka leaf diseases, Panama disease Race 1 and burrowing nematodes and grow surprisingly well under many circumstances with minimal fertiliser application. Additionally they often have cold tolerance and Panama disease TR4 resistance, both of which have been obtained without seeking such characteristics intentionally. The strong foundation laid by the diploid breeding continues to set the program apart from all other conventional breeding programs.

When FHIA took charge and funding became available from international donor agencies the breeding emphasis broadened and largely shifted to producing disease resistant hybrids that could be used by subsistence farmers in developing countries.

In more recent times the current plant breeder, Juan Fernando Aguilar, has made major progress in Cavendish breeding (Aguilar Moran, 2013). Cavendish had generally been considered as sterile so the semi-dwarf Gros Michel type, Highgate, had been used instead in crosses. By making a large number of Cavendish pollinations they were able to develop tetraploid Cavendish females for recurrent breeding which allowed the creation of second generation Cavendish triploid hybrids (Figure 7). These investigations were begun in 2003 and also included the development of beta carotene rich bananas, and disease resistant higher yielding forms of Sugar/Silk, Lady Finger, Gros Michel and Sucrier (Figure 8). This work was funded by Chiquita and so they



Figure 6. FHIA-18 is resistant to TR4

held the IP on the hybrids developed. In 2011 Chiquita had 6-7 Cavendish hybrid selections undergoing major field evaluations in Honduras. When I visited Honduras at that time the agreement FHIA had with Chiquita prevented my access to see this material. About all we know is that the selections were resistant to black Sigatoka and had the taste of Cavendish but the fruit is very different in shape. We have no information on their Panama disease TR4 resistance.

Accessibility for research/commercial activities

The issue of access to FHIA hybrids is determined by the funding of the breeding efforts. About 4 years ago DAF was licenced by FHIA to manage the screening and commercialisation of a range of their earlier hybrids including High Noon (SH-3640.10) and FHIA-18 (SH-3480, Bananza) (Figure 6). The rights to these in Australia had previously been held by ANFIC who were no longer progressing any banana activities. The outputs of more recent activities have been funded directly by Chiquita and more recently a consortium of banana entities including an Australian banana production and marketing company. Accessing genetic material from these later FHIA activities is highly unlikely as the IP is held completely by the funders who have invested seeking sole control of any resulting progeny. Also we have seen the acquisition of Chiquita by two Brazilian groups in 2014 followed in 2015 by the demise of the banana research division of Chiquita. In 2015 the CEO of Chiquita, Mr. Andrew Biles and the Director of Production, Wagner Beig were made aware by the outgoing Director of Research Ronald Romero of DAF's interest in evaluating the Chiquita financed hybrids for resistance to Panama disease TR4 in Australia. I contacted them in 2015 by email but did not receive a reply.

Given that investment in a conventional breeding program is very expensive, can take several years and comes with no guarantee of delivering a hybrid suitable for export-style commercial production the preferred option remains to access any freely available hybrids that should come along and evaluate them. Thus keep a watching brief. Maybe some international aid monies may become

available to FHIA due to the worsening Panama disease TR4 crisis and any hybrids developed might be accessible.



Figure 7. FHIA triploid Cavendish hybrid – about as close as we could get are these photos presented by FHIA breeder, Juan Fernando Aguilar



Figure 8. High yielding Sucrier hybrid developed by FHIA

4.2 Guadeloupe - CIRAD

Inception and breeding methodologies

Since the inception of modern day conventional banana breeding in the 1920s in Trinidad, the basic approach had been to cross wild species or fertile diploid cultivars (male parent) onto existing popular triploid cultivars (female parent) to produce hybrids which hopefully combined disease resistance with acceptable agronomic and organoleptic qualities. However, this had a number of drawbacks including inefficient production of hybrids, the poor agronomic, organoleptic and postharvest qualities of the end product tetraploids and most importantly it precluded recurrent selection.

Stover and Buddenhagen (1986) published a radical paper on banana breeding where they suggested resynthesizing new triploids by first doubling the chromosome number of suitable diploids, using the chemical colchicine, followed by crossing with other diploids. Since successful breeding is a numbers game the beauty of this approach was it meant that highly fertile diploid parents could be used which ensured the production of many progeny for assessment and the incorporation of disease resistance genes from several sources to promote the durability of the disease resistance. This approach formed the basis of the CIRAD (French Agricultural Research Centre for International Development) program which commenced breeding in the late 1980s.



Figure 9. CIRAD 01 from the French program in Guadeloupe is in quarantine

Program objectives

The main emphasis of the breeding program was to develop hybrids with Sigatoka leaf disease resistance to reduce the need for fungicide application. Within a few years several interesting disease resistant hybrids had been developed. CIRAD needed to know their reaction to Panama disease SR4 so the hybrids were sent to Australia in the late 1990s.

During the field evaluations in Australia some unusual symptoms were confirmed to be due to Banana Streak Virus (BSV) despite the strict quarantine processes for importation and testing. What was eventually discovered, for the first time in science, was that the BSV strain present was actually integrated into the DNA of the hybrids and was activated by stresses including the tissue culturing process. Fortunately it was found that not all parents used in the breeding program were carriers of this latent form of BSV so CIRAD refocused its activities to the ‘clean’ parents.

Subsequently a group of BSV free hybrids were developed with the best of these, CIRAD 01, being advanced to 6 multi-locational grower evaluations in Guadeloupe and nearby Martinique. However, the French West Indies growers are not fully satisfied with CIRAD 01. They desire a hybrid less prone to wind damage and have concerns about the smaller bunches which increases costs of production/tonne of fruit produced. Additionally there are issues with extreme fruit sensitivity to mechanical damage which were presented at the ISHS-ProMusa symposium in Montpellier in October 2016. Nevertheless cooperating growers see that it has potential, with one Guadeloupe grower increasing his planting to more than 5ha, having received positive support from the local market regarding smaller fruit size and its overall taste. Also CIRAD 01 can more readily be grown without pesticide/fungicide application. CIRAD 01 is included in the 6 hybrids Queensland DAF received from CIRAD which will be evaluated in a range of Australian environments. Major emphasis is now being placed at several stages in the breeding program on the development of dwarf selections.

Accessibility for research/commercial activities

Queensland DAF has received 6 CIRAD hybrids to be evaluated for pest and disease resistance and agronomic attributes as part of the new plant protection program. Collaboration between Queensland DAF and CIRAD should continue to be fostered for the synergies it brings to the R&D efforts and the associated benefits to the respective banana industries. Indeed collaboration with CIRAD should be extended to cover-cropping practices and the development of integrated systemic resistance/systemically acquired resistance for control of Panama disease and nematodes.



Figure 10. CIRAD 02, as with most French selections is taller than Williams

4.3 Brazil - EMBRAPA

Inception and breeding methodologies

Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA translated= Brazilian Agricultural Research Corporation) is a state-owned research corporation affiliated with the Brazilian Ministry of Agriculture. The banana breeding program is situated at Cruz das Almas, about 150 km by road inland from Salvador de Bahia and began in 1982.

Program objectives

The EMBRAPA program primarily has had the target of producing disease resistant (Panama disease Race 1 and Sigatoka) forms of the popular Lady Finger (Prata) and Sugar/Silk (Maca) types. The majority of their hybrids are Pome (AAB, Lady Finger) tetraploid hybrids and are typically resistant to yellow Sigatoka and Panama disease Race 1.

Despite breeding bananas for 33 years, there has been limited uptake of hybrids from their program. At the ISHS-ProMusa symposium in October 2016 EMBRAPA announced they had just entered into an association with Dole, with emphasis placed on breeding Cavendish type bananas. However, there seems to be some doubt over whether there is such an association. As the work on Cavendish has only recently been commenced it will be several years before we might have any idea of how successful this will be.

Accessibility for research/commercial activities

As part of BA10020 Banana Plant Protection Program a MOU was sought with EMBRAPA which included access to banana germplasm/hybrids for evaluation in Australia. The process began nearly 4 years ago but has not yet been signed off due to complicated approval processes in Brazil.

Potentially the involvement of Dole in the EMBRAPA program is likely to make access to varieties for testing more difficult than the existing situation.

The majority of hybrids potentially available from EMBRAPA are Pome (AAB, Lady Finger) hybrids. We have evaluated several of these in Australia in the past including PC 12.05; PV 03.44; PA 03.22 and subsequently JV 42.41; PA 12.03; PV 42.53; PV 42.81 and PV 42.320. I have described some of the hybrids as tasting quite good but none have matched the taste of Lady Finger. Most are tall like Lady Finger (PA 03.22 and PA 12.03 are intermediate height) but unlike Lady Finger several were very prone to pseudostem snapping (2-3 m above ground) in trials at South Johnstone (Figure 11). All are much lower yielding than equivalent hybrids from the FHIA program.



Figure 11. Several Lady Finger hybrids from EMBRAPA are very prone to pseudostem breakage – snapping clean off under moderate winds whilst the Australian industry standard – Lady Finger sustained no damage

Approaches have also been made for additional non-hybrid selections from Brazil included with hybrids that would be received from EMBRAPA. These included:

- 2 Panama disease tolerant Prata selections including 'Catarina' from EPAGRI to be coordinated through Marcio Sonogo
- IAC 2001, a yellow Sigatoka tolerant Cavendish (Figure 12)
- 2 high yielding Dwarf Lady Finger (Prata Ana) with pendulous bunches (one ex Sitio Barreiras, Ponto Novo and the other ex-Mossoro) (Figure 13). They have no particular disease resistance but pendulous bunches can mean significantly increased marketable packout.

Disappointingly after all this time Edson Amorim indicated to me in October 2016 that these additional selections could not be coordinated by EMBRAPA.



Figure 12. IAC 2001 is a Cavendish selection with Sigatoka tolerance which has been sought from Brazil



Figure 13. Prata with pendulous bunch sought from Brazil

4.4 Taiwan - TBRI

Inception and breeding methodologies

Taiwan began exporting bananas to Japan in the early 1900s, exports peaking in the mid-late 1960s at about 400,000 t/year. However, from 1970 the export industry declined rapidly due primarily to competition from Philippines fruit and to a lesser extent by the occurrence of Panama disease affecting Cavendish which had been first observed in 1967. It wasn't until 1990 that Panama disease TR4 was first identified in Taiwan. However, it is considered to have been there very much longer based on the preceding disease situation in the Cavendish export industry but because both Panama disease SR4 and TR4 are present in Taiwan there is uncertainty regarding strain identification for the period before VCGs became available. In 2013 export production was only 7,000 t. Production for the Taiwanese domestic market is about 300,000 t annually (<http://www.fao.org/faostat/en/#home>). Despite Panama disease TR4 being widespread in Taiwan, the very susceptible Cavendish cultivar Pei Chiao still accounts for about 50% of production. This is possible because of the annual cropping system employed for bananas followed by following with vegetables and rice.

Interestingly in their pursuit of a solution to the Panama disease problem they eventually opted for genetic resistance in Cavendish via somaclonal variation. This was after investigation of:

- Field management practices - field sanitation, soil fumigation and liming which did not give successful control, and rotation with paddy rice which was effective for only 1-2 years
- Importation of 22 Cavendish and related cultivars, none of which showed resistance
- Contemplation of conventional breeding but considered very difficult to come up with a Cavendish replacement.

So in 1985 the Taiwan Banana Research Institute (TBRI) began disease screening of somaclonal variants of Pei Chiao (Giant Cavendish). More than 10 resistant clones were identified by 1988. These have been further selected over time for improved agronomic/fruit quality attributes.

Program objectives

The TBRI program has focused solely on selecting for resistance to Panama disease TR4 in Cavendish varieties.

Accessibility for research/commercial activities

Some of the original resistant clones such as GCTCV 119 and GCTCV 215 (GCTCV = Giant Cavendish Tissue Culture Variant) had very poor agronomic characteristics – small bunches, slow cycling, weak pseudostems and high mat disorder (Figure 14). However, later selections have greatly improved in these respects. Nevertheless, for the selections we have evaluated there is typically a trade-off in some plant or fruit characteristic which reduces their competitiveness with Williams in the absence of Panama disease TR4. Notable amongst these is slower plant cycling, susceptibility to stresses and higher incidence of off-types. We currently have 3 new Taiwanese selections in quarantine including Tai Chiao #5, the most popular Panama disease TR4 resistant selection in Taiwan (Figure 15).



Figure 14. GCTCV 119 – one of the initial TR4 tolerant selections from Taiwan



Figure 15. Tai Chiao #5 selection from Taiwan is about to be released from quarantine

In a world under threat from Panama disease TR4 it seems so far that it is only the Cavendish selections from Taiwan which come close to the specifications required for export-style production. To date also Taiwan has demonstrated its desire to work with other countries by supplying several of its tolerant selections. We already have 8 selections from TBRI in Australia but the 5 we have so far evaluated are not without issues in yield, agronomic features and postharvest qualities. Other selections that could be considered for importation include superior selections of Formosana (GCTCV 218) and GCTCV 105. However, in recent months access to their selections has undergone a major paradigm shift. TBRI are now seeking large sums of money (how many \$?) from potential recipients of any further germplasm to help keep their research institute afloat.

Yield – Bunch Weight/Year

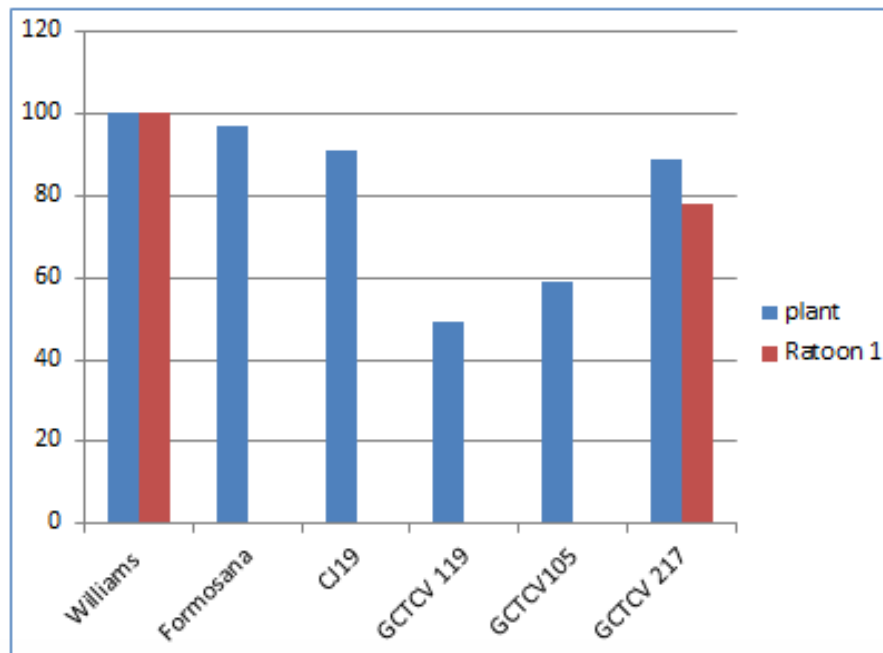


Figure 16. Typical yield trade-off with TR4 resistance. Ratoons tend to decline more in yield compared to Williams

4.5 China Groups

Inception and breeding methodologies

There are various groups involved including Guangdong Academy of Agricultural Sciences (GDAAS, Y. Ganjun), Chinese Academy of Tropical Agricultural Sciences (CATAS, Dr J. Li) and the Academy of Agricultural Sciences in Yunnan and Guangxi which have been active in the past 20 years since Panama disease TR4's first detection in China. Dr Andre Drenth, QAAFI has been endeavouring to source Panama disease TR4 resistant selections, including Guijiao No. 5, from the Guangxi academy.

A range of non-conventional breeding strategies are being employed including chemical-induced (EMS) mutagenesis, genetic engineering and the selection of somaclonal variants resulting from the tissue culturing process and recurrent selection of these following further tissue culture propagation (Chen et al., 2011; Chen et al., 2013) So far it would appear that Panama disease TR4 resistant selections have been derived from the latter strategy.

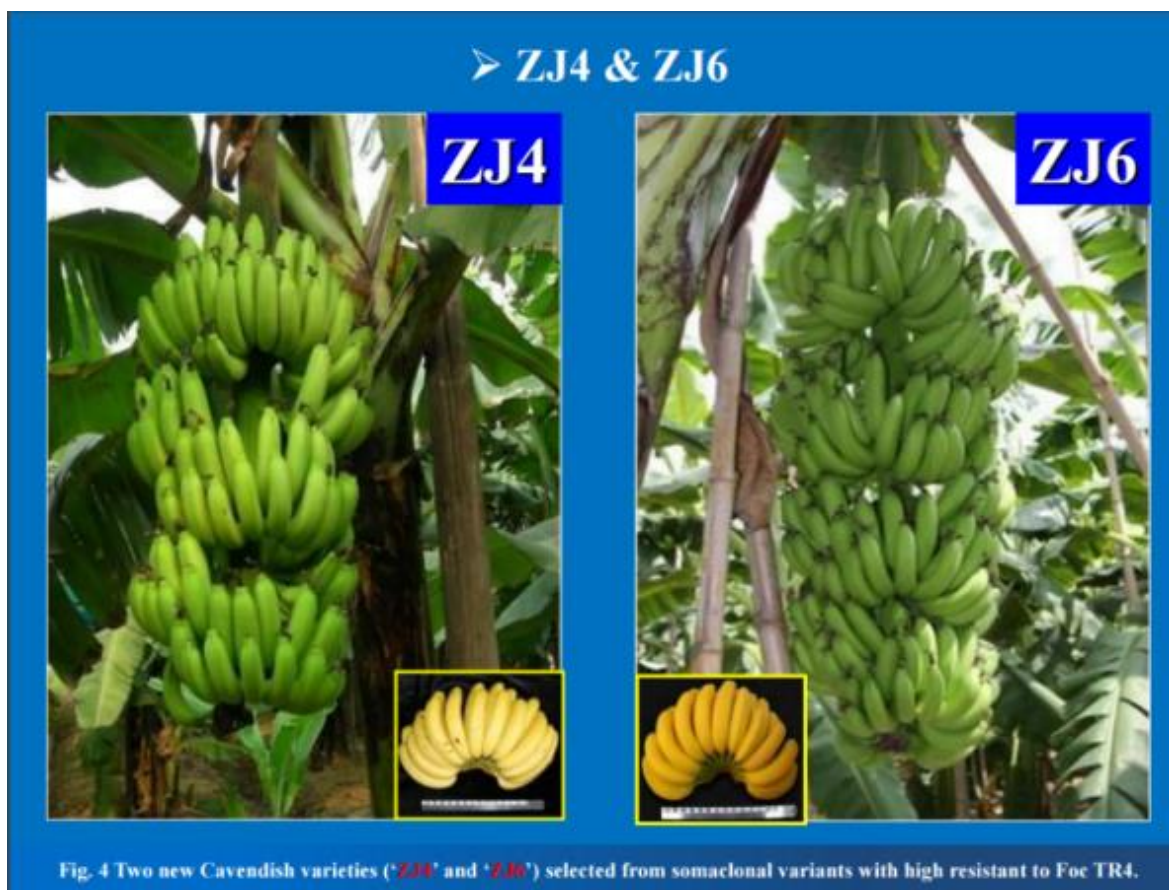


Figure 17. ZJ4 and ZJ6 – TR4 resistant Cavendish selections from China

Program objectives

The plant improvement efforts in China are concentrated on developing Panama disease TR4 resistant Cavendish and to a much lesser extent Panama disease TR4 and Race 1 resistant Ducasse.

Accessibility for research/commercial activities

On the visit to mainland China by Naomi King and Stewart Lindsay in 2012 the following selections were recommended for importation – Cavendish selections Zhongjiao No 3 (ZJ 3), Yuefeng No 1, and the Ducasse selection Zhongfen No 1. However, it seems there has not been much progress made in importing them and more relationship building would appear to be needed and in the context of a collaborative project with mutual benefits including the exchange of germplasm. More recently Stewart Lindsay returned with a brochure from BAPNET indicating 2 other Cavendish selections ZJ 4 and ZJ6 were recommended (Figure 17). This is supported by a PowerPoint presentation by Dr Yi Ganjun, 'Strategy for combating Fusarium wilt in bananas through conventional breeding, GMO's and cultivation technology.' Both ZJ4 and ZJ6 are resistant and compared very favourably on other characteristics with the susceptible industry standard Baxi (Giant Cavendish). While China would appear to have some germplasm that is worth evaluating in Australia there is little demonstration that they are about to part with it and have expressed publicly at BAPNET that they are seeking to maximise the possible IP associated with new varieties.

4.6 The Philippines

Since the arrival of Panama disease TR4 in the early 2000s the main plant improvement approach has been screening germplasm for resistance and further selection of any superior variants within that germplasm. Discussions going back over 4 years ago with Dr Gus Molina indicated that GCTCV 219 (selected out of GCTCV 119 in the Philippines) and a Dwarf Ducasse selection are tolerant to Panama disease TR4 (Figure 18). Despite assuring us that these resistant selections would be made available to us via Bioversity International (and the International Transit Centre – ITC) it would appear, that like China, they are in no hurry to part with them. Some of the more important recurrent selection activities are being undertaken at the privately owned Lapanday Food Corporation operations, which is likely to preclude access.



Figure 18. GCTCV 219

4.7 Africa

The programs in Africa are primarily focussed on seeking resistance to black Sigatoka in plantains and East African Highland Bananas both of which are used for cooking purposes. So currently these programs have no particular value in meeting our needs in Australia.

The International Institute for Tropical Agriculture (IITA) commenced plantain breeding in Nigeria in the late 1980s. CARBAP commenced breeding plantains at much the same time as IITA's program. Both programs are primarily seeking resistance to black Sigatoka. IITA started breeding East African Highland Bananas (EAHB) in Uganda in the latter half of the 1990s.

Cote d'Ivoire (CNRA) has plantain breeding and selection as a priority based on diploid crosses onto tetraploids obtained from FHIA, IITA and CARBAP but so far has very few results (pers. comm. Kodjo Tomekpe Jan 2017).

4.8 India

The breeding effort in India is focussed on non-Cavendish banana types and consists of cross breeding and non-conventional approaches. More importantly however is that discussions over several years have made it abundantly clear that they are not about to part with any banana germplasm. Additionally the crossbreeding program has been plagued by Banana Streak Virus infection.

4.9 USA – Armstrong Atlantic State University

Ortiz et al (2013) provide some details but essentially the breeding is insignificant. Richard Wallace is seeking to develop/introduce edible and ornamental bananas suited to the 'humid subtropical' climate of Georgia, USA (about 32 degrees north but colder than equivalent in NSW). Really about a narrow window for cold tolerant and short-cycle cultivars.

4.10 Israel

Rahan Meristem, the largest exporter of tissue culture banana plantlets, has several Cavendish selections with superior agronomic characteristics (<http://www.rahan.co.il/en/banana/?lang=en>). A few years ago an Australian growers' study tour visited Central America and the Caribbean and saw the Cavendish clone Gal, selected by Rahan Meristem (Figure 19). The growers were particularly keen to evaluate it in Australia as it has good bunch and plant characteristics. DAF had been in

negotiation with Rahan Meristem since the commencement of the first Banana Plant Protection Program project seeking their Cavendish selections Gal, Jaffa and Adi (9001 & 9168). However, various quarantine issues delayed the finalization of any agreement for importation and testing. Eventually Rahan Meristem decided to import their selections here privately to better capitalize on their IP should the selections perform well in Australia. These selections have recently cleared quarantine. Based on the MTA which DAF has we are entitled to [evaluate them in the three Australian trial sites as long as they are not provided elsewhere](#). Whether DAF goes ahead with this depends on industry feedback which we await. These selections have no particular resistance to pests and diseases. However, Rahan's R&D division is currently engaged in collaborative research with The University of Wageningen, aimed at developing resistance to Panama disease TR4 which may be genetic modification (Plants resistant to cytoplasm-feeding parasites <http://google.com/patents/US8716554>).



Figure 19. Gal and other elite Cavendish selections have been imported to Australia privately by Rahan Meristem



Figure 20. Gruesa – the most popular Cavendish selection in the Canary Islands has recently cleared quarantine

4.11 Canary Islands

Four Cavendish selections including Gruesa have recently been released from quarantine (Figure 20). They are high yielding Dwarf Cavendish selections from the Canary Islands which may have some application particularly in terms of shorter fruit than Williams that may better match supermarket specifications. Some are also less prone to choking and/or more cold tolerant. We are unaware of them possessing any particular pest and disease resistance.

4.12 Genetic Modification

Australia

The Queensland University of Technology have screened genetically modified Cavendish against Panama disease TR4 in the Northern Territory but no data or results have been published to verify

successful achievement of resistance. Verbal reports from QUT are that the results, in terms of Panama disease TR4 resistance, have been encouraging. Should the work prove successful there however remains the bigger hurdle of obtaining permission to commercially grow the plants in Australia along with convincing consumers to purchase genetically modified bananas. Given the complete absence of GM fresh fruit and vegetables in the Australian marketplace and the known reluctance of the public for it, any suggestion to the big Australian retailers that they accept locally-produced GM lines runs a significant risk of rejection accompanied by demands by those retailers for importation of non-GM fruit from elsewhere to meet consumer demand. Research will be ongoing in this area but it seems nothing will be ready for possible commercialisation for quite a few years yet. If those hurdles can be overcome it should be equally possible then to build Panama disease Race 1 resistance into desired varieties like Lady Finger and Ducasse.

One source of Panama disease TR4 resistance used in QUT's program is from the wild banana – *Musa acuminata* ssp. *malaccensis* (Figure 21). Thus their program could be described as cisgenesis, which is genetic modification of plants with natural genes, coding for an (agricultural) trait, from the crop plant itself or from a sexually compatible donor plant that can be used in conventional breeding. The gene belongs to the conventional breeder's gene pool. Results from a 2013 online survey (Delwaide, et al. 2015) suggested that in the EU not all GMOs are the same in consumer's eyes. Consumers had a significantly higher willingness to pay a premium to avoid consuming rice labelled as GM compared to rice labelled as cisgenic. Despite the first GM trials being undertaken as early as 1993, Panis (2016) concluded that the application of GM to bananas is still in its infancy based on limited published scientific papers and reports on confined field trials being scarce in number.



Figure 21. *Musa acuminata* ssp. *malaccensis* the source of TR4 resistance in QUT's GM Cavendish

More recently gene editing (genome editing; e.g. CRISPR = Clustered regularly interspaced short palindromic repeats) has become available. The approach is said to be more precise and could have better prospects of consumer acceptance but that remains to be seen. In such cases people's perceptions can be more important necessarily than facts. Anyway, it is early days yet with safety/regulatory guidelines in Australia still to be determined. So far the Office of the Gene Technology Regulator (OGTR) has released a discussion paper in October 2016 titled 'Options for regulating new technologies'.

Progress is being made by GM for Panama disease TR4 but no suitable GM approach for Sigatoka leaf disease resistance has yet been identified (Dale et al., 2017). But should it come, it will likely

provide the only real prospect of delivering the much needed leaf disease resistance in a Cavendish banana.

International programs

The other GM programs around the world either have Africa (cooking bananas) as a focus or there is very little prospect of cooperation with Australia.

4.13 Mutagenesis

The International Atomic Energy Agency (IAEA) commenced a Coordinated Research Project (CRP) in 2015 entitled 'Efficient screening techniques to identify mutants with disease resistance for coffee and banana'. This CRP is currently a network of 14 groups (8 banana/6 coffee) from 13 countries. IAEA's own lab in Vienna, Austria is also involved in mutation induction and detection in bananas. The main objective of the project is to adapt and develop screening protocols that are suitable for mass screening of mutant lines to identify rare plants showing enhanced resistance to disease.

Queensland DAF commenced its own mutagenesis program in project BA14014 Fusarium wilt Tropical Race 4 Research Program seeking Panama disease TR4 resistant Cavendish mutants with the first plants recently established in the field in the Northern Territory for screening in June. We are not currently part of the IAEA CRP but there should be value in DAF becoming a Research Agreement Holder in the CRP. Areas in particular we can benefit from are:

- The various synergies that come with being part of a larger group tackling a topic.
- Improved efficiencies through validation of disease screening methods/development of rapid screening techniques
- A better understanding of mechanism of Panama disease TR4 disease resistance of variants – genetic or epigenetic? It is not just about locating resistant plants but delving deeper to build a platform for greater advances.

Becoming a Research Agreement Holder would give us access to all information, data and research results gathered during the course of the CRP but likewise our information would be freely available to other participants and other relevant authorized parties. The next Research Coordination meeting from 29 May to 2 June 2017 in Lisbon, Portugal has now come and gone without our participation. The value of belonging is thus diminishing as the CRP draws to a close in November 2019.

4.14 Other

There is an opportunity to further pursue the collection and selection of within subgroup variation and not just for Panama disease TR4 resistance. The approach can include collecting naturally occurring variation (Australia and overseas) and also encouraging growers to be on the lookout for their own selections and propagating from their better performing plants. This includes active surveying within tissue culture plantings for off-type/somaclonal variation for traits of interest (Figure 23). This is not confined to just disease resistance. The screening of diversity is a key to discovery. The extra dwarf Cavendish, known as Dwarf Parfitt, had no particular commercial value, but was included when screening for Panama disease SR4 resistance in the 1980s, and was found to be resistant (Figure 22). Dwarf Parfitt was then irradiated and about 30 tolerant lines with improved agronomic features were selected from around 500 plants that went to the field (Smith et al. 2006). So it would be a good idea to progressively screen more and more germplasm for Panama disease TR4 resistance in the NT or overseas, possibly as an ACIAR project component. This can probably be done to a satisfactory level more efficiently using less plants to keep costs down based on biometry advice following the current NT trial. Information from such screening may provide starting points to support other banana improvement programs including cross-breeding.



Figure 22. Dwarf Parfitt (pictured) was the ‘ugly duckling’ resistant to SR4 ‘transformed’ through mutagenesis into DPM25.



Figure 23. Rossi Lady Finger and a broad range of Lady Finger diversity should be screened for TR4 and Race 1 resistance

5. Options/viability of partnering

Honduras – FHIA

DAF was quoted US\$50,000/month for 10 years (around A\$8 Million) to proceed on developing a pest resistant Cavendish hybrid for evaluation under Australian conditions.

Taiwan – TBRI

A closer association with the TBRI program was discussed on the 2016 Study Tour by DAF and ABGC staff at which 2 key aspects were discussed.

The evaluation of clones from the Australian mutagenesis program was canvassed if screening sites in the NT weren’t available. Apparently through official Taiwanese channels it takes about 2 years to get the irradiated plants over there but only a fraction of the time is required if virus indexed in Australia first and an agreement is in place with TBRI to evaluate for research only and data confidential. This is a slower option than screening in the NT (add another 2 years clearing quarantine coming back into Australia for any resistant selections), but TBRI has the capacity to screen much larger numbers than would be possible in the NT, should the need arise, and presumably with enhanced cost efficiency. TBRI are willing but details of the fee were not determined on the visit.

The second matter was Australia co-investing in development of somaclones from Williams Cavendish. A 5-year program at A\$100,000/year has been suggested by TBRI to generate the somaclones – basically starting with Williams Cavendish (very susceptible to Panama disease TR4), multiply to 20,000 plants and plant in 1 ha block at TBRI and identify resistant plants to multiply to 500 plants (by TC) to verify resistance. There is some conjecture if the output numbers are sufficient to offer a high chance of success and further discussion is still required. TBRI has the capacity to

screen much larger numbers of plants from TC &/or mutagenesis than is possible in Australia. Originally it was envisaged that closer association with TBRI would further improve our access to their latest resistant selections but this is now unlikely to be the case with recent policy decisions taken in 2017 by TBRI to restrict access to their germplasm so as to maximise the return on the IP held in their resistant lines.

Guadeloupe – CIRAD

Collaboration between Queensland DAF and CIRAD should continue to be fostered for the synergies it brings to the R&D efforts and the associated benefits to the respective banana industries. Indeed collaboration with CIRAD should be extended to cover-cropping practices and the development of integrated systemic resistance/systemically acquired for control of Panama disease and nematodes.

6. Identification of current varieties/progeny worthy of investigation

Table 2 Summary of breeding program outputs and their current status

Program/Country	Variety	Product style	Current status
FHIA, Honduras	FHIA Cavendish hybrids	Cavendish triploid hybrids	IP held by Chiquita; no access possible
	FHIA non-Cavendish hybrids	Triploid and tetraploid hybrids of Silk, Lady Finger, Gros Michel, Sucrier	IP held by Chiquita; no access possible
CIRAD, Guadeloupe	CIRAD hybrids (CIRAD 01, 02, 03, 04, 05 & 06)	Triploid hybrids	6 hybrids imported by DAF, 4 (CIRAD 03, 04, 05 & 06) have cleared quarantine, expected in field trials in 2018
EMBRAPA, Brazil	Tropical (YB42.21) Princesa (YB42.07)	Silk tetraploid hybrids	Currently negotiating access with EMBRAPA
EPAGRI, Brazil (state-based institution)	Panama disease Race 1 tolerant Prata selections (Catarina)	Lady Finger selections	Difficulty negotiating access via EMBRAPA; currently no access
Agronomic Institute, Sao Paulo Agribusiness Technology Agency	IAC 2001 – yellow Sigatoka tolerant Cavendish	Cavendish selection	Difficulty negotiating access via EMBRAPA; currently no access
TBRI, Taiwan	Improved GCTCV 218, Improved GCTCV 105, Tai Chiao #3, Tai Chiao #5, Tai Chiao #7	Cavendish selections	3 Tai Chiao selections imported by DAF, recently cleared quarantine, expected in field trials in 2018
Guangdong Academy of Agricultural Sciences, PR China; Guangxi Guangdong Academy of Agricultural Sciences,	Zhongjiao 3, Yuefeng 1, Zhongjiao 4, Zhongjiao 6 and Zhongfen 1; Guijiao	Cavendish selections Ducasse selection (Zhongfen 1); Cavendish selection	Currently no access

Philippines	GCTCV 219 Dwarf Ducasse	Cavendish selection and Dwarf Ducasse selection	Currently no access
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7. Conclusions

Our best prospects of delivering Panama disease TR4 resistance in a Cavendish banana appears to lie with somaclonal variants and mutagenesis. To promote and facilitate good outcomes for our DAF programs becoming a Research Agreement Holder in the IAEA CRP project would appear to be advantageous. However, the value of this is diminishing as the CRP draws to a close in November 2019. An important limitation in Australia is our restricted screening capacity for Panama disease TR4 resistance. Additional offshore screening would be desirable and Taiwan has potential to provide this. A proposal from Taiwan to multiply Williams Cavendish in tissue culture and then identify resistant somaclonal variants is currently available. Such an investment of A\$100,000 per year (for 5 years) and other R&D already in place are modest relative to the predicted losses which Panama disease TR4 would cause of \$138 million per year (Cook et al. 2015). Originally our potential access to Taiwan's existing pool of selections was also expected to be enhanced by such a relationship but recent developments at TBRI have greatly curtailed such a benefit.

If the current mutagenesis program in BA14014 is successful there could be sufficient interest in applying this same approach to obtaining a Panama disease Race 1 resistant Lady Finger and perhaps even Ducasse. But first we would need to locate some variation in Panama disease resistance in those particular subgroups as found previously for Cavendish with Dwarf Parfitt.

The pursuit of superior somaclonal variants via tissue culture multiplication or mutagenesis need not be confined to just those with Panama disease TR4 resistance. Other features such as plant height, finger length, bunch angle etc. could also be potentially obtained by this route (Pestana et al., 2011).

Table 3. Key Cavendish varieties - tolerant to Panama disease TR4 in NT trials, or under trial

Variety	Disease rating§	Status
Formosana (GCTCV 218)	susceptible	available
CJ19	susceptible	available
GCTCV 119	slowly susceptible	available
GCTCV 215		under trial
GCTCV 105		under trial
GCTCV 217		under trial
§ Williams rating – very susceptible		



Figure 24. GCTCV 217 on trial in the NT



Figure 25. GCTCV 105 on trial in the NT

Realistically given the past record of hybrids from cross-breeding programs we cannot expect their products to fit easily into the existing supply chains. An exception would be the tetraploid Ducasse hybrid FHIA-26 which is very similar in almost all respects to its female parent Ducasse which is encouraging for cross-breeding efforts. Breeding is a numbers game - the more progeny that can be generated the greater the likelihood of Panama disease resistance and required agronomic and organoleptic qualities aligning (Daniells, 2011) so it is possible to hold out some hope for cross-breeding. However, unless there is some major game changer in terms of severe restrictions on chemicals applied and method of application there will be insufficient incentive to contemplate such disruption to the supply chain which past hybrids have indicated. Thus a type of 'watching brief' on cross-breeding programs to monitor their progress and only source small numbers of selected hybrids for evaluation under Australian conditions would be most appropriate rather than direct investment into such breeding programs. Likewise a close watching brief is required on the 'new GM' (gene editing etc.). There must be a very clear signal from the marketplace that products from such technology are totally acceptable before investing.

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Appendix 10 – Develop resilient crop management options

Tropical pastures soil microbial characterisation for the suppression of *Fusarium oxysporum* f. sp. *cubense*.

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Introduction

The use of cover crops and increasing plant species diversity has been suggested as one method of altering soil organisms and increasing pathogen antagonists (Djigal et al. 2012; Ratnadass et al. 2012; Ratnadass et al. 2006; Van der Putten et al. 2006; Wardle et al. 2004). The use of cover crops in banana production systems has not been widely examined, but was reported as having a detrimental effect on crop production in sub-tropical banana production (Johns 1994). However, cover crops were identified as an innovation worthy of further consideration reducing pesticides and improving natural control of pests and diseases in Guadeloupe (Blazy et al. 2009). Furthermore, where vegetative groundcover was used between banana plants there was an increase in the activity of predatory nematodes (Djigal et al. 2012). However, the impacts of changing the mix of plant species in an agricultural production system are not always straight forward, resulting in greater soil food web complexity and regulation of pests and diseases (De Deyn et al. 2004; Djigal et al. 2012; DuPont et al. 2009; Sánchez-Moreno and Ferris 2007).

The aim of this work was to determine if pasture species may be used in rotation bananas to increase soil microbial activity and diversity leading to suppression of Fusarium wilt (Panama disease) (*Fusarium oxysporum* f. sp. *cubense*).

Materials and methods

Site and sampling

Soil from the top 0-10 cm was collected from multiple sites of a long-term field experiment investigating different pasture species at Walkamin Research Station in north Queensland. 19 pasture plots (Fig 1) were sampled and compared to two bare soil plots.



Leucana leucocephala

Chloris gayana cv. NuCal



Arachis pintoi cv Amarillo

Arachis glabrata

Fig 1. Examples of pasture plots sampled for soil microbial characteristics for suppression of *Foc*.

Soil microbial analysis

Soil nematode communities, CLPPs and enzyme activity assays were used to gauge the functional diversity of the soil microbial community under different soil management practices (groundcover and N fertilisation application rates). Soil nematodes were extracted using a modified Baermann funnel technique, described elsewhere (Whitehead and Hemming 1965), and assessed according to their trophic groups (parasites, fungivores, bacterivores, omnivores and predators) and functional guilds (Yeates and Bongers 1999). Nematode indices were calculated from the total abundance of nematodes to determine the bacterivore to fungivore ratio ($B/(B+F)$), Shannon diversity index (H') (Yeates and Bongers 1999), enrichment (EI), structure (SI) and channel indices (CI) (Ferris et al. 2001).

Whole-soil sample CLPPs were assessed in duplicate using the MicroResp™ system as described by Campbell et al. (2003). The MicroResp™ system consists of a deep-well microplate housing soil (incubated at 45% MWHC for 7 days) and added aqueous carbon sources, sealed to a colorimetric CO₂-trap and incubated for a further 6 h. The respiratory response to 15 carbon sources was tested and selected according to the recommendations of Campbell et al. (2003) including; 5 carbohydrates (L-arabinose, D-fructose, D-Galactose, D-glucose and N-acetyl-D-glucosamine), 3 carboxylic acids (citric acid, oxalic acid and L-malic acid), 5 amino acids (L-alanine, DL-aspartic acid, γ -aminobutyric acid, L-lysine hydrochloride and L-arginine) and one phenolic acid (protocatechuic acid). Fumaric acid was selected due to its role as a signalling molecule in banana roots (Yuan et al. 2015). CO₂-trap absorbance was measured at 590nm (ThermoFisher Multiscan Spectrophotometer) immediately prior to sealing to the soil microplate and following the 6 h incubation.

Bioassay

A novel bioassay system was developed to determine the recovery of *Foc* from soil. 50 g of soil from each pasture plot was placed in a 250 mL sterile container and maintained at 40% field capacity. Triplicate container systems were used for each pasture and bare soil control. Each individual container was inoculated with 40 000 spores of *Foc* Race 1 (VCG 0124) recovered from *Foc* Komada's media and allowed to incubate at 26 C for 7 days. After the seven day incubation period a pseudostem piece of a Race 1 susceptible banana cultivar (*Musa* AAA, cv Highgate) for a further 7 days. Following the second incubation period the pseudostem piece was removed and the decomposing end in contact with the soil was removed. The pseudostem piece was surface sterilised with a 1% bleach and ethanol solution. The pseudostem piece was cut in three sections at 1 cm

increments away from the base of the pseudostem piece. From each section, seven small sections, approximately 1 mm², were placed on Komada's medium and the fungal growth recorded for the number of positive pieces after 5 and 7 days, to calculate a percentage recovery of *Fusarium* from the soil.

Statistical Analysis

The relative respiration from the different carbon sources using the MicroResp system was calculated by the change in absorbance relative to water. Carbon substrates with a relative respiration greater than 1 stimulated microbial activity relative with water whereas carbon substrates with a relative respiration less than 1 reduced microbial respiration. Cluster analysis dendrograms were constructed from Euclidian similarity matrices. All analysis was done using Genstat 16 (VSN International).

Results and discussion

The differing pasture species demonstrated differences in soil microbial communities, using both the soil nematode community and substrate utilisation profiles (Fig 2). Low numbers of genera of plant parasitic nematodes were found under most pasture species, except *Bothriocloa* which had high numbers of *Pratylenchus* sp. and *Lablab*, which had high numbers of *Rotylenchulus reniformis*. *Leucena leucocephala* tended to have a relatively greater abundance of total nematodes particularly members Cephelobidae, Pangrolaimidae, Aphelenchidae and Dorylaimidae nematodes, resulting in relatively high numbers of nematodes belonging to several functional guilds; Fu2, Ba1, Ba2 and Om4 (Fig 2). One of the *Chloris gayana* plots had the greatest abundance of soil nematodes; however, these were mostly Pangrolaimidae nematodes belonging to the Ba1 guild, which are described as enrichment opportunists.

The MicroResp carbon utilisation profiles under the different pasture species demonstrated greater utilisation of citric and oxalic acid under *Leucena* and *Heteropogon* (Fig 2). There was poor utilisation of L-arginine by all pasture species and generally the utilisation of amino acids was lower than carboxylic acids and carbohydrates.

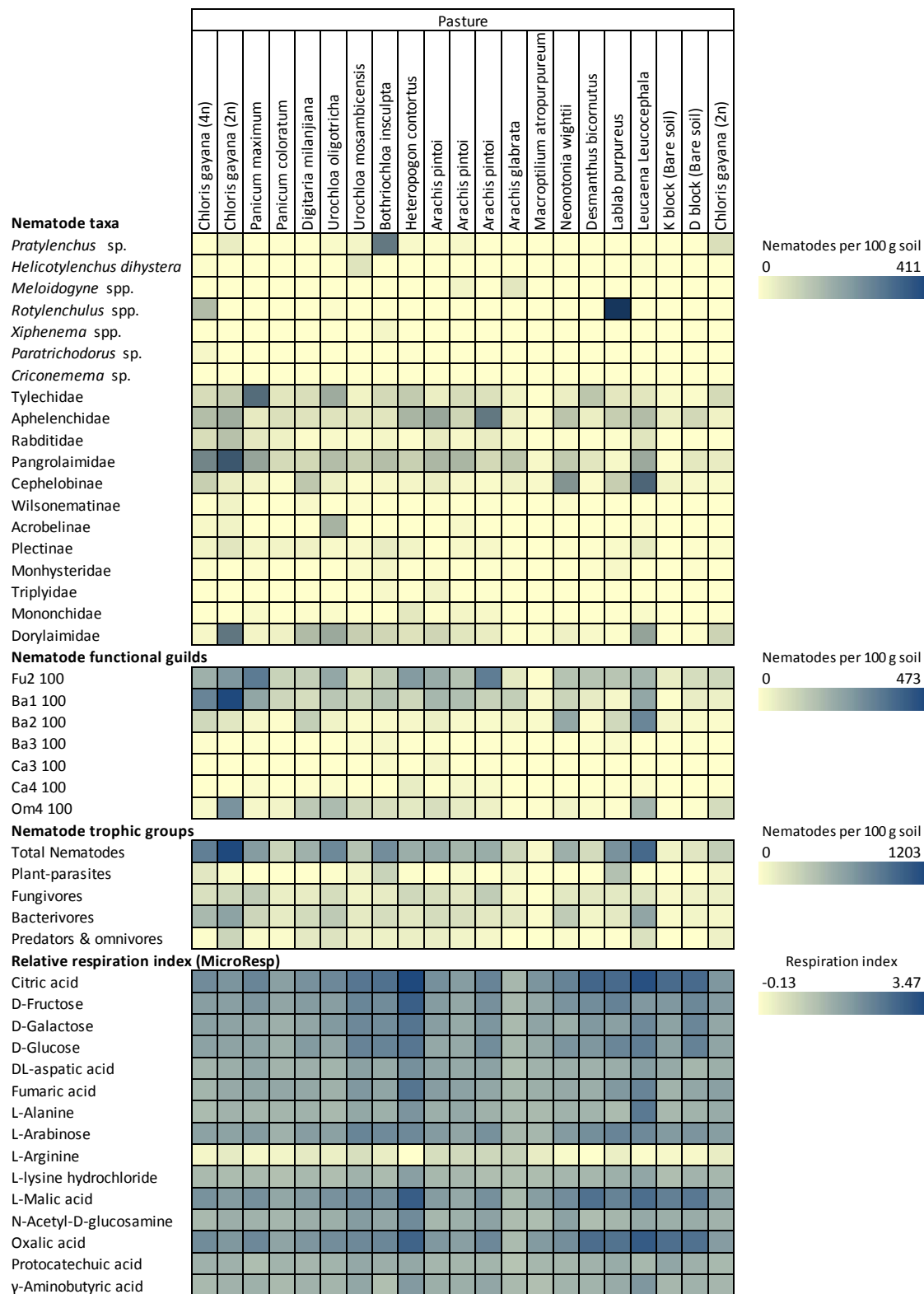


Fig 2. Heat map of the soil nematode community classified by family, functional guild and trophic groups and the substrate induced relative respiration of 19 tropical pasture compared to two bare soil controls.

A cluster analysis was used to determine the similarity in nematode community composition between pasture plots. Thirteen pasture plots had a similarity in nematode community structure greater than 90% to the bare soil plots (Fig 3). Six pasture species had a similarity less than 90%, which included *Chloris gayana* 4n and 2n, *Leucaena*, *Urochloa*, *Lablab* and *Bothriocloa* (Fig 3).

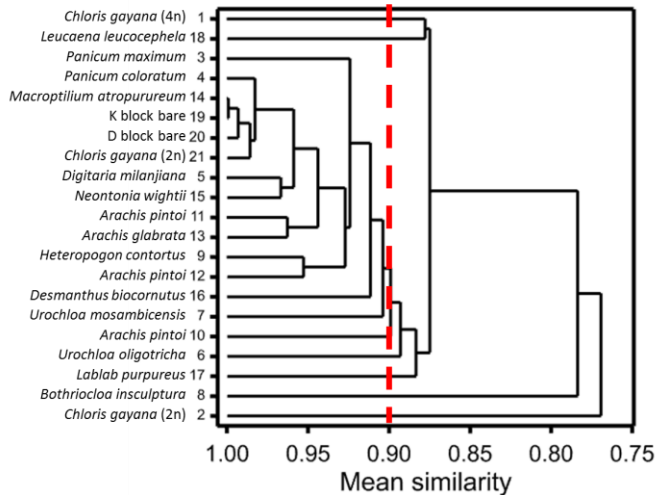


Fig 3. Cluster analysis dendrogram of 19 tropical pasture compared to two bare soil controls based on identified nematode taxa.

A cluster analysis of the utilisation of carbon substrates under each pasture plot indicated that only *Leucaena* and *Heteropogon* had a mean similarity less than 90% relative to the bare soil plots, whereas the other 17 pasture plot soils had a mean similarity in carbon utilisation profiles greater than 90% relative to the bare soil plots (Fig 4).

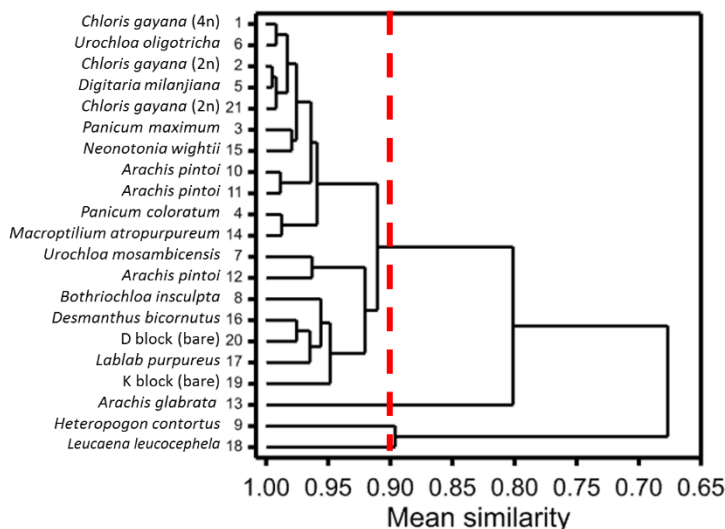


Fig. 4: Cluster analysis dendrogram of carbon utilisation profiles of 19 tropical pasture compared to two bare soil controls based on relative respiration index determined using the MicroResp system.

Four pasture plot soils completely suppressed the recovery of *Fusarium* from a baiting bioassay of the soils from the 19 pasture plots and two bare soils control. *Fusarium* was not recovered from *Leucena leucocephala*, *Dismanthus bicornutus*, *Arachis pintoii* and *Chloris gayana* plots (Fig 5). Conversely, *Fusarium* was recovered from 100% of banana pseudostem pieces of *Neonotonia wightii* (Fig 5). Both the bare soil controls had a 40% recovery of *Fusarium* from pseudostem pieces. There was some variation in the recovery *Fusarium* from pasture species that had multiple entries, such as *Chloris gayana*, which ranged from zero recovery to 50% and *Arachis pintoii* which had a range in *Fusarium* recovery from zero to 90% (Fig 5).

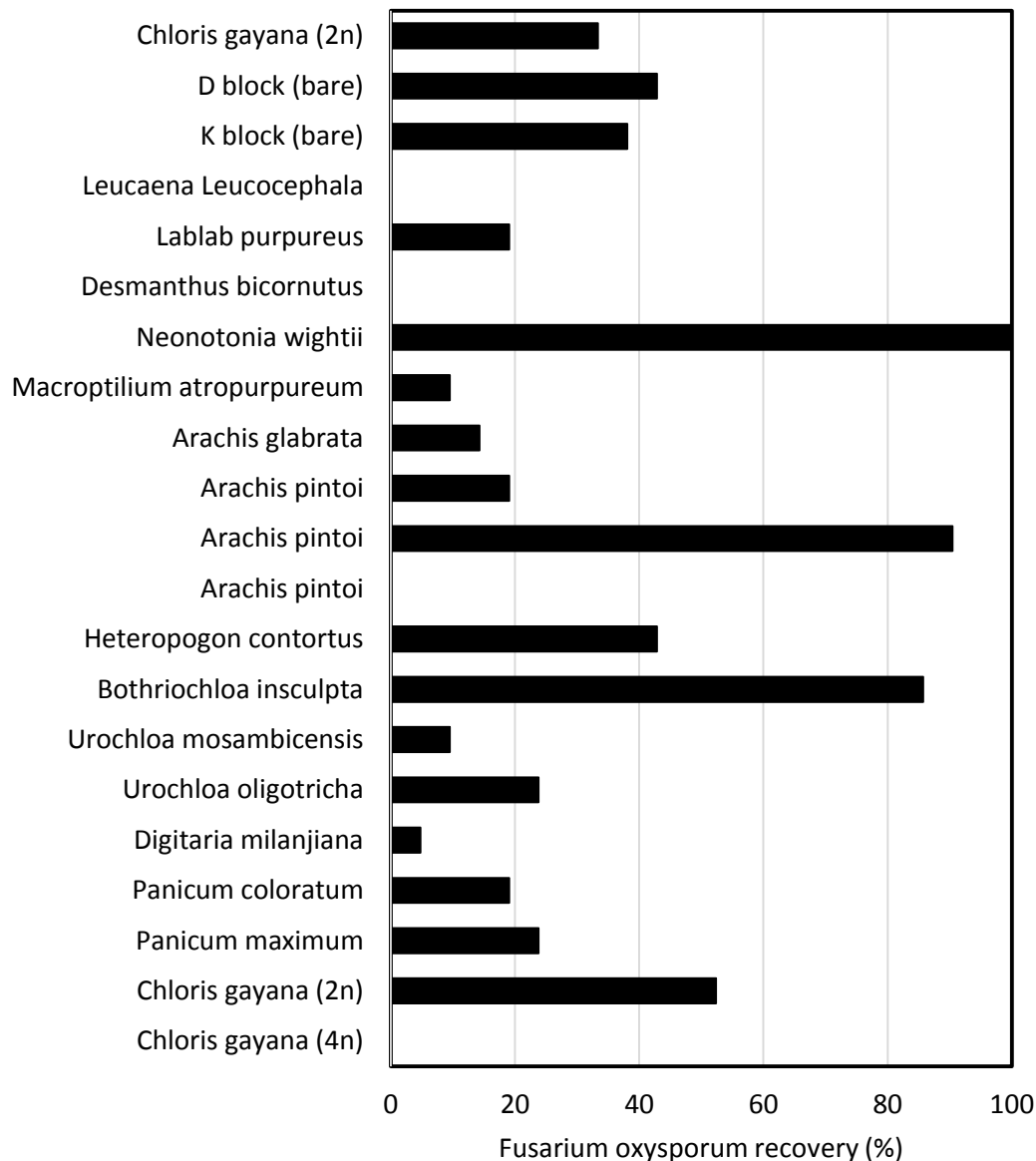


Fig 5. Recovery of *Fusarium oxysporum* when added to soil previously growing 19 tropical pastures compared to two bare soil controls.

Summary

Leucena leucocephala was found to be a tropical pasture species worthy of further investigation for suppression of *Fusarium* wilt of bananas. Soil taken from *Leucena leucocephala* plots completely suppressed the recovery of *Fusarium* from soil and also altered the soil microbial activity using

nematode community structure and MicroResp substrate utilisation profiles. Therefore, the change in soil microbial activity under *Leucena leucocephala* could possibly be leading to the suppression of Fusarium. The suppressive activity of *Leucena leucocephala* is worthy of further investigation for rotation with banana in soils that are infected with *Fusarium oxysporum* f.sp. *cubense*, the casual organism of Panama disease in bananas.

Soil from other pasture species *Dismanthus bicornutus*, *Arachis pintoii* and *Chloris gayana* also suppressed recovery of Fusarium; however the microbial activity under these pasture species was greater than 90% similar to bare soil. The similarity in nematode community structure and carbon utilisation between the bare soil with *Dismanthus bicornutus*, *Arachis pintoii* and *Chloris gayana* indicated that there was little change in soil microbial activity.

There was a range in recovery of Fusarium and microbial activity under *Arachis pintoii* and *Chloris gayana*, which both had multiple entries. This indicated factors other than just the pasture species were important in the recovery of Fusarium.

The survey of pasture species for suppression off Fusarium and changes in soil microbial activity should be viewed as a rapid screening and requires further validation by more thorough experimentation. However, the survey was able to find novel rotation crops with potential to be used in rotation with bananas for further study such as *Leucena leucocephala*.

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Assessment of the influence of root exudates on chlamydospore germination

Initial experiments on root exudates characterised the utilisation of *Fusarium oxysporum* f. sp. *cubense* Race 1 (*Foc* R1) on known exudate compounds using the MicroResp™ system (Figure 1). The carboxylic acids, which are the most common root exudates, resulted in the greatest activity of *Foc* R1, with citric acid being utilised the highest by *Foc* R1. Fumaric acid, which has been found previously to promote *Foc* growth on bananas, was significantly ($p < 0.05$) utilised less than citric acid. It had been hypothesised that fumaric acid could act as marker for activity of *Foc*, but this does not appear to be consistent, with the initial assay. The sugars tended to be utilised less than the carboxylic acids, except glucose which had a microbial utilisation equivalent to citric acid. The amino acids, as a group of exudates, tended to be utilised less than the other carbon sources, particularly L-arginine, which tended to inhibit the growth of *Foc* R1 (Fig 1).

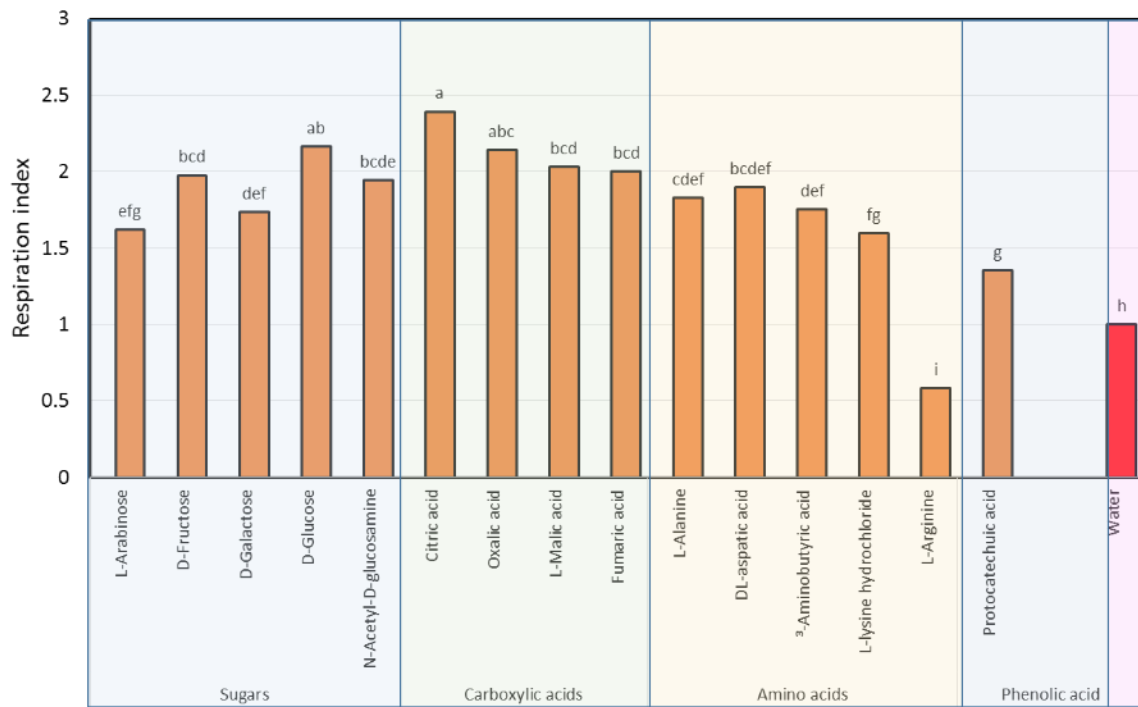


Figure 1: Utilisation by *Fusarium oxysporum* f. sp. *ubense* Race 1 of carbon compounds commonly exuded from the roots of plants relative to a water control.

Solute samplers (Rhizon) have been installed in pots in a glasshouse experiment to provide preliminary information to determine if exudates can be detected in soil solutions (Fig 2). Extractions solutions will be subjected to HPLC analysis to determine if specific compounds can be detected in the soil solutions. Furthermore, the extracted solutions will be tested for stimulation or suppression of Foc growth.



Figure 2. Solute samplers installed in pots growing bananas to determine if root exudates can be detected in soil solutions

Dr Paul Dennis from the University of Queensland has applied for a research fellowship to determine in more detail the role of banana root exudates in the *Foc* infection process. The outcomes from this fellowship application are still pending. If successful, this would enable root exudates to be investigated in much more depth than originally intended.

Sequencing data report for changes in groundcover and nitrogen management of bananas at two sites in north Queensland

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Introduction

Banana production in Australia is limited to the domestic market producing 370,000 tonnes and worth close to \$600 million annually. Northern Queensland currently accounts for more than 90 % of Australian banana production (<http://abgc.org.au/banana-industry-2/our-industry/key-facts/>), which is largely based on Cavendish cultivars. Since the majority of the banana industry is located in a confined geographic area on the coast between latitudes 15–18°S, it is highly vulnerable to impacts of natural disasters (especially tropical cyclones, Lindsay 2016) and disease epidemics. Furthermore, there is increasing pressure to improve and demonstrate practices that protect environmentally sensitive areas, such as the Great Barrier Reef.

Fusarium wilt (FW), caused by *Fusarium oxysporum* f. sp. *ubense* tropical race 4, Foc TR4 (VCG 1213/16), is increasingly impacting on banana production around the world, causing devastation of plantations and threatening the livelihoods of smallholders and independent banana producers, particularly those growing Cavendish (*Musa* AAA) in monoculture. In mid-February 2015, stem and corm samples were taken from two Cavendish plants (cv. Williams) in the Tully region of north Queensland and subsequently identified to be TR4 (VCG 01213/16) (O'Neill et al. 2016). There are no completely TR4 resistant banana cultivars that achieve the same productivity and market acceptance as the current Cavendish cultivars (Heslop-Harrison and Schwarzacher 2007).

Commercial banana production has tended to move toward an industrial approach of maximising profitability through increased production. Monocultures of genetically uniform cultivars selected for high production have intensified selection of soil borne pathogens (Heslop-Harrison and Schwarzacher 2007; Ploetz 2015). An ecological approach suggests that in genetically uniform monocultures, there is a decline in productivity due to a negative plant-soil feedback, where monocultures of genetically similar plants promote soil microorganisms that are deleterious to their own growth (Vukicevich et al. 2016). Increases in soil borne diseases are further exacerbated through the use of high analysis fertilisers as the principal source of plant nutrients, and routine application of pesticides. (Chellemi et al. 2015).

Enhancement of indigenous antagonistic organisms to suppress plant pathogens through general suppression have been developed through changing the design of agriculture production systems (Doornbos et al. 2012; Dore et al. 2011; Malezieux 2012; Ratnadass et al. 2012; Stone et al. 2004). The use of cover crops and increasing plant species diversity has been suggested as one method of altering soil organisms and increasing pathogen antagonists (Djigal et al. 2012; Ratnadass et al. 2012;

Ratnadass et al. 2006; Van der Putten et al. 2006; Wardle et al. 2004). The use of cover crops in banana production systems has not been widely examined, but was reported as having a detrimental effect on crop production in sub-tropical banana production (Johns 1994). However, cover crops were identified as an innovation worthy of further consideration reducing pesticides and improving natural control of pests and diseases in Guadeloupe (Blazy et al. 2009). However, the impacts of changing the mix of plant species in an agricultural production system are not always straight forward, resulting in greater soil food web complexity and regulation of pests and diseases (De Deyn et al. 2004; Djigal et al. 2012; DuPont et al. 2009; Sánchez-Moreno and Ferris 2007). Furthermore, the lack of vegetative groundcover and high nitrogen status of the soil can result in loss of soil biological functions such as disease suppression (Damour et al. 2012; Pattison et al. 2014).

The hypothesis for this research was that suppression to FW in bananas could be induced through soil management by altering interactions in the soil ecosystem. The specific aims were to identify soil management practices that could change the soil ecosystem and induce suppression to FW. Further to develop soil indicators allowing management practices to be assessed for their suppressive potential to FW and impacts on banana production.

Materials and Methods

Experimental design

Site 1

The field experiment established on a commercial banana plantation at East Palmerston near Innisfail, north Queensland in a red ferrosol soil (64% sand, 18% silt and 18 % clay, 2.3 % C, pH 7.2, CEC 15 $\mu\text{mol}/100\text{g}$ soil). The site had a long history of banana production, but prior to planting had been in fallow, with two years of Rhodes grass (*Chloris gayana*) and four months of Canola (*Brassica napus*) grown on the site. Bananas (cv. Williams, Cavendish *Musa* sp. AAA) were planted on the site on November 4, 2014 in a single row formation, with 3.5m between rows and 2.0 m between plants.

Site 2

The field experiment established on the South Johnstone Research Station near Innisfail, north Queensland in a dermasol soil (47% sand, 20% silt and 33 % clay, 1.7 % C, pH 5.9, CEC 7.8 $\mu\text{mol}/100\text{g}$ soil). The site had previously been used for banana production two months prior to the trial being established. Bananas (*Musa* AAA cv. Highgate and Hom Thong Mokho, *Musa* sp. AAA) were planted on the site on March 24, 2015 in a single row formation, with 5 m between rows and 1.5 m between plants.

Treatments

Two groundcover treatments, either bare or vegetated and three fertiliser treatments; farm practice 350 kg N/ha/crop cycle as urea, low N (220 kg N/ha/crop cycle as urea) and low N+E (220 kg N/ha/crop cycle as Entec DMPP (3,4-dimethylpyrazole phosphate) treated urea) were imposed on the trial site. The treatments were replicated four times in a randomised block, with 12 plants per plot.

The bare soil treatments were maintained free of vegetation by the use of herbicides (glufosinate) applied 2-monthly. The groundcover was based on *Arachis pintoi*, which was planted between banana plants as runners. However, other species were allowed to grow, and were cut every two months to maintain low growth and prevent interference with crop maintenance operations.

The nitrogen and potassium were applied monthly as a granular fertiliser. The amount of fertiliser applied in treatments receiving 220 kg N depended on the stage of growth of the plants with the

fertiliser dose increasing from 11.5 to 38.3 kg N/ha/month as plant biomass increased. Crop cycles were completed in 10 months for the initial crop and then 8 months for each subsequent crop cycle. The 350 kg N/ha/crop cycle, was applied at a fixed amount each month. Trace elements were applied as a liquid fertiliser every two months.

Soil Sampling and DNA extraction

Five samples from each treatment plot (6 x 4) were collected every 6 months from the trial establishment date. Soil samples were taken from the root zone, 10 cm from the base of five individual banana plants using a 10 cm diameter soil corer to a depth of 10cm and mixed as a composite soil sample. Soil samples were transferred to -20°C storage. DNA was extracted from 250 mg of thawed soil using the Power Soil DNA Isolation kit (MO BIO Laboratories, Carlsbad, CA) according to the manufacturer's recommendations.

PCR amplification and sequencing of phylogenetic marker genes

Bacterial and archaeal PCRs were performed on 1.5 µl DNA samples, in 1X PCR Buffer minus Mg²⁺ (Invitrogen), 100 µM of each of the dNTPs (Invitrogen), 300 µM of MgCl₂ (Invitrogen), (Invitrogen), 0.625 U Taq DNA Polymerase (Invitrogen), 250 µM of each primer, made up to a total volume of 25 µl with molecular biology grade water. The primers were 926F (5'-AAA CTY AAA KGA ATT GRC GG-3')²⁰ and 1392wR (5'-ACG GGC GGT GWG TRC-3')²⁰. The forward and reverse primers were modified on the 5' end to contain the Illumina overhang adapter for compatibility with the P5 and i7 Nextera XT indices, respectively. Thermocycling conditions were as follows: 94°C for 3 min; then 35 cycles of 94°C for 45 sec, 55°C for 30 s, 72°C for 1 min 30 sec; followed by 72°C for 10 min. Amplifications were performed using a Veriti[®] 96-well Thermocycler (Applied Biosystems). PCR success was determined by gel electrophoresis, which also facilitated visual confirmation of amplicon size and quality.

Amplicons were purified using Agencourt AMPure magnetic beads and subjected to dual indexing using the Nextera XT Index Kit (Illumina) as per the manufacturer's instructions. Indexed amplicons were purified using Agencourt AMPure XP beads and then quantified using a PicoGreen dsDNA Quantification Kit (Invitrogen). Equal concentrations of each sample were pooled and sequenced on an Illumina MiSeq using 30% PhiX Control v3 (Illumina) and a MiSeq Reagent Kit v3 (600 cycle; Illumina) according to the manufacturer's instructions.

Processing of sequence data

Primer sequences were removed from each fastq file using the QIIME v1.9.1 script `multiple_extract_barcodes.py`.²³ The header line of each sequence was then modified to contain a sample ID using a custom bash script and each file was quality filtered using the QIIME script `multiple_split_libraries.py` with the homopolymer filter deactivated. The forward reads from each sample were then concatenated into a single file. For bacteria and archaea, 16S rDNA sequences chimera checked against the 10_2013 release of the GreenGenes database²⁴ using UCHIME ver. 3.0.617.²⁵ Sequences were clustered at 97% similarity using UCLUST v. 1.2.22. GreenGenes²⁴ (10_2013 release) taxonomy was assigned to representative 16S OTU sequences using BLAST+ v. 2.2.30. The mean numbers of observed OTUs, the estimated total number of OTUs (Chao 1;²⁹) and Simpson's Diversity Index³⁰ values were calculated using QIIME. We rarefied the data to 1,200 16S sequences per sample for all comparisons of diversity.

Statistical analyses

To determine whether the treatments significantly affected the mean numbers of observed OTUs, the estimated total number of OTUs (Chao 1) and Simpson's Diversity Index values we used analysis of variance (ANOVA) with Tukey's Honest Significant Difference (HSD) tests for post-hoc comparisons of means. For all comparisons of community composition, we used Hellinger³¹ transformed OTU relative abundances. Permutational multivariate analysis of variance (PERMANOVA)³² was used to determine whether the treatments significantly affected community composition. Partial redundancy analysis (RDA) was then used visualize compositional differences between samples that was attributable to the factors deemed to be significant using PERMANOVA after first accounting for variation attributable to sampling date. In other words, the effect of time was removed so that the effects of ground cover and N treatment could be visualized more clearly. OTUs that discriminated between treatments were identified by vector fitting using the envfit function in the R package vegan. OTUs that were found to be significantly correlated ($P < 0.05$) with the treatments were then superimposed on the RDA ordinations. All analyses were performed using R.

Results and discussion

Differences between sites

Soil microbial communities at both experimental locations were dominated by representatives of the archaeal phylum: Crenarchaeota, and the bacterial phyla: Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Gemmatimonadetes, Nitrospirae and Proteobacteria (Figs. 1 and 2). Nonetheless, at the OTU-level microbial community composition differed significantly between locations (Table 1). While this difference accounted for just 15% of variation between samples it was the strongest predictor of differences in community composition, with sampling date being significantly associated with another 6% of variation. For this reason all subsequent analyses were performed within sites.

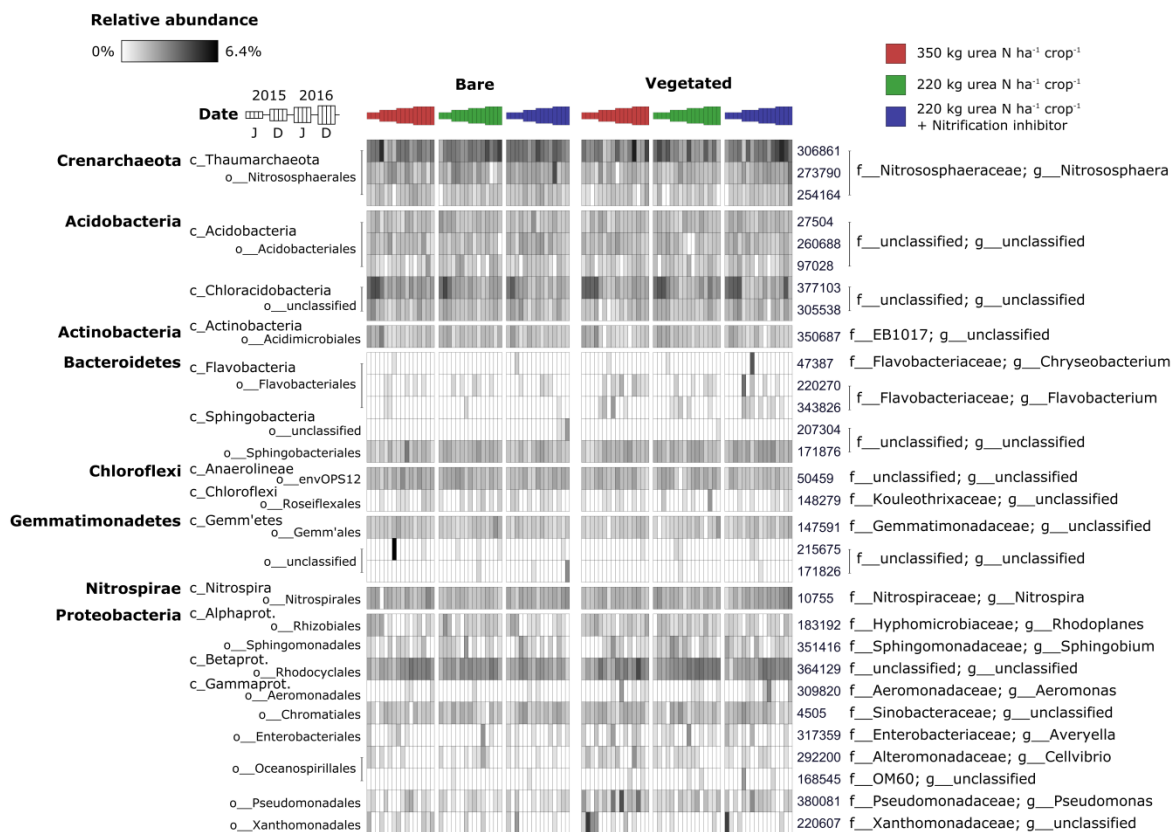


Fig. 1 Heatmap summarising the relative abundances of dominant ($\geq 1\%$ in any sample) soil bacterial and archaeal populations at site 1. The numbers of the right are OTU IDs and are consistent with other figures.

Table 1 PERMANOVA output indicating significant differences between locations and sampling dates as well as marginally significant effects of ground cover and N treatment.

	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Location	1	10.91	10.91	30.07	0.153	0.001 ***
Date	5	4.32	0.86	2.38	0.060	0.001 ***
Ground_Cover	1	0.50	0.50	1.38	0.007	0.069 .
N_Fert	2	0.92	0.46	1.27	0.013	0.079 .
Location:Ground_Cover	1	0.41	0.41	1.13	0.006	0.192
Date:Ground_Cover	5	1.87	0.37	1.03	0.026	0.307
Location:N_Fert	2	0.87	0.44	1.20	0.012	0.125
Date:N_Fert	10	3.69	0.37	1.02	0.052	0.366
Ground_Cover:N_Fert	2	0.74	0.37	1.02	0.010	0.338
Location:Ground_Cover:N_Fert	2	0.75	0.37	1.03	0.010	0.343
Date:Ground_Cover:N_Fert	10	3.63	0.36	1.00	0.051	0.443
Residuals	118	42.79	0.36	0.60		
Total	159	71.39	1.00			

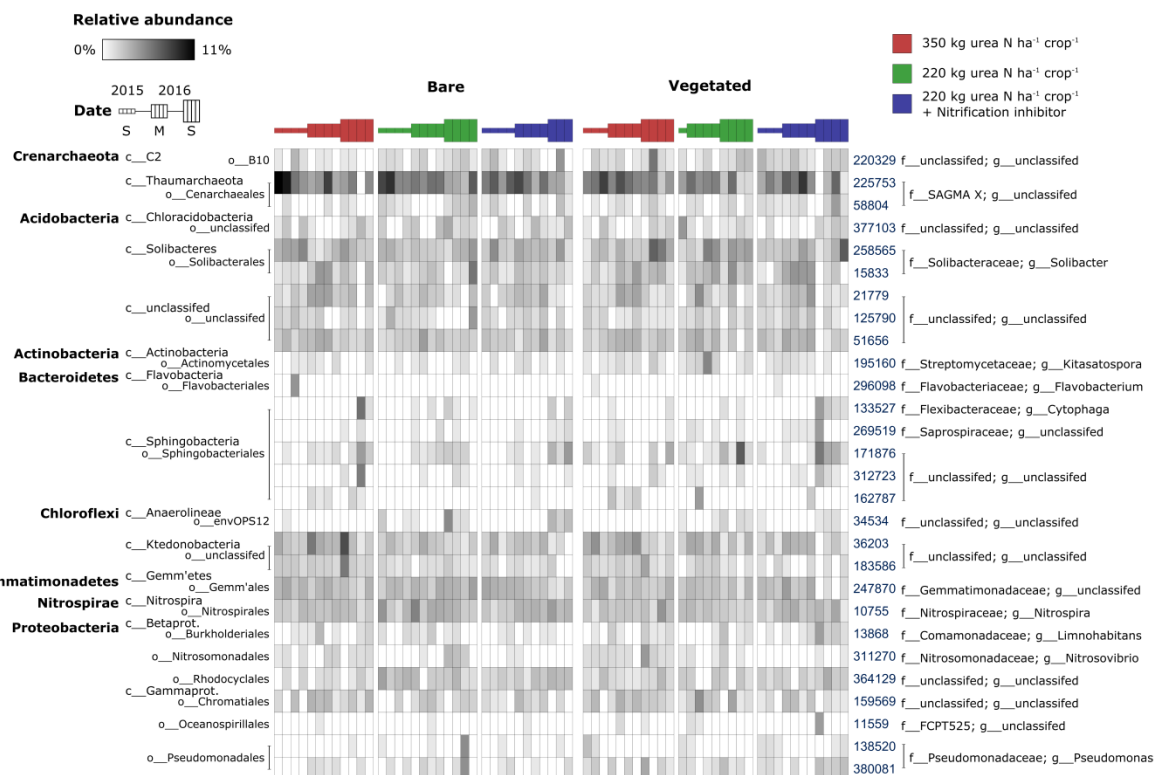


Fig. 2 Heatmap summarising the relative abundances of dominant ($\geq 1.5\%$ in any sample) soil bacterial and archaeal populations at site 2. The numbers of the right are OTU IDs and are consistent with other figures.

Site 1

Sampling date was the strongest predictor of soil microbial community composition at site 1 (explaining 6.6% of compositional variation between samples), followed by N treatment (2.4%) and

ground cover (1.4%) (Table 2). Given that sampling date simply reflects temporal variation in microbial community composition we repeated the analysis, this time removing variation attributable to sampling date such that the effects of ground cover and N treatment can be assessed with the residual variation (Table 2). This analysis revealed a significant effect of N treatment and ground cover as well as their interaction (Table 2).

Table 2 Site 1 PERMANOVA output for a maximal model containing all treatments (sampling date, ground cover and N treatment) and their interactions, as well as a final model containing ground cover, N treatment and their interaction after removing variation attributable to sampling date.

Model	Predictors	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	
Maximal	Date	3	2.304	0.76812	2.15107	0.06633	0.0001	***
	Ground_Cover	1	0.489	0.48885	1.36901	0.01407	0.0003	***
	N_Fert	2	0.839	0.41934	1.17432	0.02414	0.0021	**
	Date:Ground_Cover	3	1.082	0.36059	1.0098	0.03114	0.3375	
	Date:N_Fert	6	2.147	0.35779	1.00196	0.06179	0.4315	
	Ground_Cover:N_Fert	2	0.757	0.37842	1.05973	0.02178	0.0779	.
	Date:Ground_Cover:N_	6	2.129	0.35487	0.99379	0.06129	0.5795	
	Residuals	70	24.996	0.35709	0.71947			
Total	93	34.743	1					
Final	Ground_Cover	1	0.49	0.49	1.31	0.014	0.0001	***
	N_Fert	2	0.84	0.42	1.13	0.024	0.0002	***
	Ground_Cover:N_Fert	2	0.76	0.38	1.02	0.022	0.0257	*
	Residuals	88	32.66	0.37	0.94			
	Total	93	34.74	1.00				

Partial RDA was then used to visualise differences in the composition of microbial communities that are attributable to ground cover and N treatment after removing variation attributable to sampling date (Fig. 3). OTUs that were significantly associated with the treatment clustering were then superimposed on the ordination to visualise sample by taxa relationships (Fig. 3). This analysis separated the community associated with bare and vegetated plots on the primary axis and the N treatments on the secondary RDA axis. Interestingly, the high and low N treatments were clustered together and were separated from the communities associated with the low N treatment with the nitrification inhibitor (Fig. 3). The low N treatment with the nitrification inhibitor was significantly associated with the two representatives of the archaeal genus *Nitrososphaera*, members of which are known to perform ammonia oxidisation, and a member of the bacterial genus *Nitrospira*, which contains populations that perform complete nitrification (i.e. comammox).

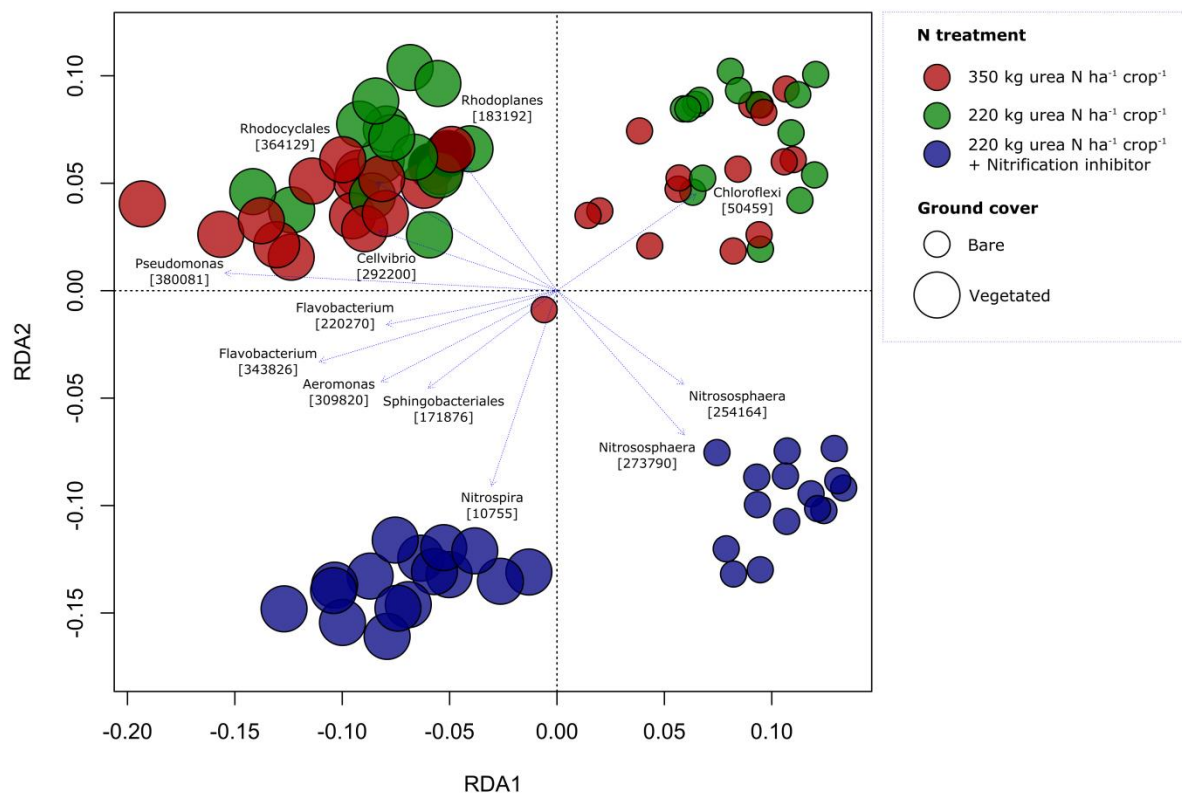


Fig. 3 Redundancy analysis ordination highlighting differences in the composition of bacterial communities that are attributable to ground cover and N treatment at site 1.

Site 2

Sampling date was also the strongest predictor of soil microbial community composition at site 2 (explaining 7.8% of compositional variation between samples), followed by N treatment (3.7%) and ground cover (1.6%, albeit marginally significant) (Table 3). As for site 1, we repeated the analysis, this time removing variation attributable to sampling date such that the effects of ground cover and N treatment can be assessed within the residual variation (Table 2). This analysis revealed a significant effect of N treatment, but indicated that ground cover was not significant (Table 2).

Table 3 Site 2 PERMANOVA output for a maximal model containing all treatments (sampling date, ground cover and N treatment) and their interactions, as well as a final model containing N treatment after removing variation attributable to sampling date.

Model	Predictors	Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)
Maximal	Date	2	2.0125	1.00626	2.71406	0.07818	0.0001 ***
	Ground_Cover	1	0.4214	0.42139	1.13655	0.01637	0.0997 .
	N_Fert	2	0.9551	0.47756	1.28806	0.0371	0.0047 **
	Date:Ground_Cover	2	0.784	0.39202	1.05736	0.03046	0.1937
	Date:N_Fert	4	1.5398	0.38494	1.03825	0.05982	0.2272
	Ground_Cover:N_Fert	2	0.7294	0.36472	0.98372	0.02834	0.5187
	Date:Ground_Cover:N_Fert	4	1.5023	0.37557	1.01299	0.05836	0.3608
	Residuals	48	17.7964	0.37076	0.69136		
Total	65	25.7409	1				
Final	N_Fert	2	0.95	0.47	1.20	0.037	0.0013 **
	Residuals	63	24.79	0.39	0.96		
	Total	65	25.74	1.00			

Partial RDA was then used to visualise differences in the composition of microbial communities that are attributable to N treatment after removing variation attributable to sampling date (Fig. 4). OTUs that were significantly associated with the treatment clustering were then superimposed on the ordination to visualise sample by taxa relationships (Fig. 4). This analysis separated the community associated

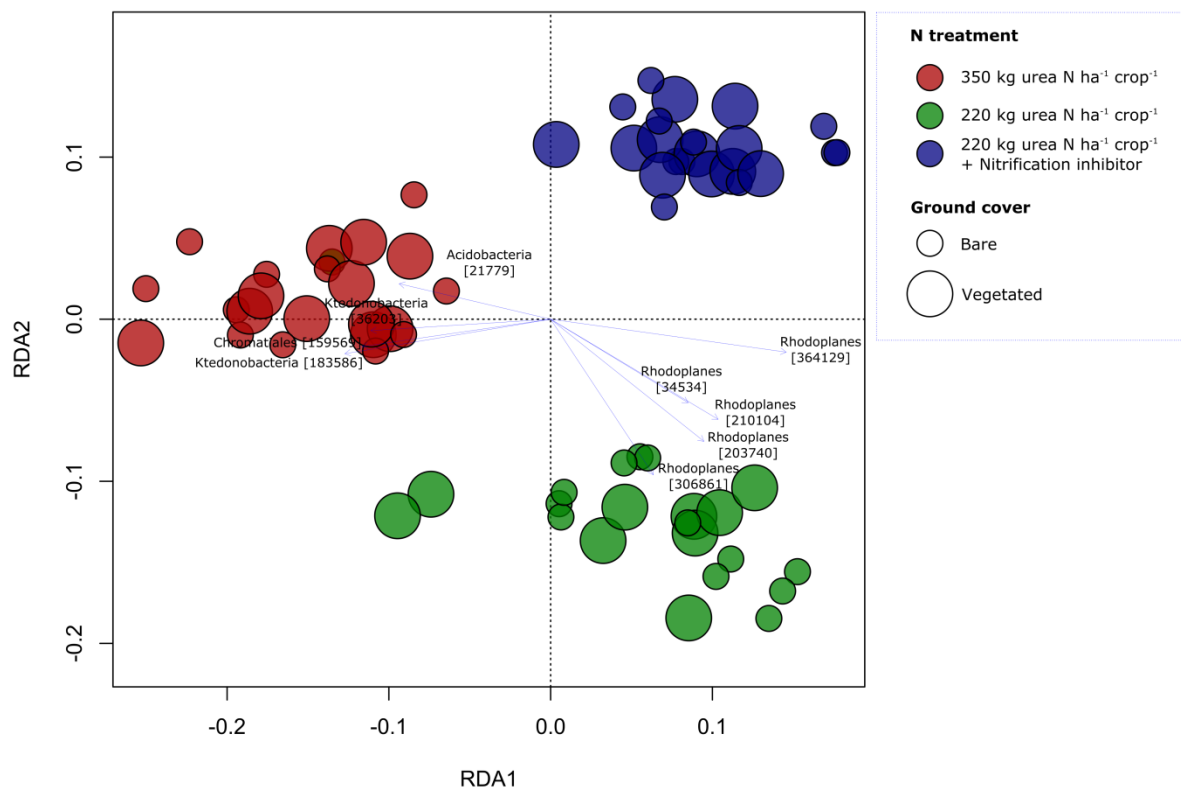


Fig. 4 Redundancy analysis ordination highlighting differences in the composition of bacterial communities that are attributable to ground cover and N treatment at site 2.

Conclusion

The use of Next Generation Sequencing using the Illumina MiSeq platform could significantly ($p < 0.001$) distinguish between banana soil management. At site 1 groundcover and nitrogen treatments could be separated, largely due to bacterial and archaea taxa associated nitrogen cycling. However, one *Pseudomonas* OTU, which are putative pathogen antagonistic bacteria was found to be associated with vegetated groundcover that was not associated with the bare soil treatment. Therefore, further analysis to determine the association between bacterial and archaea OTUs is warranted. At site 2 bacteria and archaea could distinguish between nitrogen treatments but not between vegetated and bare groundcovers. The differences in OTUs that characterised treatments at site 1 and site 2 indicated that generalisations could not be made in the response of bacterial and archaea taxa. Due to the large volume of data generated using NGS MiSeq analysis further analysis and correlations are required to link disease suppression with changes in the soil microbial community.

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Appendix 11 - The assessment of physiological methods for early, quantifiable stress and disease detection in banana plants

Katelyn Ferro

Background

Fusarium oxysporum f. sp. *ubense* Tropical Race 4 (TR4) was detected on a banana farm in north Queensland in March 2015, with a second incursion being confirmed in July 2017. These circumstances present a major risk to the viability of the \$600 million Australian banana industry. Surveillance for infected plants continues through regular on-ground monitoring of external symptoms of *Foc* TR4, which include lower leaf yellowing and pseudostem splitting. By the time these symptoms appear, the disease has already developed to an irremediable point and there is a high inoculum load in the plant material. Therefore pre-symptomatic detection could minimise potential disease spread by identifying plants before inoculum levels build and the risk of spread through soil movement is increased. Furthermore, plants suffering from abiotic stress have a reduced capacity to tolerate the presence of diseases and they are likely to succumb to infections more rapidly. Early stress detection can allow amelioration of environmental or nutritional issues in order to minimise disease potential.

The first objective of this study was to use rapid plant physiological assessment methods (proline accumulation in leaf tissue, chlorophyll fluorescence, chlorophyll content, stomatal conductance and thermal imaging) to elucidate the potential of effectively detecting plant stress (induced from abiotic factors) and disease (from *Foc* Race 1 infection) prior to the appearance of visual symptoms. The second objective was to use these tools for quantification of the severity of the stress and objectively measure leaf function and plant health.

The investigation was carried out in three parts: 1) a glasshouse pot trial which involved applying stress treatments to 'Williams' (Musa AAA) plants; 2) a glasshouse pot trial in which Dwarf Ducasse (Musa ABB) plants were inoculated with *Foc* Race 1; 3) a field trial where seven varieties with various susceptibilities to Race 1 were monitored on an infected property for their symptom expression. The results from the first pot trial were discussed in the BA14013 March Milestone Report, while the other two investigations are presented below.

PART 1: Monitoring the physiological response of Dwarf Ducasse plants infected with *Foc* R1 – pot trial

1. Methods

1.1. Plant material and growth conditions

This experiment examined the physiological response of Dwarf Ducasse plants to three treatments: inoculation of plants with *Foc* R1 (VCG 0124); exposure to fusaric acid (a phytotoxin secreted by *Foc*); and an untreated control. Based on the method performed by Dong et al. (2012), the roots of banana tube stock (originating from tissue culture) were soaked in a 2 mM fusaric acid solution for 48 hours. Plants belonging to the two other treatments were soaked in water for the same period. Following this, they were planted into 140 mm (2.5 L) pots filled with a 1:3 mixture of coarse river sand and soil (dermosol). Plants in the *Foc* R1 treatment were inoculated with colonised millet seeds as per a standard protocol developed by a DAF Senior Plant Pathologist, Wayne O'Neill. This involved gently loosening the top surface of the soil and incorporating 5 mL of seed. The 18 pots (six replications of three treatments) were arranged in a complete randomised block design on benches in the South Johnstone Research Station glasshouse. Liquid fertiliser was applied approximately

every 10 days and plants received overhead sprinkle irrigation for 10 min twice a day at 08:00 and 14:00. The trial was run from March to August 2017 (22 Weeks). Temperature and humidity were monitored in the glasshouse using a HOBO® data logger throughout the length of the trial; Photosynthetically Active Radiation (PAR) was measured from June using a Spectrum LightScout Quantum Light Sensor.

1.2. Stress response assessment

Stress assessments were performed prior to soaking plants in the fusaric acid or water solutions (week 0) and then at regular intervals (on average, every two weeks) following treatment application until 22 Weeks After Treatment (WAT). An effort was made to perform assessments at similar times in the day (and in order of replication) to minimise the effect of meteorological factors.

1.2.1 Agronomic characteristics and symptom development

To evaluate plant growth and development, plant height (cm), leaf emergence, and leaf area were measured. Leaf area was calculated using the equation proposed by Dépigny et al. (2015):

$$AFA = \left[\left(\frac{NLL-2}{2} \right) \times LA_{L1b} \right] + \left[\left(\frac{NLL-1}{2} \right) \times LA_{L3} \right] + (1.5 \times LA_{L1t})$$

Where, NLL is the Number of Living Leaves and LA is leaf area (calculated by $L \times W \times 0.8$) of the leaves of interest (newest fully emerged leaf (L1t), the third leaf (L3) and the lowest leaf in the canopy (L1b)).

External *Foc* symptom development was evaluated throughout the experimental period, while internal vascular discolouration of the pseudostem was destructively analysed at the end of the trial. The symptom rating systems adapted from Orjeda (1998) were used for these assessments (Appendix A).

1.2.2 Pigment concentration

The concentration of chlorophyll, anthocyanins, flavanols and the Nitrogen Balance Index (NBI) of each plant was measured using the optical sensor Dualix Scientific™. Calibration of this device permits the readings to be converted to pigment content in $\mu\text{g}/\text{cm}^2$. Four readings (two from each side) were taken from the third youngest leaf lamina throughout the experiment (Ravi et al. 2013), and on the second youngest (n-1) leaf from 8 WAT. Data from every leaf was collected on the last five assessment dates.

1.2.3 Chlorophyll fluorescence

Chlorophyll fluorescence was analysed using a FluorPen FP 100-MAX (Photon Systems Instruments). Three readings were taken from the left-hand side of the third leaf from each plant at each assessment date, and from the n-1 leaf at 8 – 22 WAT. Light-withholding clips were used to dark-adapt the leaves for 30 minutes prior to measuring fluorescence, which was done by exposing the dark-adapted leaves to a saturating light pulse ($3000 \mu\text{mol m}^{-2} \text{s}^{-1}$) of 1 ms duration. The minimum (F_o) and maximum (F_m) fluorescence values were used to calculate the fluorescence ratio F_v/F_m ($F_v = F_m - F_o$) and therefore detect changes induced by the stress treatments on the maximum quantum yield (QY) of PSII (an indicator of photosynthetic efficiency). It is suggested an optimal F_v/F_m value for most C3 plant species is around 0.83 (Björkman & Demming 1987, cited in Rolf & Scholes 2010).

1.2.4 Proline content

As per the method outlined by Shabnam et al. (2015), a 0.25 g sample of leaf tissue was taken from the third leaf. The sample was homogenised in 5 mL of sulfosalicylic acid, centrifuged, and then

filtered through Chux cloth. A 2 mL sample of supernatant was combined with 4 mL acid ninhydrin and placed in a boiling water bath for 30 min. The samples were cooled in an ice bath for a few minutes before pipetting each solution into the wells of a 96 well microplate and reading absorbance at 510 nm. Proline concentration was then calculated using a standard curve.

1.2.5 Stomatal conductance

Stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) was measured using a leaf porometer (model SC-1, Decagon Devices, Inc.). The instrument was calibrated prior to use on each assessment date following the manufacturer's guidelines. Two readings (from the proximal half of one side and the distal half of the other side) were taken on the third and n-1 leaves.

1.2.6 Thermal and NDVI Imaging

Thermal photos were taken of plant pseudostems using a SEEK thermal camera for Android, and NDVI photos were captured using a digital camera (Canon PowerShot SX280 HS) fitted with a NDVI lens. Each camera was positioned 30 cm from the pseudostem and horizontally aligned with a marker five centimetres above the soil surface. A white polystyrene and a brown cardboard background were used for the NDVI and thermal photos, respectively. Photos were captured in the glasshouse, with ambient and plant temperatures monitored using digital probe thermometers. An example of the set up can be seen in Figure 1.1.



Figure 1.1: Potted Dwarf Ducasse plants were placed in front of a plain cardboard background and 30 cm from the camera (not pictured) as the standard set-up for taking NDVI pictures of the pseudostem. A piece of polystyrene was placed behind for the thermal photos.

Image processing was performed by Joe El-Hayek (Mapping Officer, Biosecurity Queensland). Raw NDVI images were run through the NDVI processing tool in ESRI's Image Analysis toolkit with bands 3 (red) and 1 (infrared). A polygon outline was used to isolate and clip the pseudostem from the rest of the image, and the Raster Calculator Tool was applied to eliminate cells outside the pseudostem and below a certain NDVI (0.2 value) for non-plant objects (Figure 1.2). An average NDVI value for each pseudostem was then computed using these processed images. Raw thermal images were processed following the same procedure as the NDVI process to separate the pseudostem and re-scaled to make them as close as possible to the size of the NDVI images. A Fuzzy Overlay analysis used the OR method to sum the highest values (assigned to pixels) of these two images to highlight areas on the pseudostem which had both high temperature and low NDVI values – possibly identifying the point of infection at which the pseudostem xylem vessels were being affected by the fungus.

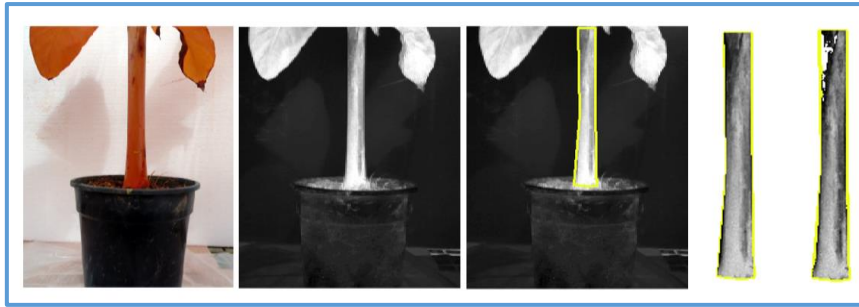


Figure 1.2: A depiction of how the pseudostems were isolated from the background of the NDVI image using ESRI's Image Analysis toolkit.

1.2.7 Statistical analysis

All statistical tests were performed using the software package GenStat 16th edition. A repeated measures analysis were carried out to compare the treatments to each other at a single point in time and across the trial period. Fisher's protected LSD tests (95% confidence interval) were performed to explore significant differences. Linear regression analyses were carried out to model potential correlations between the variables.

2. Results and Discussion

2.1. Agronomic characteristics and symptom development

The treatments did not have an effect on the plants' height, leaf emergence rate, or leaf area. As expected, there were significant differences ($p < 0.001$) over time as the plants continued to grow. All plants reached an average height of 33.5 cm. There was also no treatment effect on the rate of leaf emergence. Individual leaf area was calculated from Estimated Total Leaf Area divided by the total number of leaves. At first, the average area decreased slightly from the initial assessment (154 cm²) to 3 WAT (137 cm²), but then increased gradually to an average leaf size of 338 cm² by 22 WAT. There were significant changes in the total leaf area over time, however this did not occur in a chronological fashion. This was a result of variation in the total number of functional leaves on the plant, which generally fluctuated between 3 – 7 leaves. In saying that, the lowest leaf area was recorded prior to treatment application (455 cm²) and the highest was at the final assessment date 22 WAT (1709 cm²). Panama disease interferes with plant growth and development as it colonises the vascular system and inhibits water translocation (Ploetz 2015), so it would be expected that the agronomic performance of plants inoculated with R1 would be hindered, which was not the case. The lack of treatment effect is discussed in more detail below.

No external *Foc* symptoms were detected in the 22 week trial. Observed lower leaf yellowing was attributed to natural senescence of the older leaves as it was not symptomatic of chlorosis induced by *Foc* infection. No pseudostem splitting was observed in any of the treatments; there were no abnormalities in new leaf development, nor were the petioles displaying signs of damage or shattering. There may have been minor nutrient deficiency issues at certain stages of the pot trial, resulting in necrosis at the tips of some leaves, and residual damage from mites and aphids which were present on some plants prior to the start of the trial.

Plants were destructively analysed for internal infection symptoms after the trial ended in week 22. Sampling of the pseudostem five centimetres above the soil surface and at soil level did not reveal signs of vascular discolouration in any of the treatments. The corms were removed from the soil and sliced into quarters. Possible infection was identified in the lower quarter (Figure 1.3) of one of the

Foc R1 infected plants. This sample was further analysed by South Johnstone Research Station’s plant pathologist David East, who was only able to isolate and identify *Fusarium proliferatum* and *F. solani* but did not recover any *F. oxysporum*. He also took samples from the lower corm of two other *Foc* R1 inoculated plants and one of the control plants: all produced the same results as the first sample. This finding suggest that the inoculation treatment was ineffective, rather than plants being infected but asymptomatic.

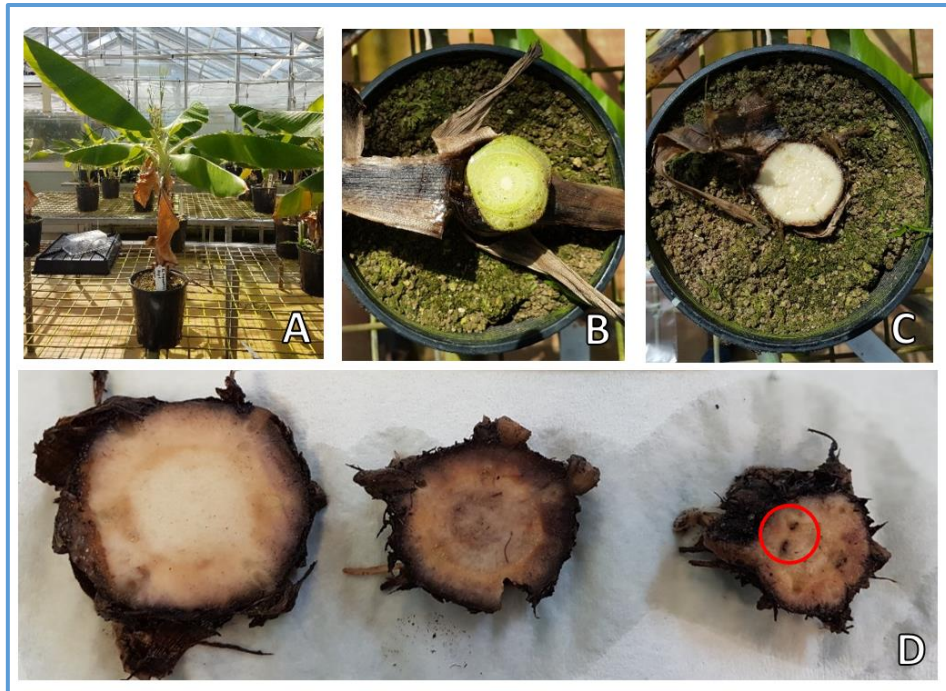


Figure 1.3: Internal and external rating of a R1 inoculated Dwarf Ducasse plant. A) Intact plant examined for symptoms; B) Pseudostem cross-section 5 cm above soil surface; C) Pseudostem cross-section flush with soil level; D) Corm cross-sections in top, middle and lower quarters. The red circle indicates suspicious discoloration which was later sampled for pathological analysis (no *Fusarium oxysporum* was recovered)

The lack of symptom development created difficulties in exploring the objective of this investigation, which was to test the effectiveness of several proximal sensing tools for early disease detection. The absence of diseased plants has resulted in data which does not offer clear answers to this question. As there was no treatment effect, the environmental influence on these ‘healthy’ plants was investigated instead. The average minimum, maximum and mean ambient temperatures the week preceding each assessment date were correlated to the physiological data collected. On the 24th July (17 weeks into the experiment) the glasshouse roof was cleaned to remove a thick layer of algae and mould. Following this event, the PAR increased by about five-fold (Figure 1.4). PAR data was only collected from June so could not be used to correlate with the data obtained from the proximal sensing tools, and only one assessment was performed after the roof was cleaned (22 WAT). Nonetheless, some of the data suggests that this change in PAR had a noticeable effect on plant performance, as discussed below.

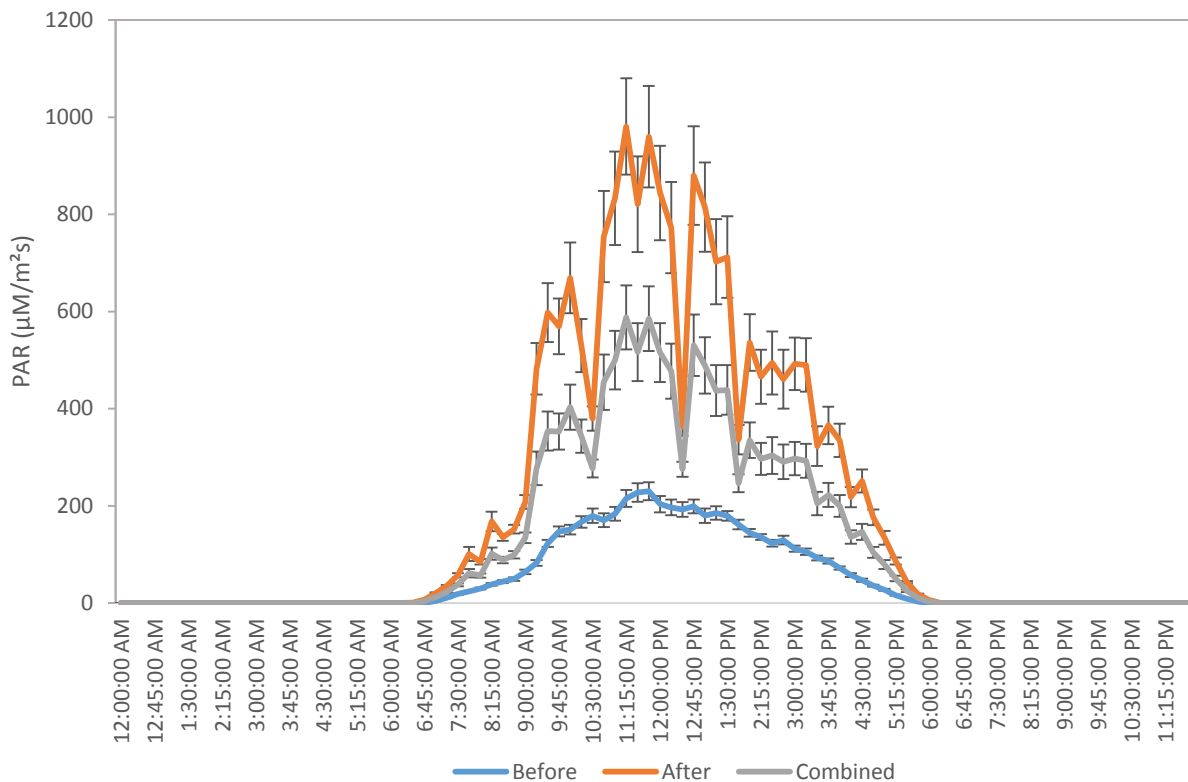


Figure 1.4: The average daily photosynthetically active radiation available to plants growing in the SJRS glasshouse before and after the roof was cleaned on the 24 July. The vertical bars display the standard error of the mean.

2.2. Leaf pigment concentrations

2.2.1. Chlorophyll

There were significant differences in Chl concentration at the different assessment dates, however these did not follow a pattern over time. In the third leaf, the concentration of $39.7 \mu\text{g}/\text{cm}^2$ at 16 WAT was significantly higher than at any other point in time. The initial ($25.7 \mu\text{g}/\text{cm}^2$) and final ($25.8 \mu\text{g}/\text{cm}^2$) concentrations were similar and were also the lowest readings from the trial. Increasing water stress can result in decreased leaf Chl content as chloroplasts are damaged from reactive oxygen species (Jajic et al. 2015). As a visual observation, the lamina of leaves appeared to fold to a greater degree at 22 WAT than in previous weeks. This phenomenon occurs because banana leaves are paraheliotropic and fold to reduce excessive light interception, as well as in response to soil moisture conditions (Thomas & Turner 2001). Soil moisture was not measured in this investigation, however it is likely that at 22 WAT plants were suffering from water stress more than at any other point in the trial due to reduced irrigation (done in an attempt to induce symptom development) and exposure to significantly more PAR during the day. An alternative explanation for the reduction in chlorophyll content at the final assessment date is that the plants had acclimated to an environment with higher light intensity and less Chl content was needed to reach photosynthetic capacity (Cessna et al. 2010). There were weak positive correlations between the Chl content and QY values of the third ($r^2 = 0.27$) and n-1 ($r^2 = 0.43$) leaves. If the leaf Chl concentration had reduced in response to water stress then it would be plausible that QY was negatively impacted for the same reason, supported by the findings in other studies which noted how photosynthetic efficiency can decline significantly as PAR increases (Hu et al. 2013).

The correlations between Chl content and the temperature data were poor. Overall, the Chl concentration in the third leaf (33.6 $\mu\text{g}/\text{cm}^2$) was statistically higher ($p = 0.002$) than the concentration measured in the n-1 leaf (32.0 $\mu\text{g}/\text{cm}^2$). However, the concentration in the third leaf was not higher than the n-1 leaf at all assessment dates due to Time ($p < 0.001$) and the Leaf x Time interaction ($p = 0.036$) also being significant sources of variation. The lower concentration of chlorophyll in the older leaf may be explained by Niinemets (2007), who noted that within-canopy variation in photosynthetic rates is chiefly driven by the degradation of Chl for nitrogen re-allocation from lower to upper canopy leaves as senescence is induced.

There were also significant differences ($p < 0.001$) between the chlorophyll concentration in the third leaf, the n-1 leaf and the average concentration of the whole plant at different assessment dates. This suggests that neither the third nor the n-1 leaf are a consistently accurate representation of whole-plant chlorophyll levels, however they can probably be used as estimates in cases where taking several measurements from each individual leaf is impractical.

2.2.2. *Flavonols*

Like the Chl measurements, there were significant differences ($p < 0.001$) in the Flav concentrations in both the third and n-1 leaves throughout the experimental period, however no trends are discernible. The leaf Flav concentrations do not correlate with the change in Chl concentrations over time ($r^2 = 0.009$). The initial concentration prior to treatment application was significantly higher (0.61 $\mu\text{g}/\text{cm}^2$) than at the final assessment date when flavonols were at their lowest (0.30 $\mu\text{g}/\text{cm}^2$). Flavonoids play several roles in protecting plants from stress (Mierziak et al. 2014) and have also demonstrated to possess antifungal properties (Galeotti et al. 2008). It is possible that simply measuring the concentration of flavonols is not an effective method of detecting stress in plants. There are a multitude of flavonoid classes each with different functions, so performing a more detailed analysis on the presence of particular compounds may be necessary in order to determine their relationship with banana stress response.

2.2.3. *Anthocyanins*

Anthocyanins are a minor pigment in Dwarf Ducasse banana leaves and present in very low concentrations. The first two assessment dates (pre-treatment and 1 WAT) show significantly higher levels of anthocyanin content in the third leaves than at the following assessment dates, but no correlation was observed between the concentration of this pigment and the other pigments measured. Furthermore, there were no notable correlations between anthocyanin concentration and the temperature data. While anthocyanins are induced in plant tissues in response to stress, not all anthocyanins have the same function (Kovinich et al. 2015). As was suggested with flavonols, it may be necessary to profile the particular anthocyanins present.

2.2.4. *Nitrogen Balance Index*

Again, time ($p < 0.001$) was the only source of variation in the NBI values. There was almost a two-fold increase in NBI between pre-treatment (45.0 NBI) and at 22 WAT (85.7 NBI). This increase did not follow a linear trend, and the latter value is only statistically similar to 80.1 NBI measured at 6 WAT. NBI is an estimate of leaf nitrogen content (Cerovic et al. 2015). Regular fertilisation of plants should mean that nitrogen deficiency was not a cause of stress in the present trial. Nitrate reductase and glutamate synthetase play a key role in nitrate assimilation and carbohydrate metabolism in plants and can be adversely impacted by high temperatures and drought stress (Xu & Zhou 2006). As a consequence, despite adequate fertiliser application, assimilation of nitrogen can be impeded. In theory, the measurements at the final assessment date should have displayed the lowest NBI as it was at this time they were assumed to be under the most stress (based on the climatic data).

Conversely, the average temperature for the preceding week and the NBI values of the n-1 leaf had a strong positive correlation ($r^2 = 0.80$). However, this relationship should be viewed with caution as it uses the average NBI values from only the last six assessment dates; more data needs to be collected for its validation. Furthermore, the NBI values from the third leaf correlated very poorly with any of the temperature comparisons and does not support the notion that these two variables are related.

2.2.5. Chlorophyll Fluorescence (F_v/F_m)

The negative correlation between QY measured on the third leaf and the maximum temperature average from the preceding week was one of the closer correlations ($r^2 = 0.46$) observed from the data (Figure 2.1). It suggests that the higher the maximum temperatures were one week prior to the assessment data, the lower the QY values are. Several studies have examined the decrease in fluorescence as stress conditions imposed on plants intensify (e.g. Zobayed et al. 2007, Razavi et al. 2008, Cowley & Luckett 2011, Batra et al. 2014, Ni et al. 2015). This physiological response is primarily a protective mechanism which aims to limit photochemical damage by deactivating light-harvesting antennae and increasing heat dissipation to remove excess energy (Ni et al. 2015). As a consequence, photosynthetic efficiency declines as less of the incoming solar radiation is being utilised for photosynthesis.

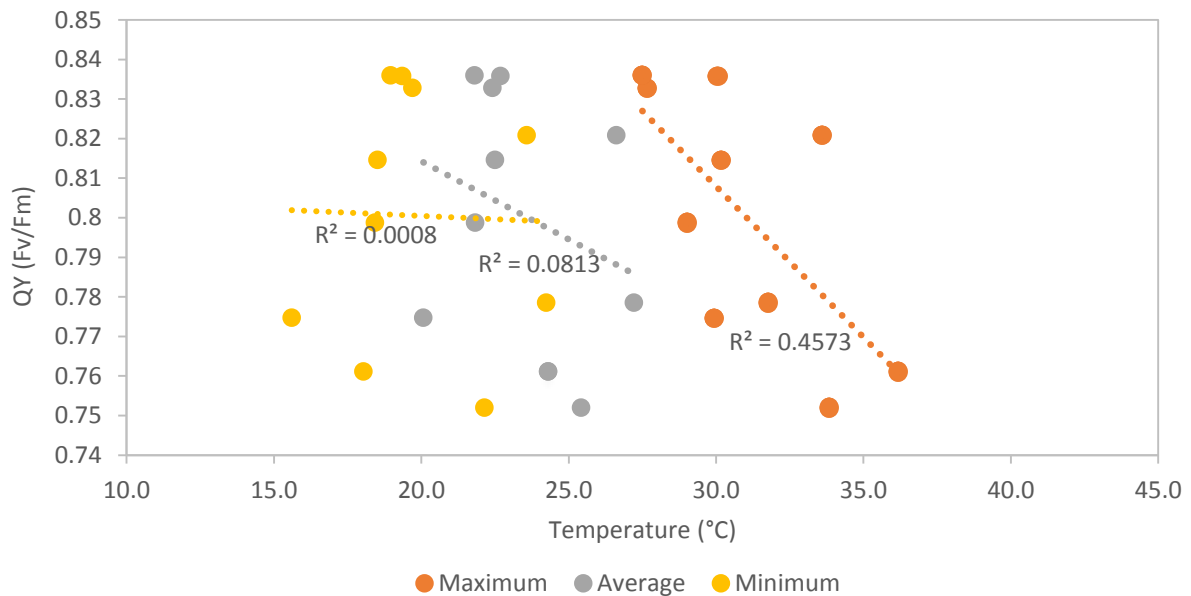


Figure 2.1: The mean values of the maximum, average and minimum temperatures one week preceding each assessment date and the corresponding QY value from the third leaf at the respective date. QY values are an average of all plants in the trial as there was no treatment effect.

QY values were the lowest at 3 WAT (0.75) and 22 WAT (0.76), where temperatures reached an average maximum of $33.8^{\circ}C$ and $36.2^{\circ}C$, respectively. In addition, these two weeks saw the temperature reach just above $41^{\circ}C$, the highest recorded temperatures throughout the trial. Some of the lowest maximum temperature averages corresponded with the QY values indicative of ‘non-stressed’ plants. Values at 8 (0.83), 14 (0.84) and 16 (0.84) WAT were statistically similar and correlated with some of the lowest maximum temperature averages of $27.6^{\circ}C$, $27.5^{\circ}C$ and $30.0^{\circ}C$, respectively.

The QY data from the n-1 leaf displayed a similar relationship, with the maximum temperature averages a week prior to the assessment dates potentially accounting for around half of the variation ($r^2 = 0.51$). There was a better correlation ($r^2 = 0.53$) between the minimum temperature averages and the QY values in the n-1 leaf than in the third leaf. The average temperature range

(that being, the difference between the average maximum and minimum temperatures for the week) had an even stronger correlation ($r^2 = 0.76$) with the QY values from the n-1 leaf (Figure 2.2), again supporting the concept that the decline in photosynthetic efficiency is a result of increased climatic stress. This relationship with temperature range was not as strong for the QY values measured in the third leaf ($r^2 = 0.33$), suggesting that more data is required to confirm the accuracy of the correlation in Figure 2.2.

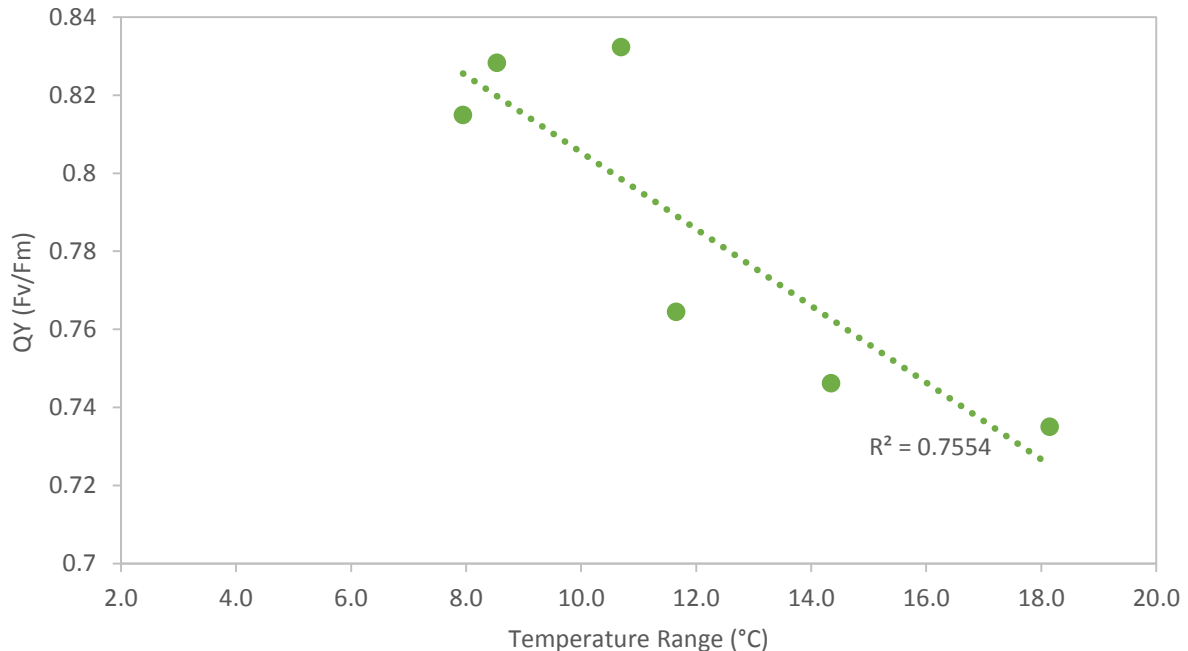


Figure 2.2: The temperature range (average maximum – average minimum) one week prior to the corresponding QY values taken on the n-1 leaf.

Comparing the available data from 8 – 22 WAT, the QY values recorded from third leaf differ significantly ($p < 0.001$) from those recorded from the n-1 leaf. A significant Time and Time x Leaf interaction were also observed. Only half of the QY values from the two leaves were statistically similar at the same assessment dates, and in all cases (apart from 22 WAT) the average values for the n-1 leaf were lower than those observed from the third leaf. As the plants were small with an average of five leaves, it is unlikely that shading and canopy architecture would have had a major impact on QY values (Niinemets et al. 2007). Although readings were taken from ‘functional’ leaves (meaning, > 50% green) and the n-1 leaves were not displaying signs of senescence, it is still possible that leaf-age contributed to this minor (yet significant) difference in QY values (Nath et al. 2013). Dong et al. (2016) was unable to detect significant differences in the photochemical efficiency of photosystem II (Fv/Fm) until after slight chlorosis was visible in the lower leaves (ten days after inoculating hydroponically grown ‘Gros Michel’ with *Foc*). Therefore it is possible that even if the inoculation treatment had worked, this measurement could not offer pre-symptomatic detection.

Several studies have found that the OJIP chlorophyll a fluorescence transient is a better parameter than Fv/Fm for evaluating plant stress (de Oliveira et al. 2009, Christen et al. 2007). It is a more sensitive measurement as it follows the fluorescence transient from the intensity at 20 μ s (F0), to the intensity at 2 ms (the J step), then at 30 ms (the I step), and finally at Fm (the P step) (Zushi et al. 2012). The fluorescence intensity at 300 μ s is also required to calculate the initial slope (Mo) of the relative variable fluorescence kinetics (Strauss et al. 2006). From these fluorescence measurements several biophysical parameters that quantify the energy flow through PSII can be derived. Christen et al. (2007) used this method to detect the vascular clogging esca disease in grapevines up to two

months prior to the appearance of foliar symptoms in situ. Although it takes longer to perform and analysis of this fluorescence data is much more complicated, future work should consider using this parameter instead of F_v/F_m .

2.2.6. Proline

There were a number of difficulties in estimating the level of proline in leaf tissue samples. The primary issue was the inability to create an up-to-date standard curve for calculating proline concentrations from absorbance readings. Three attempts were made but the prepared solutions did not display a colour change upon being heated in the hot-water bath. Consequently, the standard curve from the previous pot trial (discussed in the March milestone report) was used. The mean concentration of proline in the plants differed across the assessment dates, however these fluctuations were not found to correlate with any of the other measured variables. Despite the multitude of other studies which were able to demonstrate that the accumulation of proline appears to have a key role in stress tolerance (Kaur & Asthir 2015), the present investigation was unable to produce findings to support this concept. Ismail et al. (2004) observed a 66% increase in the concentration of proline in banana leaf tissue after plants were exposed to water stress (72% leaf relative water content). Their study, along with many of the others present in the literature, use the Bates et al. (1973) for estimating proline in leaf tissue. The present investigation followed the method by Shabnam et al. (2015) which was published as a more rapid, accurate and environmentally friendly protocol. It is not certain whether the unexpected results truly reflect the proline levels in the banana leaves, or if they have been influenced by issues with the method execution. Perhaps future work should revisit the Bates et al. (1973) protocol as it has been used more extensively.

The average temperature range from the preceding week appeared to have a loose correlation ($r^2 = 0.56$) with leaf proline levels; as the difference between the minimum and maximum temperature increased, so did the concentration of proline (Figure 2.3). However, removing the final assessment date from this data set reduces this correlation dramatically ($r^2 = 0.063$) and much more data over a greater range of temperatures should be examined.

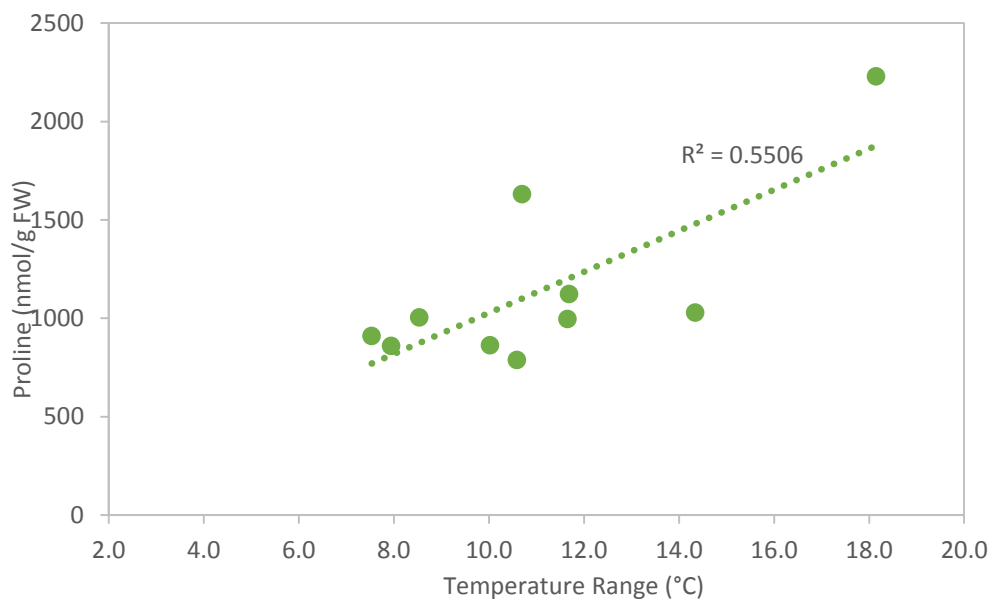


Figure 2.3: The temperature range (average maximum – average minimum) one week prior to the corresponding proline concentration values of the third leaf tissue.

2.2.7. Stomatal Conductance

Collecting stomatal conductance data proved to be much more time consuming than anticipated. As a result, the data was collected over periods of up to five hours throughout the day which may have had an impact on stomatal conductance which responds readily to diurnal factors (Robinson & Bower 1988). Also due to time constraints, conductance readings were only performed on seven out of the eleven assessment dates, consequently reducing the available data for analysis.

The cleaning of the glasshouse roof had a substantial impact on the amount of PAR available to the plants and also ambient temperature. There was a significant drop in stomatal conductance 22 WAT (the only assessment done after the roof was cleaned), which correlates with increased maximum temperatures and greater PAR. Stomatal conductance ranged from an average of 243 – 491 mmol m⁻² s⁻¹ in the assessment dates before the roof was cleaned, dropping to 44 mmol m² s⁻¹ at 22 WAT. These values are consistent with the findings of Ismail et al. (2004), where well-watered field-grown banana plants maintained stomatal conductance readings between 250 – 430 mmol m⁻² s⁻¹ and water stressed plants had readings of around 75 mmol m⁻² s⁻¹. Like with the QY values, the study by Dong et al. (2016) did not identify changes in stomatal conductance from the leaves of Foc infected banana plants until symptoms became visually observable. This indicates that measuring this gas exchange parameter may not offer pre-symptomatic disease detection but can still be useful in quantifying the level of stress (particularly water stress) that a plant is under.

2.2.8. Thermal and NDVI Imagery

A combination of thermal and NDVI imagery was used in an attempt to compensate for the shortcomings arising from only using one imaging method. The combination was hypothesised to provide a more accurate approach for detecting Foc infection as it decreases the likelihood of obtaining a false positive result. Unfortunately, re-sizing the thermal photos to fit the NDVI images resulted in overly pixilated images; the isolated pseudostems did not fit together due to differences in the nature of the camera quality, set-up and configuration. This prevented images from being overlaid as planned and limited the ability to perform a Fuzzy Overlay analysis. Another point to mention is that the figure for the temperature appearing on the thermal image has obscured some of the true data points after Fuzzy membership processing, as seen in Figure 2.4.

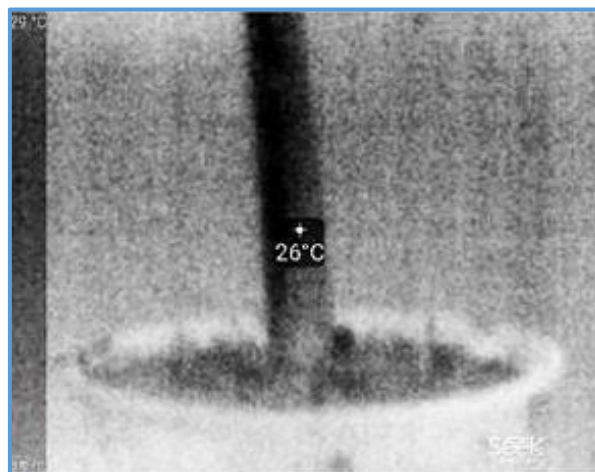


Figure 2.4: An example of how the temperature reading provided by the thermal camera interferes with image processing.

In the course of this research it was deemed that a simultaneous image capture method in which both images are captured at the same resolution, image scale and time would be required to

produce the necessary raw images required for successful processing. This may need to wait until: a) better resolution thermal cameras are made available at a consumer price point, and; b) a redesigned simultaneous image capture system to accommodate both cameras is configured.

PART 2: Monitoring the agronomic characteristics and chlorophyll activity of seven banana varieties grown in soil infected with *Foc* R1 (VCG 0124/5)– field trial

3. Methods

3.1. Trial establishment

Banana plants originated from tissue culture material provided by Sharon Hamill (Senior Principal Scientist, Maroochy Research Station). Seven varieties with varying susceptibilities to *Foc* Race 1 in tropical and sub-tropical environments were chosen for analysis. The varieties and their suspected susceptibilities are as follows:

- Williams (W): resistant
- Pisang Gajih Merah (PGM): susceptible in the sub-tropics
- Hom Thong Mokho (HTM): slowly susceptible in the sub-tropics
- FHIA-02 (F-2): slowly susceptible in the sub-tropics
- High Noon (HN): slowly susceptible
- Lady Finger (LF): susceptible
- Dwarf Ducasse (DD): very susceptible

They were planted in August 2015 on a known R1-infected property in South Johnstone, Queensland (Figure 3.1). The layout comprised of four single rows of eight replications (only seven reps for PGM and W, and six for HN). Plant variety was randomised within each replication. The property owner maintained the trial site through irrigating, fertilising, de-leafing, and bagging bunches as per his normal farm operations.



Figure 3.1: The field trial site on a South Johnstone farm with a known history of *Foc* Race 1.

3.2. Plant growth, yield and disease incidence

Data collection commenced in January 2016, five months after trial establishment. Plant growth and expression of symptoms were made at monthly intervals. Plant height (m) and leaf emergence were recorded to monitor the rate of growth. Plants were visually evaluated for leaf yellowing and pseudostem splitting based on a rating system originally described by Orjeda (1998) but adapted by Pattison et al. (2014) (Appendix B). Yield data (bunch weight, number of hands, number of fingers on the third and $n-1$ hand, and middle finger length of the third and $n-1$ hand) was collected upon harvest. After harvesting or at plant death, ratings were performed on the extent of internal vascular tissue discolouration using the methods described by Orjeda (1998) (Appendix B).

3.3. Monitoring for early disease development

The Dualex Scientific™ was used to measure the pigment content in every leaf in the canopies of the Williams and Dwarf Ducasse plants. Time limitations prevented this data being collected from every variety, so these two were chosen based on their resistance and susceptibility to R1, respectively. Three readings were taken per leaf to calculate an average. The idea was to quantify chlorophyll concentration in the leaves and elucidate subtle changes and patterns which may arise prior to symptoms becoming visually observable. In addition, one reading was taken from the third leaf of every plant to allow for varietal comparisons.

Chlorophyll fluorescence measurements were taken from February 2017 using the FluorPen FP 100-MAX (Photon Systems Instruments). Light-withholding clips were used dark-adapt the leaves for 30 minutes prior to measuring fluorescence. Measurements were taken from the same section of leaf as the pigment concentration readings.

4. Results and Discussion

4.1. Plant growth, yield and disease incidence

A summary of the agronomic data for the parent and 1st ratoon plants is outlined in Tables 4.1 and 4.2. All parent plants, with the exception of two HTM and one W, had been harvested (or died) by the end of the 24 month trial. Five out of the eight DD plants died before making it to bunching, and one had bunched but died before it was ready for harvest. Two DD survived through to harvest but the bunch sizes were meagre (2.8 and 13 kg) compared to what could be expected under better growing conditions (30.9 kg) (Daniells 2000). Seven of the 1st ratoon DD plants died before bunching, and the other survived to the end of the trial but had not thrown a bunch. One Lady Finger in the parent crop cycle expressed severe Fusarium wilt symptoms and died before bunching, with another succumbing to the disease before its bunch could be harvested. In the 1st ratoon cycle, three of the Lady Finger's died before bunching. None of the other varieties expressed external symptoms of *Foc* infection.

The average height at bunching of the PGM plants was 3.8 m for the parents, and 4.5 m for the 1st ratoon crop – almost one meter taller than any of the other varieties. This variety also had one of the fastest cycling times. Parent plants bunched at around 12.8 Months After Planting (MAP), and the 1st ratoon bunch came through around 3.1 months after the parent was harvested. The majority of 1st ratoon plants (and one 2nd ratoon plant) had bunched when the trial ended in September 2017, however were not ready for harvest.

Several issues arose with the trial design throughout the experimental period. For example, a large clumping bamboo (which bordered the first row of banana plants) grew substantially over the two year trial. It is likely that its root system and extensive foliage was interfering with the growth of

plants by competing for water and nutrients as well as blocking sunlight (particularly, the Rep 1 LF, HTM and W which were in the closest proximity). In addition, plants in the fourth row (reps G and H) were originally planted in-between Plantain varieties belonging to the grower. This again meant that these plants were in greater competition for resources compared to the other plants in the trial. Nematodes were also present in the block, and damage to several plants caused them to topple over upon bunching. These non-uniform and suboptimal growing conditions may help explain why there are large standard error values for some of the agronomic and yield data (Tables 4.1 and 4.2). One of the HTM replications was not included in the analysis as it turned out to be a Plantain variety which had accidentally been mixed up with the planting material.

4.2. Early disease detection

As yellowing occurs first in the older leaves (Ploetz 2015), the oldest and second-oldest leaves in the canopy were examined more closely for subtle changes in chlorophyll content which may occur prior to visual detection (Figure 4.1). The plants were divided into two groups based on whether the plant had visual external symptoms (most Dwarf Ducasse developed symptoms but some remained symptomless and apparently uninfected). The graphs suggest there is no obvious signal that would help with identifying a plant is under stress and visual symptoms are soon to appear. Upon the appearance of external symptoms, there is a noticeable decline in chlorophyll in several of the plants. However, for some of them, there is insufficient data from the preceding months to isolate specific trends. Research by Dong et al. (2016) had similar findings. Through monitoring photosynthetic activities, (including stomatal conductance, Fv/Fm, and leaf Chl content) significant differences were not observed between hydroponically grown 'Gros Michel' plant inoculated with *Foc* and uninoculated control plants until slight chlorosis symptoms were seen in the lower leaves. There are no clear trends in the Chl concentration of the unaffected plants (Figure 4.1). As discussed in section 2.2.1, Chl content can be influenced by biophysical properties of the leaf, leaf age, nutritional status and climatic factors. It is therefore unsurprising that there are fluctuations in the Chl content of leaves belonging to uninfected plants. Graphs were also generated for each leaf in the canopy to assist with identifying trends prior to the appearance of visual symptoms (data not shown). For example, does the chlorophyll concentration drop in the fifth leaf and increases in the fourth leaf a week before visual Panama disease symptoms appear? No patterns could be identified within the canopy which would allow detection of *Foc* infected plants.

Table 4.1: Agronomic characteristics from the Parent crop cycle of seven banana varieties growing in *Foc* R1 infected soil.

	Growth Rate		Yield				
	Height at bunching (m)	Leaf Emergence (new leaves/week)	Bunching to Harvest (days)	Bunch weight (kg)	Hand number	Fruit count	Fruit weight (g)
Dwarf Ducasse	2.0 ± 0.2	0.6 ± 0.06	85 ± 42.4	8 ± 5.1	6 ± 1.5	83 ± 39.3	85 ± 21.2
FHIA-2	2.5 ± 0.2	0.6 ± 0.05	109 ± 6.0	13 ± 0.2	6 ± 0.2	69 ± 2.7	185 ± 19.7
High Noon	2.8 ± 0.1	0.7 ± 0.06	85 ± 6.5	25 ± 0.6	7 ± 0.6	94 ± 9.7	276 ± 18.1
HTM	3.2 ± 0.2	0.6 ± 0.04	84 ± 14.4	11 ± 0.6	5 ± 0.6	62 ± 12.8	182 ± 15.4
Lady Finger	2.9 ± 0.2	0.7 ± 0.05	125 ± 8.9	12 ± 0.3	5 ± 0.3	57 ± 4.6	212 ± 36.6
PGM	3.8 ± 0.1	0.7 ± 0.06	139 ± 10.3	19 ± 0.6	7 ± 0.6	114 ± 14.9	174 ± 17.2
Williams	2.3 ± 0.1	0.6 ± 0.05	91 ± 5.1	20 ± 0.2	6 ± 0.2	94 ± 5.2	215 ± 7.0

Table 4.2: Agronomic characteristics from the 1st Ratoon crop cycle of seven banana varieties growing in *Foc* R1 infected soil.

	Growth Rate		Yield				
	Height at bunching (m)	Leaf Emergence (new leaves/week)	Bunching to Harvest (days)	Bunch weight (kg)	Hand number	Fruit count	Fruit weight (g)
Dwarf Ducasse	1.8 ± 0.2	0.4 ± 0.05	-	-	-	-	-
FHIA-2	3.0 ± 0.2	0.5 ± 0.06	111	27	8	112	241
High Noon	3.5 ± 0.3	0.6 ± 0.03	111	36	9	149	242
HTM	2.7 ± 0.4	0.6 ± 0.06	-	-	-	-	-
Lady Finger	3.6 ± 0.4	0.6 ± 0.07	-	-	-	-	-
PGM	4.5 ± 0.1	0.6 ± 0.04	110 ± 14.0	25.1 ± 0.5	7.5 ± 3.1	133.3 ± 10.8	187.7 ± 8.1
Williams	2.3 ± 0.2	0.5 ± 0.03	-	-	-	-	-

A residual maximum likelihood (REML) repeated measures analysis was conducted on the third leaf chlorophyll data from each variety. Various covariance models were fitted with the ante-dependence model of order one being most appropriate. The ante-dependence model reflects the dependence of a measure on previous observations for the same subject without the assumption of stationarity. There was a significant main effect of both time (MAP) and variety ($p < 0.001$), as well as a significant interaction ($p < 0.001$). Due to the large number of pairwise comparisons for the interaction, separate comparisons are made between varieties at each time assessment, and between times within each variety. In Table 4.3 pairwise comparisons are made comparing the Chl concentration within each variety over time (down the columns). For varieties F-2, HN, HTM, LF and PGM, the mean chlorophyll reading at 5 MAP is significantly lower than all other times. For all varieties except HN and LF there is a significant drop in the means between 14 MAP and 16 MAP. Table 4.4 compares each variety at the different points in time. When comparisons are made between the varieties at each assessment, only 6 MAP found no significant differences. Variety DD had the lowest mean chlorophyll reading at 8 of the 12 assessments. At seven of these eight assessments, the mean for F-2, HN and PGM were significantly higher than the mean for DD.

Chlorophyll content is influenced by plant growth stage, leaf age, and edaphic or climatic factors which may cause chlorosis (Merzlyak et al. 1999; Chapman & Barreto 1997). In addition, leaf thickness and water content have been found to influence readings taken using Chl meters; these variables and may differ both between varieties and over time (Marenco et al. 2009). All these factors were likely to contribute to the fluctuating Chl content over the trial period. Differences in plants which were at the same stage of development demonstrates that inter-varietal variation is a trait which needs to be taken into consideration when comparing different varieties and when drawing conclusions on their health status based on leaf chlorophyll content

Due to the late purchasing of the fluorometer, chlorophyll fluorescence data was only collected from February 2017. The lack of long-term fluorescence data prevented the creation of graphs like what was used to analyse the Chl content data, and also made other forms of analysis difficult. The relationship between the Chl content and QY was modelled (Figure 4.2). There was a stronger non-linear correlation between the chlorophyll concentration and the chlorophyll fluorescence data collected from DD leaves ($r^2 = 0.46$) than there was from the W leaves ($r^2 = 0.16$). This was potentially due to the higher incidence of lower leaf yellowing in the DD plants which resulted in more data for leaves with lower chlorophyll concentrations to be evaluated against the corresponding QY values. This suggests that measuring QY may also assist with quantifying the stress status of banana plants which are already displaying signs of ill-health (such as leaf chlorosis).

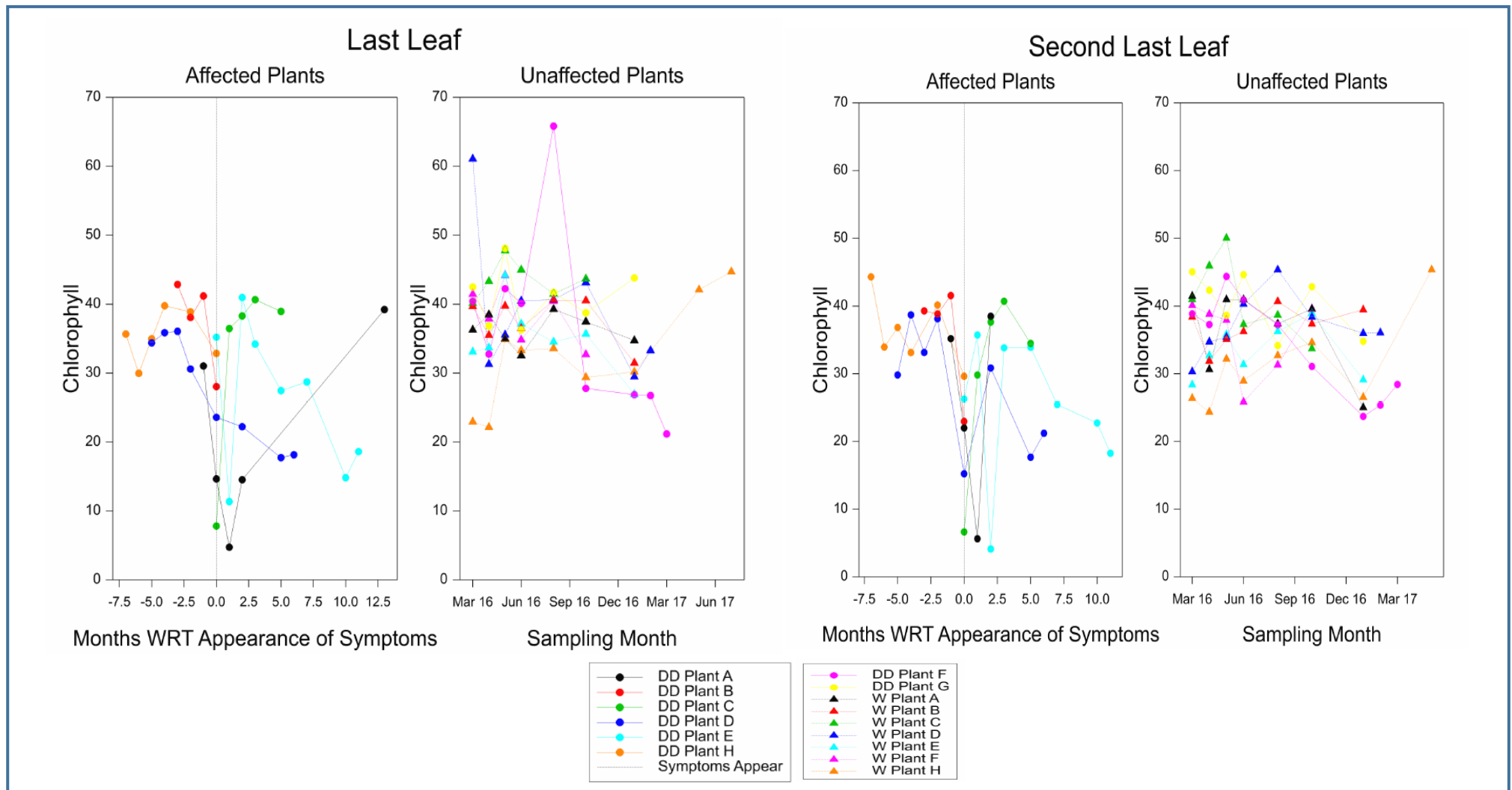


Figure 4.1: Graphs on the left are for affected plants, while healthy unaffected plants are graphed on the right for the last and second last leaves in the canopy. The points on the graph for affected plants are with respect to the month in which the symptoms first appeared. The month when symptoms first appeared is represented as 0 on the horizontal axis. Negative values on the horizontal axis are samples taken prior to symptoms appearing, while positive values represent samples after symptoms first appeared. For example, -3 represents results obtained 3 months prior to symptoms first appearing, and +3 is 3 months after symptoms first appeared

Table 4.3: Pairwise comparisons are made comparing the difference in chlorophyll content across the Months After Planting (MAP) within each variety. Values indicated the chlorophyll concentration ($\mu\text{g}/\text{cm}^2$) \pm the standard error of the mean. Values which do not share a letter in the same column are statistically different ($p < 0.05$).

MAP	Variety						
	Dwarf Ducasse	FHIA-2	High Noon	HTM	Lady Finger	PGM	Williams
5	29.3 \pm 2.2 ce	27.1 \pm 2.3 g	26.1 \pm 2.5 d	20.7 \pm 2.3 c	27.6 \pm 2.2 g	27.6 \pm 2.3 f	26.3 \pm 2.3 e
6	37.5 \pm 2.5 ab	36.9 \pm 2.5 ef	39.3 \pm 2.9 c	37.4 \pm 2.7 ab	38.5 \pm 2.5 bcde	36.3 \pm 2.7 d	38.1 \pm 2.7 abc
7	36.7 \pm 1.9 a	35.3 \pm 1.9 f	41.4 \pm 2.2 c	39.2 \pm 2.1 ab	42.3 \pm 1.9 bcf	43.0 \pm 2.1 ce	36.4 \pm 2.1 bcd
8	31.6 \pm 2.1 bce	41.7 \pm 2.1 cde	42.4 \pm 2.4 bc	37.9 \pm 2.2 ab	35.7 \pm 2.1 de	41.0 \pm 2.2 cde	33.3 \pm 2.2 cd
9	37.5 \pm 2.9 ab	40.6 \pm 2.9 bcdef	47.0 \pm 3.4 abd	35.5 \pm 3.1 ab	35.7 \pm 2.9 ef	38.2 \pm 3.1 de	34.2 \pm 3.1 cd
10	32.4 \pm 2.4 abc	43.3 \pm 2.3 acbde	48.8 \pm 2.6 ab	39.8 \pm 2.4 ab	41.0 \pm 2.3 bcd	41.8 \pm 2.4 cde	39.0 \pm 2.4 abc
11	36.0 \pm 2.1 ab	45.3 \pm 1.8 abcd	50.6 \pm 2.1 a	36.7 \pm 1.9 ab	38.5 \pm 1.8 cde	46.7 \pm 1.9 abc	38.4 \pm 1.9 abc
12	36.0 \pm 2.4 ab	44.7 \pm 2.2 abcd	53.4 \pm 3.3 a	38.5 \pm 2.2 ab	45.4 \pm 2.9 ab	50.8 \pm 2.6 ab	42.6 \pm 2.4 a
14	31.9 \pm 2.4 abc	48.0 \pm 2.0 a	53.1 \pm 2.3 a	41.8 \pm 2.1 a	50.4 \pm 2.1 a	51.6 \pm 2.1 a	42.0 \pm 2.1 ab
16	22.2 \pm 4.2 ef	41.3 \pm 3.5 def	46.4 \pm 5.6 abc	30.8 \pm 3.8 b	48.5 \pm 3.8 ab	45.2 \pm 3.8 bcd	31.0 \pm 3.4 ce
17	28.7 \pm 4.4 abcd	46.8 \pm 3.7 abc	44.3 \pm 7.1 abc	35.9 \pm 3.9 ab	48.0 \pm 4.9 ab	54.7 \pm 4.1 a	37.3 \pm 3.5 abd
18	22.5 \pm 2.0 df	47.7 \pm 1.9 abd	46.4 \pm 3.4 abc	39.7 \pm 2.0 ab	46.4 \pm 2.4 ab	53.6 \pm 2.3 a	45.4 \pm 2.2 a

Table 4.4: Pairwise comparisons are made comparing the difference in chlorophyll content across the seven varieties at each Month After Planting (MAP). Values indicated the chlorophyll concentration ($\mu\text{g}/\text{cm}^2$) \pm the standard error of the mean. Values which do not share a letter in the same column are statistically different ($p < 0.05$).

	Month After Planting											
	5	6	7	8	9	10	11	12	14	16	17	18
DD	29.3 \pm a 2.2	37.5 \pm a 2.5	36.7 \pm bc 1.9	31.6 \pm d 2.1	37.5 \pm b 2.9	32.4 \pm c 2.4	36.0 \pm b 2.1	36.0 \pm d 2.4	31.9 \pm c 2.4	22.2 \pm c 4.2	28.7 \pm c 4.4	22.5 \pm d 2.0
F-2	27.1 \pm ab 2.3	36.9 \pm a 2.5	35.3 \pm c 1.9	41.7 \pm a 2.1	40.6 \pm ab 2.9	43.3 \pm ab 2.3	45.3 \pm a 1.8	44.7 \pm bc 2.2	48.0 \pm a 2.0	41.3 \pm ab 3.5	46.8 \pm ab 3.7	47.7 \pm ab 1.9
HN	26.1 \pm ab 2.5	39.3 \pm a 2.9	41.4 \pm ab 2.2	42.4 \pm a 2.4	47.0 \pm a 3.4	48.8 \pm a 2.6	50.6 \pm a 2.1	53.4 \pm a 3.3	53.1 \pm a 2.3	46.4 \pm a 5.6	44.3 \pm abc 7.1	46.4 \pm abc 3.4
HTM	20.7 \pm b 2.3	37.4 \pm a 2.7	39.2 \pm abc 2.1	37.9 \pm abc 2.2	35.5 \pm b 3.1	39.8 \pm b 2.4	36.7 \pm b 1.9	38.5 \pm d 2.2	41.8 \pm b 2.1	30.8 \pm bc 3.8	35.9 \pm bc 3.9	39.7 \pm c 2.0
LF	27.6 \pm a 2.2	38.5 \pm a 2.5	42.3 \pm a 1.9	35.7 \pm bcd 2.1	35.7 \pm b 2.9	41.0 \pm b 2.3	38.5 \pm b 1.8	45.4 \pm abc 2.9	50.4 \pm a 2.1	48.5 \pm a 3.8	48.0 \pm ab 4.9	46.4 \pm bc 2.4
PGM	27.6 \pm a 2.3	36.3 \pm a 2.7	43.0 \pm a 2.1	41.0 \pm ab 2.2	38.2 \pm ab 3.1	41.8 \pm b 2.4	46.7 \pm a 1.9	50.8 \pm ab 2.6	51.6 \pm a 2.1	45.2 \pm a 3.8	54.7 \pm a 4.1	53.6 \pm a 2.3
W	26.3 \pm ab 2.3	38.1 \pm a 2.7	36.4 \pm bc 2.1	33.3 \pm cd 2.2	34.2 \pm b 3.1	39.0 \pm bc 2.4	38.4 \pm b 1.9	42.6 \pm cd 2.4	42.0 \pm b 2.1	31.0 \pm bc 3.4	37.3 \pm b 3.5	45.4 \pm b 2.2



Figure 4.2: The correlation between chlorophyll concentration and chlorophyll fluorescence data collected from Williams and Dwarf Ducasse plants growing in a field trial with *Foc* Race 1 infected soil.

The fluorometer used in this trial was a relatively basic instrument, and perhaps more sophisticated models would have greater sensitivity to early changes in photosystem activity in the leaf tissue. Furthermore, the optical lens of the fluorometer developed small scratches on it simply from being used. When contacted, the representative from ICT International assured that these abrasions would supposedly not influence the accuracy of the Fv/Fm readings. As previously discussed in section 2.3, the OJIP parameters may be a better chlorophyll fluorescence parameter for detecting early stress and disease in plants.

A notable barrier to performing chlorophyll fluorescence measurements in-field is the impracticality of attaching the dark-adapting leaf clips to the leaves of banana plants. Using a ladder, it took 15 – 20 minutes to attach three leaf clips to each leaf of a mature (2 – 3 m) plant, preceded by a 30 minute wait for the dark-adaption process, and then another 15 – 20 minutes to take the readings and record the data. Perhaps the use of equipment, such as a bagging machine, could make the execution of this task more efficient.

5. Conclusions

This multicomponent investigation examined several proximal sensing methods which scrutinised the early physiological responses of banana plants to stress and disease. Measuring the concentration of plant pigments (particularly chlorophyll) assisted in determining the severity of stress or Foc infection, due to the relationship this pigment has with leaf function. Considerations need to be made for inherent differences in chlorophyll concentrations across the different banana varieties when using this variable as an indicator of plant health. Measuring chlorophyll fluorescence may also be useful for quantifying stress severity, however the instrument and method used in the present trials need to be reconsidered and improved for future work if this is to be a tool for pre-symptomatic disease detection. In this study, stomatal conductance, levels of proline accumulation and thermography could not be verified as useful measurements for stress quantification or disease detection in banana plants, and further work is required before they are worth incorporating into early detection protocols. In addition, future trials involving inoculation treatments may wish to sample plants throughout the investigation to ensure infection has occurred so that the results are able to meet the experimental objective.

The outcome of this study highlights the importance of having well-trained biosecurity staff for surveying properties at-risk of contracting Panama disease, and having farm staff who are on the lookout for suspicious plants. At this stage, visual observations of external disease symptoms (primarily lower leaf yellowing and pseudostem splitting) remains the earliest way of detecting plants infected with Foc.

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Appendix A –

The internal and external rating systems used for evaluation of *Foc* infection adapted from Orjeda (1998).

External Symptoms:

Rated on a scale of 1 – 3, where: 1 = absent, 2 = mild, 3 = severe.

- A. Yellowing of foliage
- B. Splitting of pseudostem base
- C. Changes in new leaves (irregular pale margins, narrowing, burning plus ripping of lamina and becoming more erect)
- D. Petiole collapse

Internal Symptoms:

Vascular discoloration percentage rating system.



1. Corm completely clean, no vascular discoloration.



2. Isolated points of discoloration in vascular tissue.



3. Discoloration of up to 1/3 of vascular tissue.



4. Discoloration of between 1/3 and 2/3 of vascular tissue.



5. Discoloration greater than 2/3 of vascular tissue.



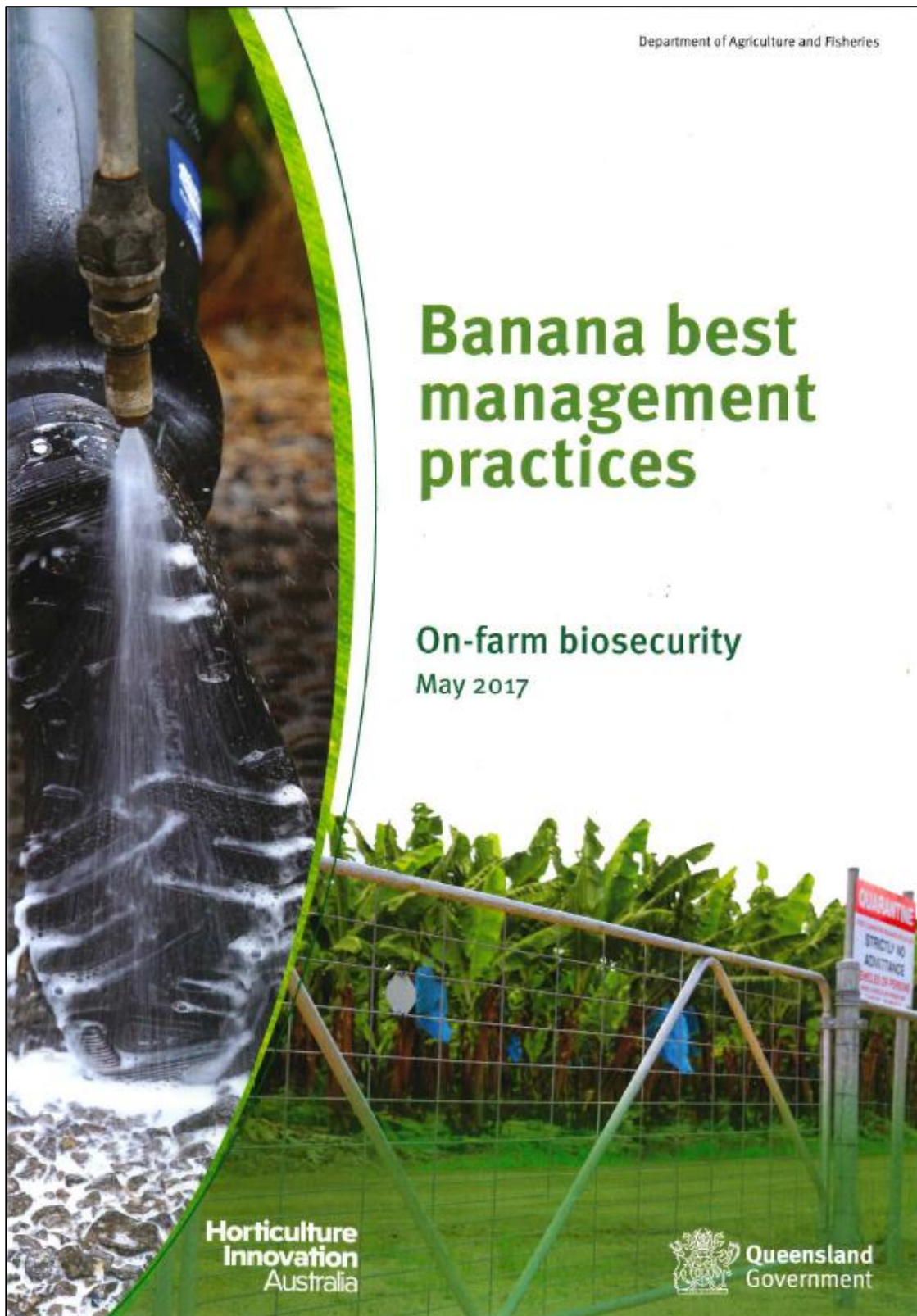
6. Total discoloration of vascular tissue.

Appendix B –

Growth, external and internal symptoms of *Fusarium oxysporum* f. sp. *ubense* ratings for banana (adapted from Pattison et al. 2014):

Banana growth and development stages	External symptoms description	Internal symptoms description
Bits	1. Plant showing no symptoms	1. No vascular discolouration
Emergence	2. Early symptoms of pseudostem splitting but no leaf yellowing	2. Isolated points of discolouration in vascular tissue of the pseudostem
15 leaf stage	3. Plant showing slight streaking and/or yellowing of lower leaves and/or pseudostem splitting	3. Discolouration of up to one-third of vascular tissue of the pseudostem
25 leaf stage	4. Plant showing streaking and/or yellowing of majority of lower leaves and/or slight streaking and/or yellowing of younger leaves and/or some pseudostem splitting	4. Discolouration of between one-third and two-thirds of the vascular tissue of the pseudostem
Bunch emergence	5. Plants showing extensive streaking and/or yellowing of most or all of the leaves and/or extensive pseudostem splitting	5. Discolouration of greater than two-thirds of the vascular tissue of the pseudostem
Bract fall	6. Mother plant dead, sucker alive	6. Total discolouration of vascular tissue of the pseudostem
Half mature bunch	7. Mother plant and sucker dead	
Mature bunch		

Appendix 12 – Banana best management practices guide: On-farm biosecurity, May 2017



Appendix 13: The economics of banana biosecurity – modelled economic scenarios for biosecurity practices and alternative production systems

Activities have been undertaken to examine costs associated with the adoption of identified biosecurity practices and to compare productivity from alternative Cavendish production systems in the presence of the disease. A detailed analysis of the costs associated with both biosecurity adoption and alternative Cavendish production systems is difficult due to gaps in our current knowledge. Adoption of biosecurity practices is influenced by the topographical and geographical nature of each individual property, owner acceptance of risk, the financial capacity of each business and access to reliable information on input levels for some practices. This makes it very difficult to make a generalised assessment of the cost of adoption as it depends significantly on the attributes of each individual property. Economic comparisons of alternative Cavendish production systems are restricted by a lack of data on disease response achieved with the alternative systems.

As a result the two assessments are done in different ways. For biosecurity practice adoption the analysis compares the capital and operating costs incurred to protect a hypothetical 56 ha property free of flooding and significant overland flow for contiguous and non-contiguous scenarios. For the alternative production systems the analysis takes the form of relative comparisons to contrast the productivity of alternative Cavendish variety scenarios (lower productivity, increasing disease mortality, additional fallowing required) with disease-free standard Cavendish systems.

The outputs of these analyses have significant implications for resource allocation and future actions, including R&D investment, in spite of their inherent limitations.

Comparing the costs of implementing biosecurity practices to manage *Foc* TR4 spread

The development of the modelled scenarios is based on the application of the principle of exclusion of all non-essential vehicles, machinery, tools and visitors and application of differential access to the property using zoning. The modelled scenarios worked from this basis drawing on specific practice examples from the Biosecurity Queensland Standards and Guidelines, the ABGC Biosecurity extension project and observed practices adopted by banana producers in FNQ region. To better reflect the reality of implementing effective biosecurity practices the hypothetical property is an amalgamation of real properties known to the author and the suite of practices chosen for the model scenarios reflect the level of risk inherent in their geography and topography.

The key changes reflected in the adoption of the suite of biosecurity practices are in significant capital investment to undertake:

- Duplication of machinery
- Fencing to restrict property access
- Installation of wash down facilities, footwear exchange facilities and foot baths
- Provision of farm specific footwear for staff and visitors
- Provision of disinfectant application equipment

Changes in operating costs and inputs largely revolve around disinfectant products and additional labour costs associated with compliance activities eg. Vehicle and machinery washing and disinfection, footwear exchange etc.

The modelled farm scenarios were based around a hypothetical 56 ha (small/medium) farm and compared a scenario of a contiguous land area with access managed at a single point to a non-contiguous land area comprised of 3 parcels separated by public roads.

The assumptions and results underlying these two scenarios are presented in Table 1.

Table 1. Assumed practices and costings for comparison biosecurity practice adoption for contiguous and non-contiguous scenarios on a 56 Ha hypothetical farm

Key Changes	Scenario – contiguous	Scenario – 3 separate parcels
Duplication of machinery	Lime spreader - \$35000 Farm vehicle - \$30000	Lime spreader - \$35000 Farm vehicle - \$30000 Bagging machine - \$100000 Tractor - \$45000 Quad bike - \$15000 Picking trailer modifications - \$1200
Fencing	Boundary fencing undertaken by contractor, 3510m at \$8/m - \$28080	Boundary fencing undertaken by contractor, 5820m at \$8/m - \$46560
Washing/boot change facilities	Compliant wash-down - \$50000 Earthworks/ballast/concrete - \$17000 Footbaths – 3 at \$1100 - \$3300 Shuttle and electric pump - \$700 Boot room (shipping container) - \$6000 Boot change points (internal) - \$500	Compliant wash-down X 3 - \$150000 Earthworks/ballast/concrete X 3 - \$35000 Footbaths – \$1100 X 6 - \$6600 Shuttle and electric pump X 3 - \$2100 Boot room (shipping container) - \$6000 Boot change points (internal) X 3 - \$1500
Provision of footwear	1.75 sets of rubber boots at \$25/pair for 30 staff - \$1320	4.3 sets of rubber boots at \$25/pair for 30 staff - \$2700
Disinfectant product	Spray application - 6000L/yr at \$0.14/L of 1% mixture of DDAC - \$840 Footbath sanitiser replacement – 3 footbaths at 100L replaced weekly at \$0.14/L - \$2184	Spray application - 12000L/yr at \$0.14/L of 1% mixture of DDAC - \$1680 Footbath sanitiser replacement – 6 footbaths at 100L replaced weekly at \$0.14/L - \$4368
Labour inputs	Wash down, 4 hours per week at \$21.45/hr - \$4462	Wash down, 22 hours per week at \$21.45/hr - \$24540
Capital costs	\$172000 \$1230/acre; \$3070/ha	\$477000 \$3405/acre; \$8512/ha
Operating costs	\$7500 \$53/acre; \$134/ha	\$30590 \$218/acre; \$546/ha

Conclusions

The economic assessment of the cost of implementing effective biosecurity practices has been difficult due to the influence of individual circumstances on each property. The ease with which the principle of exclusion can be applied and the management of access via the implementation of zones on farm significantly influences the cost of implementing biosecurity practices. The biggest influence on this is whether the property is a contiguous land area or is separated by public roads. As a result the project has tried to compare the two scenarios for a hypothetical 56 ha farm implementing effective biosecurity practices observed on a range of banana farms. An extrapolation of these results to the whole FNQ industry is presented below.

Scenario	Cost across industry
FNQ industry (11,150 ha) – contiguous scenario	\$34,230,500 capital \$1,500,000 operating annually
FNQ industry (11,150 ha) – separated scenario	\$94,909,000 capital \$6,090,000 operating annually
FNQ industry (11,150 ha) – extrapolation for 1:3 contiguous/separate scenario	\$79,740,000 capital \$4,940,000 operating annually

The results indicate the significant scale of investment required to implement biosecurity practices across the industry, the relative advantage a contiguous property has over a non-contiguous situation and the significant increase in on-going operating costs associated with washing vehicles and machinery in a separated situation. It is difficult to estimate how much of the industry falls into each situation so an extrapolated scenario where 3 out of 4 properties is non-contiguous has been also been presented.

While a direct extrapolation to the whole of the NQ industry is crude due to the inherent inaccuracies built into this assessment that have been outlined, there are a number of points to note. Firstly that the capital investment and on-going operating costs required to protect a non-contiguous farm can be a significant barrier to implementation. This is reflected in evaluation results from the ABCG Biosecurity extension project that found money/cost and time as the two main reasons provided as to why surveyed producers had not implemented biosecurity practices. Lack of knowledge about Panama disease TR4 and its risks and pathways was not reported as a major barrier. The second key point is about the scale of potential R&D investments to protect the industry. For example, if the industry opted to invest directly in a breeding program that succeeded in developing a resistant variety with acceptable production and consumer characteristics then large investments of up to \$10M could still be regarded as providing excellent value for money if it removed the need to implement practices of this nature. Unfortunately this does not address the risk associated with other exotic pest or disease incursions some of which would be mitigated by the implementation of the stated practices.

Comparison of production from alternative production systems in the presence of the disease

Direct economic comparisons of alternative Cavendish production systems are difficult because of the significant knowledge gaps for the performance and disease response of the various options. As a result an alternative approach comparing the productivity of the alternative systems has been undertaken because we have access to some local varietal production data, disease resistance data from the Philippines and cropping system information from the last NT producer.

Our current understanding of requirements for a successful disease suppressive production system is based on *Foc* TR4 inoculum reduction and suppression as well as genetic resistance. As a result the currently postulated alternative production system would use GCTCV 218 as the most productive Cavendish variety with resistance and aim for fewer crop cycles before a fallow period free of bananas. Based on experiences in the Philippines, Taiwan and China the best estimate of a the system timeline would be 4 crop cycles followed by 3 years fallow period giving a 7 year cycle of cropping and fallowing. Thus a 7 year period has been used as a basis to model all the alternative production system to allow a meaningful comparison of different crop cycle and fallow periods. Allowances for cumulative plant mortality has also been incorporated into the calculation to reflect the relative varietal susceptibility, where it is known, based on NT or Philippine data and observations.

The assessment compares productivity data for Williams Cavendish in north Queensland free of *Foc* TR4 with a number of alternative scenarios:

- Use of GCTCV 218 (Formosana) grown in a suppressive farming system that includes limited cropping cycles and long term fallows
- Use of 219 (improved selection of GCTCV 119 selected in the Philippines) grown in a suppressive farming system with a standard 1 year fallow
- Use of Williams grown in a NT-style farming system that includes limited cropping cycles and long term fallows

The assumptions that form the basis for the alternative production systems to compare are:

Williams Cavendish (*Foc* TR4 free)

- Full crop cycles for the 7 year period
- Average crop cycle length over period is 40 weeks
- Plant crop and 6.8 ratoons for 6 years
- 1 year fallow
- Planted at 1700 plants/ha, no cumulative mortality attributed to *Foc* TR4
- Average bunch mass based on trial data – 22 kg plant crop, 33 kg ratoon crop
- Marketable pack-out calculated assuming 20% reject rate and 10% attribution of bunch mass for bunch stalk
- Assume packing 13.75 kg per carton

GCTCV 218 (suppressive production system)

- Limited crop cycles for the 7 year period
- Average crop cycle length over period is 52 weeks
- Plant crop and 3 ratoons for 4 years
- 3 year fallow
- Planted at 1700 plants/ha, cumulative mortality attributed to *Foc* TR4 is 6%, 15%, 25% and 40% for the respective crop cycles
- Average bunch mass based on trial data – 20 kg plant crop, 33 kg ratoon crop
- Marketable pack-out calculated assuming 20% reject rate and 10% attribution of bunch mass for bunch stalk
- Assume packing 13.75 kg per carton

219 (suppressive production system)

- Full crop cycles for the 7 year period
- Average crop cycle length over period is 52 weeks
- Plant crop and 5 ratoons for 6 years
- 1 year fallow
- Planted at 1700 plants/ha, very limited cumulative mortality attributed to *Foc* TR4 – 2% over period
- Average bunch mass based on Philippines data –15 kg plant crop, 22 kg ratoon crop
- Marketable pack-out calculated assuming 20% reject rate and 10% attribution of bunch mass for bunch stalk
- Assume packing 13.75 kg per carton

Williams Cavendish (*Foc* TR4 present, NT production system)

- Limited crop cycles for the 7 year period
- Average crop cycle length over period is 40 weeks
- Plant crop and 1 ratoon for 1.6 years twice in period separated by fallow period
- 3 year fallow period
- Planted at 1700 plants/ha, cumulative mortality attributed to *Foc* TR4 is 7% and 20% for the respective crop cycles
- Average bunch mass based on trial data – 22 kg plant crop, 33 kg ratoon crop
- Marketable pack-out calculated assuming 20% reject rate and 10% attribution of bunch mass for bunch stalk
- Assume packing 13.75 kg per carton

Comparisons of modelled productivity

The productivity outputs from the 4 modelled scenarios are presented in Table 2.

Table 2. Comparisons of modelled productivity for alternative Cavendish varieties in suppressive production systems with disease-free Williams Cavendish

Williams Cavendish (<i>Foc</i> TR4 free)	GCTCV 218 (suppressive production system)
<ul style="list-style-type: none"> • Plant crop plus 6.8 ratoons • 374,000 kg fruit produced • 299,200 kg marketable pack-out • 21,800 cartons produced (13.75 kg) • Represents 100% of potential productivity 	<ul style="list-style-type: none"> • Plant crop plus 3 ratoons • 139,800 kg fruit produced • 111,900 kg marketable pack-out • 8,100 cartons produced (13.75 kg) • Represents 37% of standard productivity
219 (suppressive production system)	Williams Cavendish (NT production system)
<ul style="list-style-type: none"> • Plant crop plus 5 ratoons • 187,400 kg fruit produced • 149,900 kg marketable pack-out • 10,900 cartons produced (13.75 kg) • Represents 50% of standard productivity 	<ul style="list-style-type: none"> • Plant crop plus 1 ratoon, repeated after fallow • 136,800 kg fruit produced • 109,400 kg marketable pack-out • 8,000 cartons produced (13.75 kg) • Represents 37% of standard productivity

Conclusion

Modelling the productivity of alternative production systems with partially resistant Cavendish varieties reveals that none of the current alternatives can match the productivity of Williams Cavendish in a disease-free situation. This situation has been well publicised at industry field days and meetings during the incursion and reinforces the stated position that moving to the currently available alternative varieties in the absence of the disease is not recommended. The fairest comparison for alternative production systems is between each other as their implementation is only advocated in the scenario where *Foc* TR4 is widely distributed and competition with disease-free Williams Cavendish production does not occur.

In comparing the alternative scenarios it is important not to directly extrapolate cost of production from the productivity outputs for each modelled production system as are significant differences in costs due to the cost of fallowing and regular replanting. For example, the NT-style Williams and GCTCV 218 alternative production systems have similar productivity but there are additional costs for the Williams system because of the replanting cycle that increases its cost of production.

The value of the productivity modelling is to allow the manipulation of variables such as bunch mass, crop cycle times and population mortality to identify the key requirements and productivity drivers that any potential production system must achieve. For example, identifying non-host fallows and other suppressive practices that reduce the cumulative population mortality in GCTCV 218 to 10% over 6 crop cycles increases its relative productivity to 71% of the standard system, a nearly two-fold increase on the current GCTCV 218 production model.

Appendix 14: Domestic and international conference papers and abstracts

The 5th ISHS-ProMusa symposium: *Agroecological approaches to promote innovative banana production systems* Montpellier, France, 10 to 14 October 2016.

Integrating management practices to support banana production in the presence of Fusarium wilt

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Abstract

Fusarium wilt (FW), caused by *Fusarium oxysporum* f. sp. *cubense* tropical race 4, FocTR4 (VCG 1213/16), is increasingly impacting on banana production around the world. The disease threatens the livelihoods of many smallholders and independent banana producers, particularly those growing Cavendish (*Musa* AAA) in monoculture for the domestic or international supermarket trade. Depending on their circumstances, banana growers require different sets of management practices to either; protect their farms from disease incursions, to slow the spread of the disease within their farms or enable them to return to profitable banana production once decimated by FW. Currently, there are no completely resistant cultivars that achieve the same productivity and marketability as current Cavendish cultivars. Experience has shown that to do nothing or to disregard the disease leads to widespread losses and promotes the spread to previously uninfected areas. Therefore, there is a need to implement management strategies at different levels of disease threat, to manage the disease epidemics in already infected areas to slow the spread and protect uninfected areas. A successful integrated FW management strategy combines knowledge of the epidemiology of FocTR4, with knowledge of the banana production system. However, banana producing countries and even plantations differ in their production systems, so there is no easy solution to manage FW. For example, in Australia early detection, farmer awareness, on-farm biosecurity and agro-ecological practices have slowed the spread of FW. In Taiwan and the Philippines, the inclusion of somaclonal tissue culture variants integrated with sound eradication, exclusion and cultural practices has allowed banana production to continue in the presence of FocTR4. As validated improvements in agro-ecological plantation management and disease management strategies are developed, they can be incorporated into commercial production systems to allow farm viability in the presence of Fusarium wilt.

Key words: Disease suppression, farm management practices, *Fusarium oxysporum* f.sp. *cubense*, on-farm biosecurity, resistant varieties

Monitoring microbial functional and structural diversity for management of disease suppressive soils

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ABSTRACT

The activities of the soil microbiota are essential to the long-term sustainability of agricultural systems, through their influences on biological, chemical and physical processes, which drive essential ecosystem services. Moreover, changes in microbial communities are considered as precursors to changes in the health and viability of the soil environment, due to their responses to changes in agricultural management practices. Therefore, measurements of soil microbial activity and diversity are ideal indicators for monitoring soil status and the efficacy of soil management. However, due to the vast genetic taxonomic diversity of soil microorganisms analysis of microbial diversity is often problematic, time consuming and costly. Two banana experiments were established in far north Queensland, Australia, under different nutrient and groundcover management practices, to understand the dynamic changes in soil microbial communities and their effectiveness to sustain ecosystem services, such as organic matter decomposition and disease suppression. Using a functional approach soil biological communities were analysed using, soil nematode communities, community level physiological profiles and enzyme activity assays. A reduction in nitrogen fertiliser inputs was related to a greater number of fungivorous nematodes which was also linked to greater suppression of *Fusarium oxysporum* f. sp. *cubense* using a novel soil baiting bioassay. Furthermore, community level physiological profiles enabled differentiation of soils over time demonstrating a shift in soil microbial functional diversity. The results highlight how soil management can drive differences in soil microbial activity and diversity to improve soil functions in banana production.

Keywords: disease suppression; *Fusarium oxysporum* f. sp. *cubense*, microbial diversity, community level physiological profiles

Development of an integrated management system to suppress *Fusarium* wilt of bananas

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Fusarium wilt (FW) of bananas, caused by *Fusarium oxysporum* f. sp. *cubense* (Foc), has become widespread in Southeast Asia. FW has recently been detected on a single property in the main banana producing region of Queensland. Farm quarantine of the affected property and on-farm biosecurity of unaffected properties have restricted the spread of the disease. However, to ensure the viability of the Australian banana industry, management practices are required for the three scenarios that banana growers may encounter; namely: 1. no FW present, requiring practices required to remain disease free; 2. FW has been detected requiring practices to limit the spread of the disease; and 3. FW has become widespread requiring remediation practices. Training on disease epidemiology, disease spread pathways and effective farm biosecurity practices has been disseminated to 85% of banana growers. Early FW detection methods using remote, proximal sensing and soil DNA testing are under development. Eradication procedures of infected plants and techniques to enhance the decomposition of the banana pseudostems may reduce the return of Foc inoculum to the soil. Vegetated ground covers that reduce soil movement may also increase Foc antagonists, providing increased competition. The removal of alternative hosts that act as Foc refugia potentially reduces pathogen survival. In plantations where FW has become widespread, crop rotation to reduce Foc inoculum is required before replanting with clean planting material, using cultivars with enhanced resistance. The addition of FW suppressive management practices, in areas infested with Foc, will enable banana growers to remain economically viable.

Appendix 15 – Project extension and communication plan

BA14013: Fusarium wilt Tropical race 4 – Biosecurity and sustainable solutions

Extension and Communications Plan

March 2016

This publication has been compiled by <insert name/s> of <insert business group>, <insert department>.

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Background

Panama disease caused by the fungus *Fusarium oxysporum* f.sp *cubense* (*foc*) is regarded as one of the world's most destructive disease and has devastated many countries around the world. Panama Disease Tropical race 4 (TR4) which attacks the Cavendish variety, is considered to have the most economically damaging impact. In March 2015 Panama Disease TR4 was detected in north Queensland and has changed the way that banana business in this area will be managed. BA14013: Fusarium wilt Tropical Race 4 – Biosecurity and sustainable solutions is structured to prioritise research into three main scenarios in which growers may find themselves in now and into the future:

- I don't have this disease and I want to keep it off my farm
- I have had detections on my property how to I limit its spread and reduce the inoculum load
- The disease is widespread throughout my property, should I continue farming and if so how can I do this safely and economically?

The targets for this project include:

- 100% of banana growers aware of on-farm biosecurity best practice and how to prevent foc from entering their farms.
- 100% of banana growers aware of the necessary steps to prevent the spread of the disease if found on one part of the farm
- 100% of banana growers aware of how to assess their viability as banana producers and the options available should TR4 become widespread.
- 50% implementation of on-farm crop monitoring through on-farm tools, proximal and remote sensing.

The Extension and Communications Plan

2.1 Role of the extension and communications plan

The role of this extension and communications plan is to:

- Identify the key stakeholders
- Identify the key messages that will be relayed to the key stakeholders
- Describe the activities where extension and communication will occur

2.2 Objectives

The objectives of the extension and communication component of this project are to:

- Ensure that growers are informed and understand the ways in which Panama Disease TR4 can spread
- Ensure that growers understand the potential impact of the Panama disease TR4
- Ensure that growers are equipped with the most up to date information available on on-farm biosecurity practices.
- Ensure that growers are keep informed in a timely manner of the results and outcomes from experiments conducted in this project.
- Ensure that growers understand that an integrated approach to managing the disease will be required and there will be no silver bullet solution available into the foreseeable future.
- Inform Biosecurity Queensland of developments in research outcomes that could influence their operational, logistical and planning operations.

- Ensure that grower's implement practices that are scientifically validated from this project.

2.3 Issues to consider when planning communication and extension activities

Since Panama was detected in the Tully valley growers have to minimise movement on and off their properties. Consequently field walks on growers properties have ceased or undertaken with limited numbers of participants. This has changed the way that extension activities can be delivered.

Utilising electronic communication and the use of pre-recorded video are tools that are proving to be effective ways of communicating with growers. Similarly "field day" style events now need to be held in more publically accessible locations like local halls and function rooms need to be utilised.

It is a requirement that all electronic communication material is approved through Biosecurity Queensland before being publically released. This adds time to the period in which communication material is drafted and finalised. Therefore ensuring that the Biosecurity Queensland staff are aware of upcoming communication material will streamline this process.

There are agribusiness's, which are involved, in selling chemical and biological products. There are some disinfectant products that will be tested for their efficacy against the spores that cause Panama Disease TR4. There will be written communication with the manufactures of these products before results are publically released. In formal communication these disinfectant products will be referred to based on their active ingredient and commercial product names will only be provided as examples. It is not in the scope of this project to evaluate commercially available biological products.

2.4 Associated projects

There are three projects within the banana industry's portfolio of projects that need to be considered and are the key to the success of this extension and communication plan:

BA13004: National Banana Development and Extension Project

This project is the industries national extension program that coordinates the communication knowledge transfer from past, current and future projects. The project delivered the national technical information updates (roadshows) in 2014 and is co-ordinating them again in June/July 2016. It is also responsible for maintaining links with the Next Gen growers group. Industry service providers, grower associations and supply chain members.

ABGC Panama TR4 On-farm Biosecurity Extension Project

The On-Farm Biosecurity Extension project focuses on on-farm biosecurity strategies as the best option available to stop the spread of TR4 by infested soil, water or planting material through on-farm biosecurity measures. ABGC with Queensland DAF Extension staff developed biosecurity workshop modules and is responsible for delivering them to banana growers in North Queensland. Additionally, the Extension team also co-ordinated the Panama TR4 field day (November 2015), one-on-one farm visits and subsequent on-farm biosecurity reports.

BA13003: Communications project for the banana industry

The project produces, manages and distributes electronic and printed publications and other materials and provides services to communicate industry work and achievements. The project also assists with public comment required on industry issues and assists with convening major industry events, such as the Australian Banana Industry Congress. The project's major printed publication is the Australian banana industry's flagship publication, Australian Bananas magazine. It also produces other news-focused publications, such as the Banana Growers' e-Bulletin, electronic alerts and information materials such as industry videos, and fact sheets. The project also provides content and management for the industry website and facilitates the posting and management of online content for extension-focused industry projects.

Stakeholder assessment

Below is a summary of the key stakeholders, outcome required and the key messages for each respective stakeholder.

Stakeholder Group	Role in the Project	Outcome required	Key Messages for Stakeholder
Growers without detections of panama disease TR4	<ul style="list-style-type: none"> - Recipients of written material distributed through the ABGC communications project - Viewers of informative videos - Attendance at field day style events - Participate in on-on-one visits and communication 	<ul style="list-style-type: none"> -Growers implement best practice on-farm biosecurity systems. -Growers understand the requirements they will be required to meet to continue trading if the disease is found of their property. 	<ul style="list-style-type: none"> - Preventing Panama disease tropical race 4 from getting onto a property the currently the only way to continue banana farming as we know it. Therefore implementing the best on-farm biosecurity practices is the best management option. -Effective on-farm biosecurity practices need to be simple and easy to follow to ensure that they become part of everyday business now and into the future -Early detection is the key to slowing the spread of this disease. Report all suspect plants to Biosecurity Queensland
Grower/s with positive detection/s of Panama Disease TR4	As above plus:	-Grower implement best bet management practices to minimise the spread of the disease on their properties	<ul style="list-style-type: none"> - Effective on-farm biosecurity practices should be implemented to minimise the risk of spreading the disease off farm. - Early detection of infected plants is the key to slowing the spread of the disease. These plants should be destroyed using practices that rapidly degrade the pseudostem and reduce inoculum load. - Internal biosecurity should be implemented to minimise spread to uninfected areas of the farm.
Growers widespread detections of Panama Disease TR4	As above plus:	- Growers make informed decisions about whether to continue production or not.	<ul style="list-style-type: none"> - Once the disease is widespread there becomes a point where it is uneconomical to continue production. - As inoculum load builds up this risk to the rest of industry increases. - Decisions need to be made as to when to cease production on a property once the disease is widespread on a property.

Australian Banana Growers Council	Support through Communications Program	- Ensure that latest outcomes and results from this project are included in communication material which is distributed to stakeholders in the industry	- Key messages as above for growers. - Specific outputs from trials within the project
Grower Reference Group	Guiding the requirements of the project	Grower reference group knowledge is used and members feel that their input is valued. Member are advocates of the project and provide advice on how best to achieve the desired outcomes	Your input is valued to provide insight into the activities in this project

Communication Activities

Resources and Activities

Key Messages

Specific key messages will vary pending results from trials within this project however the two key messages are as follows:

- Preventing Panama disease tropical race 4 from getting onto a property the currently the only way to continue banana farming as we know it. Therefore implementing the effective on-farm biosecurity practices is the best management option.
- There is no “silver bullet” solution when it comes to panama disease TR4. An integrated approach needs to be taken to managing the disease consisting on good on-farm biosecurity practices, practices which favour the growth of suppressive organisms in the soil and potentially tolerant varieties.

Branding and acknowledgments

All communication and media materials in relation to this project must have prior agreement from Agri-science Queensland (Irene Kernot or Samantha Allen) as well as Biosecurity Queensland (Simone Newman-Webster).

All communications material for public release should contain the following statement
The Fusarium wilt Tropical Race 4 – Biosecurity and sustainable solutions project is funded by Horticulture Innovation Australia Limited using the banana industry levy and funds from the Australian Government, and is supported/led/led and supported by the Queensland Department of Agriculture and Fisheries.

Due credit needs to be given to: The University of Queensland (UQ), The **South Australian** Research and Development Institute (SARDI), and Biosecurity Queensland (BQ) for their involvement in specific sections of the project which they have respectively contributed to.

Details on specific activities

The project will be represented and as results are obtained will be communicated via the following communication and extension activities:

Target Audience	Vehicles	Responsibility	Frequency
Growers, consultants and resellers	Roadshows (produced by BA 13004)	Tegan Kukulies	Once (June/July 2016)

Growers	Cassowary Coast Banana Growers Association Meetings	Tony Pattison & Stewart Lindsay	Project representative to attend 12 meetings (held monthly) and present research updates as required.
Growers	Mareeba Banana Growers Association Meeting	Stewart Lindsay and Tegan Kukulies	Project representative to attend 6 meetings (held every second month) and present research updates as required.
Consultants and Resellers	BAGMAN meeting (Banana agribusiness managers)	Stewart Lindsay, Tony Pattison and Tegan Kukulies	2-3 times per year
Growers	The Next Gen Growers group	Tegan Kukulies	3-4 times per year plus written communication
Growers and industry stakeholders	Australian Banana Growers Magazine	Stewart Lindsay, Tony Pattison and Tegan Kukulies	4 times per year
Growers and industry stakeholders	Australian Bananas Newsletter	Stewart Lindsay, Tony Pattison and Tegan Kukulies	3 times per year
Growers and industry stakeholders	Australian Bananas e-bulletins	Stewart Lindsay, Tony Pattison and Tegan Kukulies	Monthly
DAF staff	Panama TR4 Program Update	Tegan Kukulies	Monthly (TBC)
Growers and Industry Stakeholders	Banana Industry Congress	Key project members	Mid-2017 (Date and location TBC)

*Industry stakeholders include: growers, resellers consultants, transport companies, carton companies, ripeners and wholesalers.

Appendix 16 – Project extension and communication activities

Date	Activity	Stakeholder Group	Attendance
Group activities – R&D coordination			
6/4/16	UQ Fusarium Focus video-conference	Panama disease R&D staff; ABGC staff; BQ staff	30
13/5/16	UQ Fusarium Focus video-conference	Panama disease R&D staff; ABGC staff; BQ staff	40
16/8/16	DAF Panama disease R&D update seminar	Panama disease R&D staff; ABGC staff; BQ staff, NT DPI staff	40
20/9/16	Meeting with ABGC TR4 R&D Manager – update for ABGC board and CEO on project progress	ABGC Board and staff	N/A
7/12/16	Biosecurity Queensland Panama Response Program Planning Manager – update on project progress and activities	BQ staff	N/A
10/1/17	Dr E Aitken, UQ, seminar on <i>Foc</i> TR4 resistance gene marker development, South Johnstone	DAF RD&E staff; BQ staff; ABGC staff	15
14-15/2/17	Panama disease R&D Update seminar, Brisbane	DAF R&D staff; ABGC staff and board members; BQ staff; NT DPI staff; UQ staff; QAAFI staff; NSW DPI; ACIAR; HIA Ltd; JCU; UNE	50
6/3/17	BQ response and epidemiological review, Prof A Viljoen, Stellenbosch University	DAF project staff; BQ staff	15
25/9/17	Panama disease R&D Update seminar, Brisbane	DAF project staff; ABGC staff; 3 grower representatives; BQ staff; NT DPI staff; NSW DPI staff; UQ staff; JCU staff; UNE PhD student; Hort Innovation staff	35
Group activities - industry			
13/11/15	DAF/ABGC Panama disease industry field day	Banana growers, consultants,	100

		agricultural retailers, chemical company representatives	
10/3/16	Banana agribusiness managers discussion group – sanitiser R&D presentation	Consultants, agricultural retailers, chemical company representatives	25
13/4/16	Mareeba Banana Growers Association meeting – sanitiser R&D presentation	Banana growers	5
8/6/16 Mareeba 9/6/16 Innisfail 16/6/16 Tully 23/6/16 Carnarvon 5/7/16 Coffs Harbour 7/7/16 Murwillumbah	National Banana Industry Roadshow – presentations on project results: <ul style="list-style-type: none"> • Sanitiser trials – K Grice • Early detection/remote sensing – K Ferro • Soil health – T Pattison 	Banana growers, consultants, agricultural retailers, chemical company representatives	165 total
30/6 & 1/7/16	ABGC Panama TR4 industry meetings	Banana growers, industry service providers	50 total
July 2016	ABGC Chairman project progress briefing – summary of project activities for his panel discussion at PHA seminar, Melbourne	ABGC Board chairman, PHA seminar attendees	N/A
22/9/16	Banana agribusiness managers discussion group – supplementary sanitiser R&D presentation on corrosion and effective concentration monitoring	Consultants, agricultural retailers, chemical company representatives	22
4/4/17	ABGC TR4 R&D manager – discussions on new sanitiser screening results including assessment against TR4	ABGC TR4 R&D manager, and ABGC CEO and Board by extension	N/A
12/5/17	DAF Panama R&D Open Day, South Johnstone	Banana growers, consultants, agricultural retailers, chemical company representatives	109
22/6/17	Presentation at Australian Banana Industry Congress 2017 on project activities and results	Banana growers, consultants, agricultural retailers, chemical company representatives, supply chain	200+

		businesses, R&D agencies	
6/7/17	Cassowary Coast Banana Growers Association meeting – update on project activities	Banana growers, ABGC staff	18
1-2/8/17	ABGC Panama TR4 industry meetings – update on new incursion (Tully, Innisfail, Mareeba)	Banana growers, industry service providers	130 total
Conference presentations			
10-14/10/16	<p>5th ISHS-ProMusa Symposium: Agroecological approaches to promote innovative banana production systems, Montpellier, France</p> <ul style="list-style-type: none"> Integrating management practices to support banana production in the presence of Fusarium wilt (T Pattison) Monitoring microbial functional and structural diversity for management of disease suppressive soil (A McBeath) 	International banana researchers	90
14-17/11/16	<p>9th Australasian Soilborne Disease Symposium, Lincoln University, Christchurch</p> <ul style="list-style-type: none"> Development of an integrated management system to suppress Fusarium wilt of bananas (T Pattison) 	Domestic and international researchers	60
25-28/9/17	<p>Australasian Plant Pathology Society/CRC Plant Biosecurity - Science Protecting Plant Health Conference, Brisbane (pres.)</p> <ul style="list-style-type: none"> Engineering banana cropping systems to suppress soil borne diseases QA based disinfectants for effective on-farm biosecurity management of Panama disease in bananas <p>Posters:</p> <ul style="list-style-type: none"> Effects of commercial disinfectants on the survival of <i>Foc</i> Race 1 and TR4 propagules The survival of <i>Foc</i> in plants co-habiting Australian banana farms Testing the efficacy of urea as a treatment for the 	Domestic and international researchers; biosecurity agency staff and policymakers	523

	<p>destruction of <i>Foc</i> in infected soil</p> <ul style="list-style-type: none"> The assessment of physiological methods for early, quantifiable stress detection in banana plants Using <i>Trichoderma</i> to suppress Fusarium wilt (<i>Foc</i>) in banana cropping systems 		
Written material			
Australian Bananas – Issue 45, Spring 2015	<ul style="list-style-type: none"> Zoning out bad habits – making the change to effective biosecurity 	Banana growers, allied service providers, R&D staff	1000
Australian Bananas – Issue 46, Autumn 2016	<ul style="list-style-type: none"> Fielding ideas – growers share their TR4 innovations Ammonium compounds clean up 	Banana growers, allied service providers, R&D staff	1000
Australian Bananas – Issue 47, Spring 2016	<ul style="list-style-type: none"> Early detection trials DDAC test kits Panama disease R&D overview 	Banana growers, allied service providers, R&D staff	1000
Australian Bananas – Issue 48, Autumn 2017	<ul style="list-style-type: none"> Panama R&D update summary 	Banana growers, allied service providers, R&D staff	1000
Australian Bananas – Issue 49, April 2017	<ul style="list-style-type: none"> Meeting of TR4 Minds – the latest update on TR4 research 	Banana growers, allied service providers, R&D staff	1000
Australian Bananas – Issue 50, September 2017	<ul style="list-style-type: none"> Panama Open Day report 	Banana growers, allied service providers, R&D staff	1000
BQ Panama TR4 Program Update newsletter	<p>Mar/Apr 2016</p> <ul style="list-style-type: none"> Groundcover trials, reducing inoculum, weed host status survey, development of biosecurity BMP <p>May/Jun 2016</p> <ul style="list-style-type: none"> Weed host status survey, early detection, pseudostem destruction, soil ecology trials <p>Jul/Aug 2016</p> <ul style="list-style-type: none"> Panama R&D update seminar <p>Sept/Oct 2016</p> <ul style="list-style-type: none"> Disinfectant trials against TR4 from NT, new sanitiser testing in Qld, weed host trials, inoculum reduction <p>Jan/Feb 2017</p> <ul style="list-style-type: none"> Testing concentration of disinfectant solutions, disinfectant corrosion testing, detecting stressed banana plants 	Banana growers, ABGC staff, local government, utilities	400 recipients (116 growers)

DAF Fact sheets	<ul style="list-style-type: none"> • Panama Disease Tropical Race 4 Research Update – Disinfectant trials • 	Banana growers, allied service providers, R&D staff	N/A
Banana best management practices guide	On-farm biosecurity	Banana growers, allied service providers, R&D staff	142 (as at 29/9/17)
International TR4 networks/visits			
23/2/17	3 Israeli banana R&D staff visited DAF South Johnstone to discuss <i>Foc</i> TR4 R,D&E activities	DAF R,D&E staff; BQ response program staff; ABGC staff	N/A
20/6/17	Prof R Ploetz (University of Florida) & Dr F Bakry (CIRAD, France) visited DAF South Johnstone to discuss <i>Foc</i> TR4 R,D&E activities	DAF R,D&E staff; BQ response program staff; ABGC staff	N/A
27/6/17	Dr Roberto Young, Dole Honduras, visited DAF South Johnstone to discuss <i>Foc</i> TR4 R,D&E activities	DAF R,D&E staff; BQ response program staff; ABGC staff	N/A

Quaternary Ammonium products aid in the management of Foc

Screening of disinfectants products for their efficacy against Panama disease (*Fusarium oxysporum f.sp. cubense – Foc Race 1*) has resulted in products that growers can use or apply as part of their on-farm biosecurity. Testing is on-going against the Tropical Race 4 (TR4) strain in the Northern Territory, however results so far have been comparative. A number of products already have registration as general purpose disinfectants.

Quaternary ammonium (QA) products containing 120g/L of the active didecyl dimethyl ammonium chloride (DDAC – Path-X™, Steri-Max® or SporeKill®) or benzalkonium chloride (BA – Bactex CF® or Agriquat®) at ≥ 100g/L had the best activity against all spore types of Foc.

Things to remember:

- Remove all soil and organic matter before applying any disinfectant (a detergent based product can assist in this process).
- Use a DDAC (12% active) or a benzalkonium chloride with an active ≥ 10% (Table 1)
- Dilute the disinfectant product to a 1% solution (e.g. 10 ml product to 990 ml water)
- Products can be used in footbaths, drive-through dips or spraying farm machinery and equipment.
- Use products as per label specifications (contact times)
- If solutions become dirty, replace them.



Peter Trevorrow and Kathy Grice (DAF) assessing disinfectant against Foc RT1



Clean equipment thoroughly before applying a disinfectant

Don't expect a disinfectant to be your only line of defence against Panama disease

Table 1. Products registered as disinfectants and their activity in the laboratory against Foc Race 1 and TR4 with soil added (1g to 20 ml solution)

Category	Disinfectant names	Contact times – Foc R1				Contact times – TR4			
		≤ 30 sec*	5 min	30 min	24 hr	≤ 30 sec	5 min	30 min	24 hr
Quaternary ammonium	DDAC (120g/L) (1:100 dilution)	✓	✓	✓	✓	✓	✓	✓	✓
	Path-X™	✓	✓	✓	✓	✓	✓	✓	✓
	Steri-Max®	✓	✓	✓	✓	✓	✓	✓	✓
	SporeKill®	✓	✓	✓	✓	✓	✓	✓	✓
Benzalkonium chloride (1:100 dilution)	Bactex CF® (100g/L)	✓	✓	✓	✓	✓	✓	✓	✓
	Agriquat® (250g/L)	✓	✓	✓	✓	✓	✓	✓	✓
	Farmcleanse® (1:10 dilution)	✗	✗	✗	✗	✗	✗	✗	✗
Detergent based	Farmcleanse® (1:10 dilution)	✗	✗	✗	✗	✗	✗	✗	✗

* ≤ 30 sec is the time it takes to process a sample and simulates the time to walk through a footbath or drive through a dip.

Screening of disinfectants against Foc R1 conducted by DAF researchers Peter Trevorrow and Kathy Grice and testing against TR4 conducted by Vu Tuen Nguyen (Department of Primary Industry and Resources – Northern Territory). There may be factors beyond the scope of this research that has been undertaken which have the potential to influence results. Please contact Peter Trevorrow or Kathy Grice on 07 4017 0770 for further information.

Figure 2. Biosecurity Queensland Panama TR4 Program Update example – regular feature on R&D progress

Department of Agriculture and Fisheries
Biosecurity Queensland

PANAMA TR4 PROGRAM UPDATE



JULY / AUG 2016

CURRENT STATUS OF PANAMA DISEASE TROPICAL RACE 4

Detections of Panama disease tropical race 4 in Queensland are still restricted to a single property in Tully. The infested property, and a second property linked through shared machinery and family ownership, remain under quarantine but continue to trade fruit under a strict biosecurity protocol and daily supervision of farming activities by Biosecurity Queensland's Panama TR4 Program. Intensive surveillance by Program field officers is being conducted at frequent intervals on the infested property as early detection and prompt destruction of infected plants help manage inoculum build up. The Panama TR4 Program continues to work with the property owners who maintain strict on-farm biosecurity procedures.

The Panama TR4 Program has now surveyed 89.5% of the planted industry in the region. The surveillance to date has been on properties that have links to the infested property or that have been inspected in response to public reports of suspected Panama disease. The Program continues to work with industry, stakeholders and the community to deliver the highest standard of biosecurity control and containment, surveillance and engagement across Far North Queensland.

SUMMARY OF ACTIVITIES

- A suitable representative should be invited to meet officers for consent at the time of entry. Growers are urged to participate in meetings with the new Act to ensure they understand their general biosecurity obligations.
- 273 Panama education sessions have been delivered to service and lobby agencies by the Program's community engagement officers at community events in 2016.
- 285 members of the public approached Panama TR4 Program community engagement officers at community events to learn more about Panama disease since July 2016.
- One year surveys have been conducted on 250 individual banana properties by the Panama TR4 Program. These surveys include public land and residential blocks, but the vast majority are commercial banana farms in Tully, Winaloa, Atherton Tablelands and Laidlaw districts.
- Biosecurity officers have the legal powers to enter properties for the purposes of conducting surveillance for Panama disease tropical race 4. However, as part of our entry procedures under the new Biosecurity Act, officers will now seek written consent before entering properties.

DID YOU KNOW?

THE PANAMA TR4 PROGRAM HAS INVESTIGATED 348 CASES OF INTEREST FOR PANAMA DISEASE TROPICAL RACE 4. CASES ARE LINKED FROM THE INFESTED PROPERTY THROUGH A COMPROMISED TUBING PROCESSION INCLUDE CONTRACTORS AND PUBLIC EMPLOYEES.

For more information visit www.biosecurity.qld.gov.au or call 13 25 23



Figure 3. Examples of Australian Bananas magazine

PROTECTING BANANAS 20

Early detection: Look for the signs

processes in the plant can cause a variety of things to happen," Ms Ferris explained.

"In temperature might rise making it detectable through thermal imaging, or it may change the level of a certain chemical or the stability of the cell membrane in the leaf tissue meaning we can take samples and test for this in the lab.

"Stress and disease might also affect a plant's capacity to photosynthesise. So we can monitor changes using equipment, which can read the levels of gas exchange that are happening in the leaf - CO2 and water vapour, as well as the chlorophyll activity in the leaf.

"Generally these changes will be quite rapid in response to stress and can be detected before we visually see signs of them."

While Ms Ferris's research is still in its infancy, she remains confident of yielding positive outcomes based on similar research conducted on various crop species in other parts of the world.

"There was a Chinese study on Fusarium infection (Panama) in a banana growing hydroponically in a greenhouse, which was able to detect almost a three-degree temperature difference between an infested plant and a non-infested plant four days after inoculation and before the expression of disease symptoms," she said.

"That that doesn't necessarily translate to being able to detect it through thermal imaging in a field because there are so many variables that are going to influence the image. So that really needs to be refined into a method that could work in a more practical sense."

The competence of banana plants and their cropping systems has shown many challenges to researchers, who have also begun trialling high resolution satellite imagery to detect stress across on a larger scale.

"The imagery would be used to gauge how well a plant was photosynthesising - it might be possible to identify a unique spectral signature, which will potentially correspond with a Panama infested plant," said Ms Ferris.

As Australia's efforts to manage Panama Tropical Race 4 turns from reactive to preventative, an important part of research is to focus our efforts on early detection methods to respond quickly to any future incursions.

Currently the only means of identifying if a banana plant might be affected with *Fusarium oxysporum* f. sp. cubense (TR4) is by visual assessment, followed by diagnostic laboratory testing.

Early visual symptoms of the disease include leaf yellowing, wilting, stem splitting and discoloration to the pseudostem.

But now the race is on to get a jump start on this insidious disease with research currently being conducted to improve early detection techniques. Department of Agriculture and Fisheries research horticulturist Estelyn Ferris, who specialises in banana production systems, has been trialling different remote and proximal sensing methods to evaluate the health of banana plants before they show any physical signs of Panama.

"Ideally we hope to detect, assess or possibly through human surveillance," said Ms Ferris.

"This will allow the owner of the problem to be alerted as early as possible, and in the case of Panama disease, it will minimise the build-up of disease in the plant."

Ms Ferris's research has centred on studying the physiological changes of a stressed plant compared to a healthy plant, by conducting on-farm studies using Panama Tropical Race 4 infested plants as a surrogate for Tropical Race 4, with plans for additional greenhouse trials.

Sensing methods used in her research have included infrared thermal camera imaging to detect plant temperature changes. Double or SPAD meters to measure chlorophyll content levels in plant leaves, and plant-held fluorimeters to measure the photosynthetic capacity of a detached leaf.

"The disruption of normal metabolic



Estelyn Ferris, Research Horticulturist, Banana Production Systems, Department of Agriculture and Fisheries.

July 2016 | Australian Bananas magazine

Fielding ideas - growers share their TR4 innovations

Converted shipping containers, cattle tags, specially-designed spray nozzles and catwalks are just some of the items innovative banana growers are using to protect their farms from Panama Tropical Race 4 (TR4).

Banana growers met at the industry's first TR4 day at Wangan in North Queensland to share their stories on how they are managing the challenges of stopping the soil-borne disease.

Four growers spoke about the changes they have made to their farms to introduce and upgrade biosecurity measures.

First banana grower Tiger Mercedes, Wangan's Gavin Eilers and Maria Cook growers Matt Abbott and Kiri Fleetsford were also present. The TR4 day was also participated in biosecurity videos screened at the field day.

Mercedes said the field day audience of his initial struggle with working out how to introduce effective biosecurity measures to his Maria Cook banana farm.

For the first two weeks after TR4 was confirmed in the Lilly Valley "I wasn't too sure where my head was at," he said. "I didn't know what to do because I was trying to do the whole thing in one go."

Kiri's husband's automated washdown, nozzles that spray wash water and then fertiliser from a 1000-litre stubble,



his approach to biosecurity measures. "We started with the most important area. In our eyes, to the least aid for us that was walkway proofing or vermin proofing - so that was our first priority to stop animal movement. Then we added it from there to stop trucks and people and any other vehicles from crossing paths with tractors, machinery, farm machinery - we've just built on it from there."

Tiger Mercedes, a Queensland grower who previously grew Disease 1 on his farm about 16 years ago, said he had upgraded his Last Banana farm's wash down system after TR4 was confirmed in the Lilly Valley.

"When this case came up we said 'okay we'll modify this so we've got clean to clean out'."

"We've stopped traffic coming into the shed - we've got one truck that comes in a day - empty in, full out... no other vehicles."

Kiri said he had taken power and water to his farm gate to install a modified wash down system that includes a nozzled had housing a gate over nozzles that clean down the undercarriage and tyres of



Tiger told about the upgrades to his farm gate system which includes foot baths and a transport vehicles that need to come on to the farm.

The number one spray fresh water and then another saved in a 1000-litre drum.

While the system is for vehicles from transport, custom and trailer suppliers, Kiri said it had been tested out on more challenging tasks. "We actually drove a tractor through it with a heap of mud on it still having a bit of dirt at the moment so it needs a few more washes," Kiri said.

"Work vehicles get parked at the side of the property area and we've basically got a vehicle that goes from the wash to the shed."

Andrew Serra has installed a shipping container, bought in Cairns and converted into a boot room.

There is a foot bath at either end and a bench where workers exchange their boots before either their own farm boots or gumboots identified using attached cattle tags. Boots are also replaced for visitors.

Chair Diego Fleites checked all growers for attending the field day and killing on biosecurity terms.

"Certainly in the early days there were lots of questions and the questions were coming in faster than we could generate answers," he said.

"I guess today is the peak of us wanting to generate some of those answers. What I've certainly noticed is that some of the solutions that have been found by individual growers are absolutely brilliant."



Andrew Serra in his converted shipping container boot room.

Ammonium compounds clean up

Initial research into five categories of disinfectants and sanitisers has found Quaternary ammonium products such as Sporekill and Steri-Max to be the most effective against Panama spores.

The research has been carried out on Panama Race 1-culturing *Panama* sporophore f. sp. cubense chlamydo-spores. Due to quarantine restrictions, TR4 spores were not used in the research.



Queensland Department of Agriculture and Fisheries Markets plant pathologists Peter Brownlow (above) and Peter Grier outlined the research and presented their initial findings at the Wangan TR4 field day.

The tests used different dilution rates of the product to check their effectiveness in killing the spores over four different lengths of contact time - from a few seconds, similar to the contact time for footwear in a foot bath, through to 24 hours, similar to the time wash down water might stay in a holding tank.

Testing was also done with and without the presence of a similar proportion of soil to that which would be found in vehicle dips or footbaths - one part soil to 20 parts of the product solution.

Further research is continuing to investigate the influence of different soil types on the effectiveness of the products to treat waste water from wash down.

The other four categories of disinfectants and sanitisers tested were Bleach, detergent based products, Bio Bleach and bleach.

Zoning out the bad habits

What used to be normal, everyday farm practices can now put banana production at risk from TR4. Stewart Lindsay* reports on how to make the change to safer farming.

It's one of the most significant disease threats to global banana production and it's now in North Queensland.

Panama disease Tropical Race 4 of bananas, caused by the fungus *Fusarium oxysporum* f. sp. *cubense*, is spread through the movement of contaminated plant material, soil and water.

There are currently no effective chemical, biological or cultural practices that can eliminate the disease and there are no notably resistant banana varieties that are commercially acceptable.

The most promising effective biosecurity practices on your farm to prevent Panama disease is certainly the only measure in safeguard continued banana production.

Planning how to implement these practices is crucial.

Firstly, it can help you decide if, and how, to invest in TR4. And planning will also help you to determine



Washing vehicles, people and equipment between zones must be subject to a thorough wash down and disinfection.

where best to place any infrastructure you decide to introduce to your farm.

Developing a plan might initially seem overwhelming because what has been considered normal practice in the banana industry can now pose a significant risk of spreading the disease.

Excluding risks

Excluding the movement of plant material, soil and water onto your property should be the foundation of

Banana farm separation zones

Farm zone

- includes all your banana blocks
- includes all machinery and equipment associated with the field production of bananas
- is a general rule, don't let the machinery and equipment leave this zone
- no other equipment or machinery is allowed in
- movement of staff and contractors into this zone is based on thorough washing and decontamination procedures, including managing staff footwear and entry procedures
- the zone boundary must include access to the part of the packing shed where bunches are unloaded
- boundary must give access to machinery and workshop area
- a physical barrier, such as a fence, separating this zone from the other two zones will help stop accidental movement across the boundary.

Separation zone

- the area where essential farm inputs are delivered, packed fruit is collected and farm vehicles not directly involved in banana cultivation are parked
- generally, non-employees in area adjacent to the output side of the packing facility and close access to other storage and workshop area
- access is limited to essential business inputs and vehicles. These must be inspected, washed and decontaminated
- no movement between this zone and the farm zone without direct washing and decontamination to an acceptable level of cross-contamination from the separation zone
- surface runoff from this area must be intercepted and drained away from the farm zone to avoid contamination.

Exclusion zone

- for vehicles and people that do not have to enter either of the other two zones for the conduct of your banana business
- should be as being like an extension of the public road
- is for visitors and staff not directly associated with the production aspect of your business
- external vehicles should remain in this zone
- staff and visitors can only go into the other zones if they use clear footwear or wash and decontaminate their footwear.



Selecting and other physical barriers are important to restrict movement across the farm zone boundary.



"effective biosecurity is currently the only measure to ensure continued banana production"

Using property maps helps to plan your zones.

them. It can be implemented quickly with temporary barriers (pipes, logs, rocks etc) until fencing or other permanent barriers are put in place.

Fencing or other types of physical barriers are very important to reinforce the separation of zones.

Movement across zone boundaries must be managed very closely with clear procedures and appropriate facilities to wash down and decontaminate vehicles, footwear, machinery and tools.

Clear procedures must be developed and communicated to any person who needs to cross over a zone boundary. It is especially important for all staff, suppliers and contractors to be involved in complying with the procedures.

The owner or manager is ultimately responsible for the biosecurity of a banana farm and registerable for compliance with exclusion and wash down and decontamination procedures and facilities is vitally important.

**Stewart Lindsay is Principal Development Horticulturist & Team Leader at the Queensland Department of Agriculture and Fisheries, South Johnstone Research Station.*

TR4 MANAGEMENT



Leading banana researchers met recently to discuss latest applied research and technology focusing on the short- to long-term management of TR4.

MEETING OF TR4 MINDS

THE LATEST UPDATE ON TR4 RESEARCH.
WHAT'S NEW, IN PROGRESS AND ON THE HORIZON.

Last month marked two years since Panama Tropical Race 4 (TR4) was first detected in Queensland's Far North, on a farm in the Tully Valley. As the anniversary neared, leading banana researchers from across Australia met to discuss the latest applied research and breakthrough technology that continues to focus on the short- to long-term management of the disease. By Tony Pattison and Tegan Kukulics.

Since its initial detection in North Queensland, Panama TR4 has brought many challenges to banana growing, production and the industry at large.

In the two years since detection, banana growers have become much more aware of the disease, and most have implemented effective on-farm biosecurity. Practical banana research is heading toward reducing the impacts of the disease.

There is a long history of research on Panama disease throughout Australia. The use of applied research and cutting edge technology by banana researchers from the Department of Agriculture and Fisheries (DAF) (agronomy and biosecurity), University of Queensland, James Cook University, Northern Territory Department of Primary Industries and Resources, New South Wales Department of

Primary Industries and the University of New England was discussed at a recent workshop in Brisbane.

The latest research and development aimed to improve growers' confidence in disease containment, reduce disease spread and provide farm management options if the disease became widespread.

One of the first questions North Queensland banana growers asked when TR4 became a reality was, "How do I protect my property?"

Implementing effective on-farm biosecurity is paramount. New management strategies are being developed using revolutionary studies on the genetics of the Fusarium fungus, the cause of Panama disease.

Containing the disease on the single infected property has also become a priority, and improving

our knowledge of containment by establishing the most effective destruction of infected plants continues to be a primary goal.

However, in the event that Panama disease did spread, options for managing the disease and its impacts on the banana industry need to be developed. The creation of banana plants with better resistance is underway, by manipulating the tissue culture process.

When Australia's leading scientists researching aspects of Panama disease met in Brisbane in February, they discussed a range of existing research, what is new, in progress and on the horizon. Here, we take a snapshot of their innovative work.

WHAT'S NEW?

Extensive research into the use of disinfectants has shown the

quaternary ammonium (QA) group of chemicals to be very effective.

The initial disinfectant testing, which was conducted using Panama disease Race 1, is now being tested using Panama TR4 in the Northern Territory (NT). Testing so far shows that products — for example, products containing 120g/L (decyl) dimethyl ammonium chloride, a Quaternary Ammonium (QA), made up at 1% — are also effective on Panama TR4.

Further testing is also showing that detergents (such as Farmalene) can actually de-activate disinfectant products, if mixed directly. Therefore, growers should ensure they have thoroughly washed off detergents before applying disinfectant products.

Dip and compare colour test strips have also shown promise, with testing currently being finalised. Also in this space, there is good news for growers that have a shuttle or spray tank and don't regularly make up QA disinfectant solutions. Using the test strips as indicators, disinfectant solutions (1% QA products), which have been left in a sealed

TR4 MANAGEMENT



container exposed to the elements of the weather (full sunlight), have remained at a 1% solution for 12 weeks. Initial testing is also showing that these disinfectants are no more corrosive to a range of metals than water. Together, the use of colour test strips and metal corrosion tests are helping banana growers make sure chemicals are being used effectively.

Current destruction protocols of infected plants involve bagging the plants, gouging growing points and applying high rates of urea to infected plant material, as well as the soil immediately surrounding it. Results from a novel lab bioassay and a field trial in New South Wales (NSW) (using Subtropical Race 4) demonstrated that this was an effective method to quickly kill the Fusarium fungus.

Surveys of weed and groundcover species from Panama Race 1 sites in North Queensland and Subtropical Race 4 sites in NSW, identified that some weed species are alternative hosts to Panama disease. A pot trial with 18 common weed and groundcover species revealed that under controlled conditions, Fusarium could be recovered from all 18 species. Where and how the Fusarium is surviving on the weeds needs to be clarified and how this translates to the field.

WHAT IS IN PROGRESS?

There are some exciting advancements in understanding

the Fusarium genetics. Genetics is another language to most of us but, in a nutshell, there are many different Fusarium species, each with their own individual 'barcode'. Researchers are analysing the differences in these 'barcodes' to better understand their relationships with each other, and which segments may be responsible for causing Panama disease.

Under strict quarantine conditions scientists have also manipulated the Fusarium fungus to 'glow' in order to better understand where and how the fungus moves through banana plants. This research is important to help understand how the fungus moves through the banana plants, to cause disease symptoms which lead to plant death.

The first trial looking at the effect of nutrients on Panama Subtropical Race 4 development has commenced. Using a controlled aeroponic system (soilless), disease development is going to be monitored following different application rates of boron. Other nutrients will be evaluated following similar trials from this initial trial. In addition to this, an extensive soil type survey is being conducted on North Queensland banana properties' characterising differences in soil characteristics, and understanding how these differences attribute to suppression of Panama disease.

Scientists are continuing to apply mutagenesis techniques to tissue

culture banana plantlets in an attempt to develop a commercially viable banana cultivar with improved tolerance to Panama TR4. In a multi-pronged approach, firstly mutagenesis has been applied to GCTV 119 in an attempt to improve tolerance to the disease and also Gold Finger in an attempt to improve agronomic and eating quality. The first plants produced from this approach are expected to be planted in the NT soon.

WHAT IS ON THE HORIZON?

Genome sequencing and soil community profiling are just two of the methods that will be applied to help understand the role soil biology may play in suppressing Panama disease. An array of methods will be used to monitor the soil biology following varying practice changes, such as the use of different cover crops. Different cover crop options are currently being evaluated in a pot trial and this will help determine which species will be used in a cover crop field trial in the NT.

Clean planting material is a key priority for minimising the risk of spreading TR4. Negotiations are currently being evaluated in a pot trial and this will help determine which species will be used in a cover crop field trial in the NT. Clean planting material is a key priority for minimising the risk of spreading TR4. Negotiations are currently being evaluated in a pot trial and this will help determine which species will be used in a cover crop field trial in the NT.

Council. This improved system will give growers renewed assurance that tissue culture producers, that are certified under the new system, continue to supply clean disease free planting material.

The hard copy of the On-farm Biosecurity BMP developed as a 'sister' program to the industry's well adopted Banana BMP Environmental Guidelines, is nearing completion. The resource aims to set industry guidelines for on-farm biosecurity practices through the use of a self-assessment checklist, similar to that of the BMP Environmental Guidelines.

These papers have been funded by Horticulture Innovation Australia Limited using the Research and Development System levy with co-investment from the Queensland Department of Agriculture and Fisheries and funds from the Australian Government.

Planning has commenced for DAF to host a Panama R&D update event for growers at the South Johnstone Research Station in May. This interactive event will bring the industry up to date on the latest Panama research. Watch the e-bulletins for updates on this event.

See [www.abgc.org.au](#) for 2017 pics from the Meetings of TR4 MINDS gathering.

COMMUNITY EVENTS

PANAMA OPEN DAY



More than 80 banana growers, researchers and other industry leaders took part in a Panama disease open day at the South Johnstone Research Station in May.

The interactive event was designed to highlight the latest research and development focussed on Panama disease Tropical Race 4 (TR4).

Hosted by the Department of Agriculture and Fisheries (DAF), the day included exhibits and a field tour, highlighting the latest Panama studies and on-farm biosecurity practices, focussed on the short- to long-term management of the disease.

A key component of the day was the launch of the Banana Best Management Practices, On-farm Biosecurity manual, designed for growers to help manage on-farm biosecurity risks.

The manual was jointly funded by Horticulture Innovation Australia using banana research and development levies and funds from the Australian Government with co-investment from DAF.



INDUSTRY




Figure 4. National Banana Development and Extension Project – Banana Roadshow, Mareeba 9/6/16



Figure 5. National Banana Development and Extension Project – Banana Roadshow, Innisfail 10/6/16



Figure 6. Agenda for the National Banana Roadshows




National Banana Development
and Extension Program


National Banana Roadshow Series 2016

**MAREEBA, Department of Natural Resources and Mines, John Charles room,
Thursday 9th June**


9.00 AM - Welcome, overview of the day	Tegan Kukulies (DAF)
Theme 1 – Panama disease tropical race 4	
<ul style="list-style-type: none"> - Using Zoning to protect your farm: Grower examples - Biosecurity Queensland Panama TR4 Program – what’s new - Banana farming with TR4: Lessons from the Philippines and Taiwan 	Sarah Simpson (ABGC) Rebecca Sapuppo (DAF) Dr Rosie Godwin (ABGC) & Patrick Leahy
Morning Tea	
<ul style="list-style-type: none"> - The down and dirty on disinfectants for Race 1 Panama disease - Remote sensing technology: exploring a new method for early disease detection and for the evaluation of plant health - Understanding fusarium genetics - Varieties Update – 2 years on 	Kathy Grice (DAF) Katelyn Ferro (DAF) Dr Elizabeth Aitken (UQ) Jeff Daniells (DAF)
Theme 2 – Production and environmental practices	
<ul style="list-style-type: none"> - Timing is everything – crop scheduling with ethephon stem injections - Fungi & bacteria: the yin & yang of banana soils - Introduction to Matt Abbott’s Nuffield Scholarship Experiences 	Stewart Lindsay (DAF) Dr Tony Pattison (DAF) VIDEO
Lunch	
<ul style="list-style-type: none"> - Reef safe nitrogen management - Bananas, water quality and the Great Barrier Reef - Banana BMP – What’s new and why you should use it? 	Jeff Daniells (DAF) Michelle McKinlay (ABGC) Tegan Kukulies (DAF)
Theme 3 – Supply chain management	
<ul style="list-style-type: none"> - Crown End Rot of Banana: Our learnings from the first year - Implementation of the 15kg 1-Piece Carton - 3 year strategic marketing plan for Australian bananas - Evaluations 	Peter Trevorrow (DAF) Tristan Kitchener (Kitchener Partners) Elisa King (HIA) Tegan Kukulies (DAF)
2.30 PM - Program finish	




Queensland
Government



Horticulture
Innovation
Australia



Australian
Banana



NSW
Department of
Primary Industries

Figure 7. Panama R&D Open Day flyer

Panama R & D Open Day

South Johnstone DAF Research Station, Friday 12th May 2017

8:30 am – 12:30pm

Topic/Activity	
Welcome and overview of the day	
Launch of the Best Management Practices for On-farm Biosecurity	
Disinfectant facts: reminder of effective products, results from testing of products in the Northern Territory, corrosion and longevity results, demonstration on how to use test strips to test concentrations.	<i>Kathy Grice & Shanara Veivers</i>
Soil health: groundcover and nitrogen trial results, nematode microscope demonstration, soil biology measurement demonstration.	<i>Tony Pattison & Anna McBeath</i>
Rapid Destruction: explanation about the validation of the use of urea in the destruction process, demonstration of a plant injected with fungus to increase rate of plant degradation.	<i>David East</i>
Proximal and Remote Sensing: showcase remote and proximal sensing tools, demonstration of equipment for detecting 'unhealthy' plants before visible symptoms appear	<i>Trevor Parker & Katelyn Ferro</i>
Tolerant Varieties: tour of varieties which have 'tolerance' to Panama disease tropical race 4, description of the mutagenesis process which is being taken in an attempt to develop a resistant cultivar.	<i>Jeff Daniells</i>
Grower insights on Panama disease tropical race 4 in the Philippines	
TurningPoint evaluation and Wrap Up	
Lunch + Exhibition of other Panama R & D	


Activities in the Paddock

For catering purposes and since strict on-farm biosecurity practices will be implemented to enter the paddock please RSVP with your shoe size to, Tegan Kukulies on 0459 846 053 or email tegan.kukulies@daf.qld.gov.au by Friday the 5th of May.

Figure 8. Printed media associated with the Panama R & D Open Day

FACEBOOK.COM/INNADVOGATE SATURDAY MAY 6 2017 LIFESTYLE 09

Rural insight



South Johnstone Open Day to highlight latest developments

Panama research hot topic

BANANA growers will soon have access to a new tool to help tackle on-farm biosecurity risks, such as Panama disease tropical race 4 (TR4), which is currently posing a risk to the \$600 million banana industry.

The Australian Banana Growers' Council is encouraging local banana growers to attend a unique Panama disease open day at South Johnstone next week.

Hosted by the Department of Agriculture and Fisheries (DAF), the day is designed to highlight the latest research and development focused on Panama disease Tropical Race 4 (TR4).

The interactive event will include a field tour and exhibits at the DAF research station ASGC, Chair Stephen Lowe said the half day event would provide a unique opportunity for growers to learn about some of the current applied research and development focusing on the short-to-long-term management of TR4.

"All growers know of the constant threat TR4 poses and must be aware of the extensive history of research and development that has been, and continues to be, carried out by researchers Australia wide," Mr Lowe said.

OPEN DAY EXHIBITS

- Future varieties and the strategies being used to develop them
- Soil health practices
- Disinfectant demonstrations
- Options for remote and proximal sensing
- Understanding the effect of urea in the TR4 destruction process

"The open day is designed to show growers first-hand some of the latest Panama advances and increase their confidence in the future containment and management of this disease."

A key component will be the launch of the new Banana Best Management Practices, On-farm Biosecurity manual - the latest tool designed for growers to help tackle on-farm biosecurity risks.

Queensland DAF's Team Leader of Banana Production Systems Stewart Lindsay said the manual was a valuable resource for all banana farming businesses.

"This manual is designed to help growers implement effective on-farm biosecurity practices," Mr Lindsay said.

The open day will be held on Friday, May 12, at the South Johnstone DAF Research Station (8.30-12.30pm).

Australian Banana Growers' Council chairman Stephen Lowe and deputy chairman Ben Franklin will be present at next Friday's Panama disease open day at South Johnstone.

IN BRIEF

ORGANIC IS GROWING
THE Australian Organic Annual Report reveals Australia holds the world's largest amount of organic managed farmland (53%) but it's still not enough to meet local demand. The report supports evidence of a growing consumer consciousness at check-out, and increased education of the benefits of organic food. Australia's organic industry is now valued at more than \$1.4 billion and by 2018, the total value is anticipated to exceed \$2 billion, given the current growth trajectory. A third of organic operations are in New South Wales, a quarter in Victoria and a fifth in Queensland.

MAREEBA FIELD DAYS
THE future of agriculture in the Mareeba and Tablelands region will go on display at this year's Rotary FNQ Field Days. Mareeba District Fruit and Vegetable Growers Association president Joe Mors said the Field Days provided the perfect platform to promote agriculture across North Queensland and farmers could look forward to seeing the latest and greatest developments in the industry. "The ongoing Field Days will reveal some of the new technology now being used, such as robotics and drones," he said. Rotary FNQ Field Days will be held between Wednesday, May 24, and Friday, May 26, at Mareeba's Kerriebe Park.

PSYCHOLOGY REBATE
THE Australian Psychological Society has welcomed the Government's recognition of the needs of people in rural and remote Australia with the announcement that people living in these areas will be able to claim a Medicare rebate for online videoconferencing consultations with psychologists. APS President Anthony Cicchello, says the move will provide greater equity of access to evidence-based psychological care for Australians living in small country towns, remote and very remote Australia.

Innisfail Advocate, Saturday May 6th 2017

24 WEDNESDAY MAY 10 2017 CAIRNSPOST.COM.AU

Rural insight

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New weapons swing into action to fight against threat of Panama disease

No rest in war on pest

TOM VOLLING

THE TR4 detection, isolation and disinfection are some of the new weapons added to the fight against Panama disease.

The Department of Agriculture and Fisheries (DAF) has announced the Panama disease open day on Friday at the DAF research station, where they will unveil the four pillars of research and development since November 2012.

DAF Banana Production Systems Team Leader Stewart Lindsay has worked with the Far North Queensland banana industry for the past 17 years.

"We have released the disinfectant, the new variety of banana, the new tool to detect the disease, such as the handheld scanner, and the manual for on-farm biosecurity," he said.

"The scanner is a good job - they don't know how useful it can be," Mr Lindsay said.

"Banana growers are just like everyone else in society. You've got some people who are very proactive and some who aren't at all. I would say most have done what they can already do."

"Maybe they are just a bit sceptical because they have done such a good job - they don't know how useful it can be."

"Science, industry and management practices are always the things that will be discussed at the open day."

Mr Lindsay will showcase a manual on the new disinfectant and the handheld scanner, and also the health of banana plants that are showing signs of Panama TR4 disease.

"The scanner has been used at a property in the Tablelands region for several years ago."

"It is a handheld scanner that can be used for on-farm biosecurity, such as the handheld scanner, and the manual for on-farm biosecurity."

"The thing with this disease is if you get it, you can't get rid of it. It's a 100 per cent disease that is really slow down the banana industry."

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STAYING UP-TO-DATE ON LATEST IN TECHNOLOGY

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Demand tipped to heat up as weather cools

FULLY banana growers are looking to begin planting in the next few weeks as the weather cools down and the risk of frost is reduced.

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Cairns Post, Saturday 6th May 2017



DIRE THREAT: Sharna Veivers, Research Horticulturalist with the Department of Agriculture and Fisheries, and Wangan banana farm supervisor Stephen Walls look at test strips used to test concentration levels of disinfectants used as part of on-farm biosecurity practices.

Fighting the Panama threat

THE Australian Banana Growers' Council is encouraging local banana growers to attend a unique Panama disease open day at South Johnstone tomorrow from 8.30am-12.00pm.

Hosted by the Department of Agriculture and Fisheries (DAF), the day is designed to highlight the latest research and development focussed on Panama disease Tropical Race 4 (TR4), which remains a constant threat to the country's \$600 million banana industry.

The interactive event will include a field tour and exhibits at the DAF research station, to see and hear about tolerant varieties and the strategies being used to develop them, soil health practices, disinfectant demonstration, options for remote and proximal sensing and understanding the effect of urea in the TR4 destruction process.

ABGC Chair Stephen Lowe said the half-day event would provide a unique opportunity for growers to learn about some of the current applied research and development focussing on the short-to-long-term management of TR4.

"All growers know of the constant threat TR4 poses to our industry and most are aware of the extensive history of research and development that has been, and continues to be, carried out by researchers Australia wide," he said.

"The open day is designed to show growers first-hand some of the latest Panama advances and increase their

confidence in the future containment and management of this disease."

A key component of the day will be the launch of the new Banana Best Management Practices, On-Farm Biosecurity manual, which is the latest tool designed for growers to help tackle on-farm biosecurity risks.

Queensland DAF's Team Leader of Banana Production Systems Stewart Lindsey said the manual was a valuable resource for all banana farming businesses, whether they already have on-farm biosecurity systems in place or are planning to implement them.

"The Australian banana industry is vulnerable to pests and diseases," he said. "This manual is designed to help growers implement effective on-farm biosecurity practice."

"It consists of a self-assessment checklist and reference materials divided into four major sections: zoning, general farm operations, crop production and fruit movement."

The manual was jointly funded by Horticulture Innovation Australia using banana research and development levies and funds from the Australian Government with co-investment from DAF.

For catering purposes and since strict on-farm biosecurity practices will be required to enter the paddock, please RSVP to the open day, with your shoe size, to Tegan Kakulites on 0439 846 053 or email tegan.kakulites@daf.qld.gov.au

Wild dog baiting

NOTICE is hereby given that Hinchinbrook Shire Council is conducting a shire wide Wild Dog Baiting Program utilising 1080 poison. The baiting program will commence today, Thursday 4 May 2017.

The Wild Dog Baiting Program is required due to high populations of wild dogs in certain areas that are impacting on livestock production.

This notice is to advise that the program is taking place and landholders participating in the campaign must comply with set procedures for baiting. This includes notification to all adjoining landholders at least seventy-two (72) hours prior to the baiting.

For further information, please contact Hinchinbrook Shire Council's Environment and Community Services Department on 4776 4607.

Rainfall Tully

Statistics 2017 - MAY

S	M	T	W	T	F	S
1	2	3	4	5	6	mm
7	8	9	10	11	12	13
14	15	16	17	18	19	20
21	22	23	24	25	26	27
28	29	30	31			

Month to Date - 56.9 mm Days - 5
Year to Date - 3523.2 mm Days - 87



WAREHOUSE DRIVE THRU PICK UP 4-6 CLIFFORD ROAD INNISFAIL

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COME AND SEE US AT THE
FNQ Field Days - MAY 24-26
We will be on Stand A21
to discuss any custom blend
requirements with you

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Growforce Fertiliser or custom Black Urea Blends



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Rural insight

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Growers learn how to fight back against TR4

A BUNCH of banana growers has dived into the latest research into the Panama disease Tropical Race 4.

About 100 banana growers, researchers and industry leaders learned about disease management at South Johnstone Research Station last Friday.

ABGC chair Stephen Lowe said the half-day event was a unique opportunity to build further industry confidence.

"Panama TR4 is a constant threat to our industry," he said.

"But it was a chance to gain insight into the groundbreaking research and development that is giving growers greater reassurance that TR4 can be managed into the future."

"Australia is recognised globally as a world leader in the fight against TR4 and this was not only an informative exercise, but was also a celebration of the scientific efforts carried out by researchers Australia-wide that is helping to protect our growers and industry."

A new Banana Best Management Practices, On-Farm Biosecurity manual, which is designed for growers to help tackle on-farm biosecurity risks, was also launched.



HANDY WISDOM: Department of Agriculture and Fisheries principal horticulturalist Jeff Daniels presenting an information session.

Industry's website upgraded

SUGAR Research Australia has unveiled a fresh website to improve growers' access to the latest industry information.

It follows investor feedback that criticized the old site for being difficult to access and navigate.

SRA chief executive Neil Fisher said the new site provided extensive information on the organisation's research.

"The new site includes information and fact sheets for growing and milling the crop, details about SRA's research investments, information for research collaborators, as well as our corporate information and publications," he said.

"The new-look website presents information in a clear and modern way for our investors and other industry stakeholders."

The SRA e-library is the home of the vast catalogue of research and other publications authored by SRA and others. The site is available at www.sugarresearch.com.au

Appendix 17 – Evaluation of the Banana Industry Roadshows 2016

The National Roadshow series undertaken as part of the National Banana Development and Extension Project in 2016. The series visited Mareeba (9th June), Innisfail (10th June), Tully (16th June), Carnarvon (23rd June), Coffs Harbour (5th July) and Murwillumbah (7th July). Project topics from BA14013 namely the research on disinfectants, remote and proximal sensing, an overall varieties update and soil health overview featured at most of the day long events. Overall 147 growers and industry stakeholders (excluding researchers) attended the 6 events. Table 1 shows the breakdown of attendance at each location.

Location	Number of attendees
Tablelands	32
Tully	31
Innisfail	33
Coffs Harbour	19
Murwillumbah	15
Carnarvon	17
Total Attendance	147

The events overall successful and this was evidenced through evaluation obtained through the turning point electronic polling system. The full evaluation results from the 2016 Roadshow events can be found in the appendix of the final report of BA13004 however the table below summarises the percentage of attendees which may or may not be considering changing a practice on their farms as a result of attending one of the roadshow events.

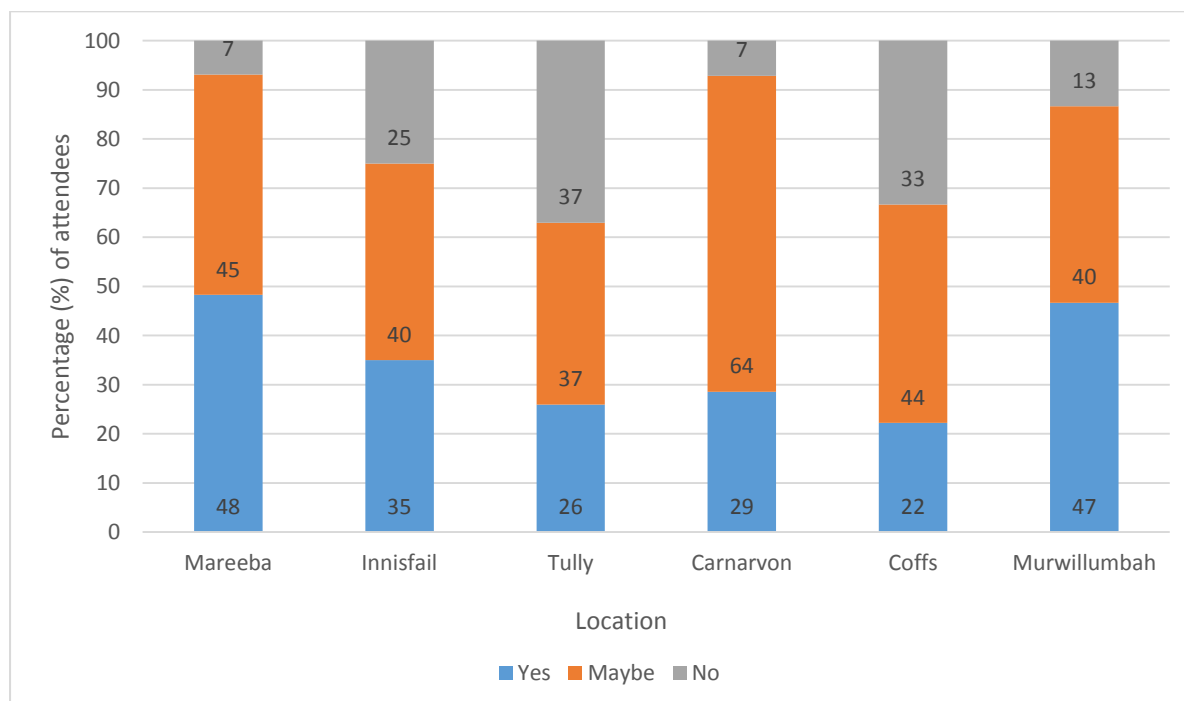


Figure 1: Breakdown of the percentage of attendees which would change something, might change something or would not change something on their farms after attending the 2016 Roadshow events

Appendix 18 – Evaluation of the Panama R & D Open Day

Attendance

In total 109 people attended and participated in the Panama R & D Open Day which was held on the 12th of May 2017 and showcased the latest research and development advances. The graph below shows the distribution of those that attended growers and industry stakeholders (e.g. private consultants, agronomists, resellers etc.) who were the main target audience made up 64% of attendees.

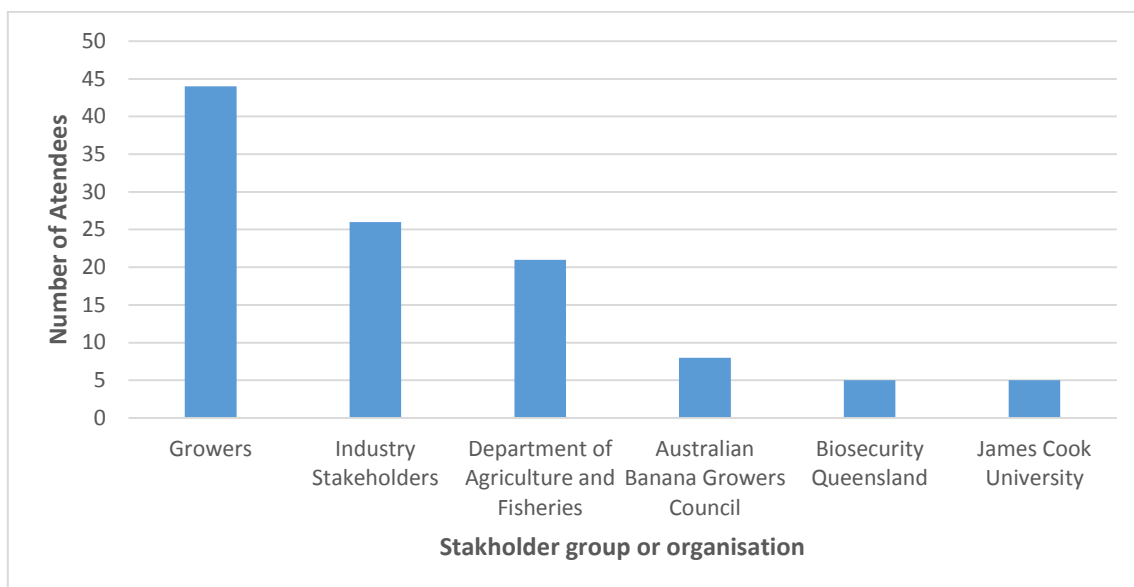


Figure 1: Distribution of industry stakeholders and representatives from organisations which attended the Panama R & D Open Day (12th May 2017)

Evaluation

TurningpointTM which is an electronic polling system was used to evaluate the Panama R & D Open Day. This survey was conducted at the completion of the day. Polling was conducted with growers and industry stakeholders and excluded researchers involved in Panama & D projects. The table below summaries the questions which were asked of the attendees and the respective percentages for replies.

Table 1: Summary of responses to the evaluation question asked at the Panama R & D Open Day	
How much to you now know about Panama disease R & D?	
1 - Nothing at all	0%
2 - Very little	0%
3 - Some idea	17%
4 - Good understand	65%
5 - I'm across it all	19%
Will you change anything after attending today?	
Yes	46%
Maybe	50%
No	4%
Would you attend an event like this again?	
Yes	59%
Yes & I would recommend it to others	39%
No	2%
How would you rate today?	

1 – Being the lowest	0%
2	0%
3	0%
4	0%
5	4%
6	4%
7	22%
8	29%
9	22%
10 – Being the highest	18%