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Final report

project

Integrated management of Fusarium wilt of bananas in the Philippines and Australia

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2 Executive summary

Fusarium wilt of bananas caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) is considered one of the most destructive banana diseases. The Tropical Race 4 (TR4) strain of the pathogen is particularly virulent on commercial Cavendish banana production, which supplies bananas to consumers around the world. Currently, there are 15 countries that have reported to have Fusarium TR4, mostly in South-East Asia.

To reduce the impacts of Fusarium wilt and improve the livelihoods of banana growers in the Davao del Norte region of the Philippines and the north Queensland region of Australia, HORT/2012/097 was undertaken. The project aimed to;

- increase knowledge of on-farm biosecurity, minimising Fusarium wilt incursions,
- develop long-term management strategies to slow the spread of the disease and
- develop options to allow smallholder producers to return to economic production.

To achieve the overall aims, three objectives were undertaken;

Firstly, was to develop options to limit losses in banana production by improving knowledge of on-farm biosecurity. On-farm biosecurity protocols were very effective in slowing the spread of Fusarium TR4 in Australia, following the outbreak of the disease near Tully in March 2015. Project work conducted in the Philippines demonstrated deficiencies in the implementation of on-farm biosecurity, due to lack of effective chemicals and poor implementation. However, this project identified how local solutions, using low cost boot scrapers constructed from wire mesh, together with effective disinfectants, could be implemented to improve farm biosecurity practices.

The second objective was to evaluate integrated crop management approaches to enable commercial banana production in the presence of Fusarium wilt. In Australia, there was strong evidence that increasing soil microbial activity was related to suppression of Fusarium wilt. To increase soil microbial activity on banana farms vegetated ground cover was adopted over an estimated 1,500 ha (10%) of the north Queensland banana industry. In the Philippines, differences between Australia and Philippine banana production systems, meant that implementation of vegetated ground covers on smallholder commercial banana farms was problematic. In the Philippines scenario, the use of a resistant cultivar, GCTCV218, was the only option to continue banana production for growers who had Fusarium TR4 on their farms. A synopsis from the field experiments, indicated that any integrated crop management system for banana where Fusarium TR4 was present, requires cultivars with resistance as a basis.

The third objective was to determine the barriers to adoption of Fusarium wilt management practices. In Australia, banana growers tended to have greater knowledge of Fusarium wilt. The greatest barrier was the financial capacity to implement biosecurity practices, which amounted to capital costs of 13% and annual running costs of 0.8% of the value of the banana enterprise. In the Philippines, Fusarium TR4 continued to spread within Davao del Norte, with the main barriers to adopting Fusarium wilt management practices around knowledge of what to do, the widespread extent of the problem and the availability of resources.

The project HORT/2012/097 focused heavily on increasing the capacity of Philippine project partners to develop solutions to Fusarium TR4, with training activities to increase the capacity to quantify soil biology and disease suppression. The involvement of banana growers as project partners allowed additional communication of project results and added value to the research outcomes. HORT/2012/097 was also used to demonstrate how international collaborative projects could benefit both partner countries and contributed to a case study for the 2017 Foreign Policy White Paper. As Fusarium TR4 continues to spread globally, the outcomes from HORT/2012/097 will continue to generate impacts on how to manage Fusarium wilt, to protect livelihoods of smallholder banana producers.

3 Background

Banana is one the world's most important fruit in terms of production volume and trade and among the world's top 10 staple foods (Dita *et al.*, 2018). Banana cultivation is often conducted by smallholder farmers with 114 million tonnes produced globally in 2017 (FAO, 2019). In the Philippines, the total area planted to banana is 456,641 ha producing 5.8 million tonnes of fruit worth US$1.6 billion per annum (FAO, 2019). In 2017, the Philippines was the 2nd largest global exporter of bananas, trading 2.67 million tonnes worth US$1,049 million, (FAOStat) and employing an estimated 350,000 people. Furthermore, up to 80 distinct banana varieties are cultivated in the Philippines for local consumption with the most popularly planted types being Saba (186,300 ha), Lakatan (57,000 ha), Latundan and Bungulan (local Cavendish selection). There was decline in the area of banana production area in 2016, which readjusted in 2017 (Fig 3.1A), but a previous decline in the quantity of banana produced in 2014 with a slow recovery since (Fig 3.1B). Concurrently, with the decline in quantity of bananas produced, there was a decline in the export value of bananas in 2014 relative to other banana exporting nations (Fig 3.1C), which had mostly recovered by 2017.

The Australian banana industry is much smaller by comparison to the Philippines, reaching as much as 16,612 ha in 2016 and producing as much as 412,972 tonnes in 2017, for domestic consumption, with no export market (Fig 3.1).

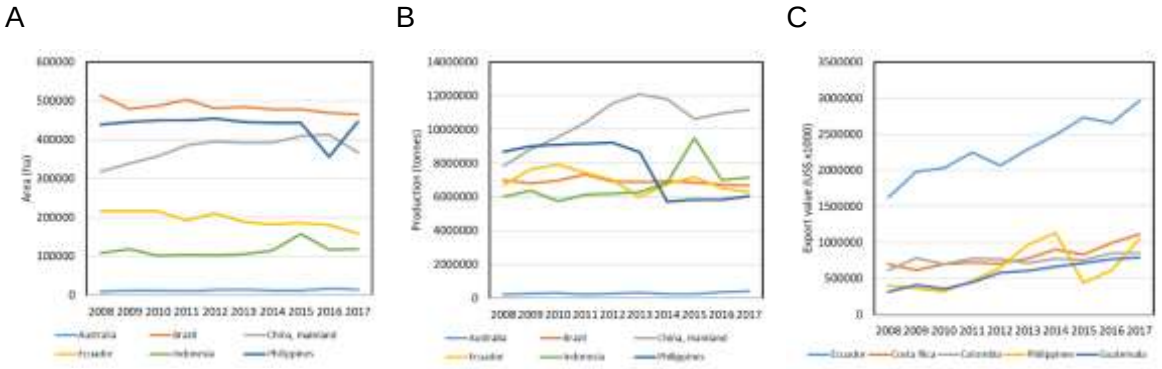

Fig 3.1. Area (A), quantity (B) and export value (C) of bananas from selected banana producing countries over a 10-year period from 2008 to 2019.

Fusarium wilt of bananas caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) is considered one of the most destructive banana diseases in history (Stover and Simmonds, 1987). The pathogen was first described on bananas in 1874 (Pegg et al., 1996), but is believed to have co-evolved with its banana host in Asia, and from there was disseminated to new areas through infected planting material (Ploetz and Pegg, 1997). Foc, is found in all Asian countries where bananas are grown, with multiple vegetative compatibility groups (VCGs) found in most countries (Mostert et al., 2017). However, it is the dominance of VCG 1213/16 known as Tropical Race 4 (TR4) that is of greatest concern to the export banana industry relying on clones of Cavendish (*Musa* AAA) (Ploetz et al., 2015). The epidemics of TR4 became a real concern in the Asian region during the late 1990's and 2000's (Molina et al., 2009) and fuelled the global spread of the disease (Fig 3.2), with an estimate that 1.7 million ha of bananas could be affected by Fusarium wilt by 2040 in a worst case scenario (Scheerer et al., 2016). The recent detection of Fusarium TR4 in Colombia (García-Bastidas *et al.*), the fourth largest banana exporting nation, has implications for Latin America and the Caribbean, with the worst case scenario indicating that wide spread of Fusarium TR4 would have a considerable economic impact on trade, food security and the economic wellbeing of banana producing countries, as well as on producers in other exporting countries and consumers in importing countries (FAO, 2019). According to the FAO, Fusarium TR4 of bananas is expected within 10 years to cause a loss of 160,000 ha of bananas, resulting in a 2% decrease in global banana

production, and a loss of 240,000 jobs from the banana sector thus increasing the retail price in developed countries by 3.2%, by 2028 (FAO, 2019). South-East Asian countries are predicted to suffer the greatest losses in the banana market due to Fusarium TR4.

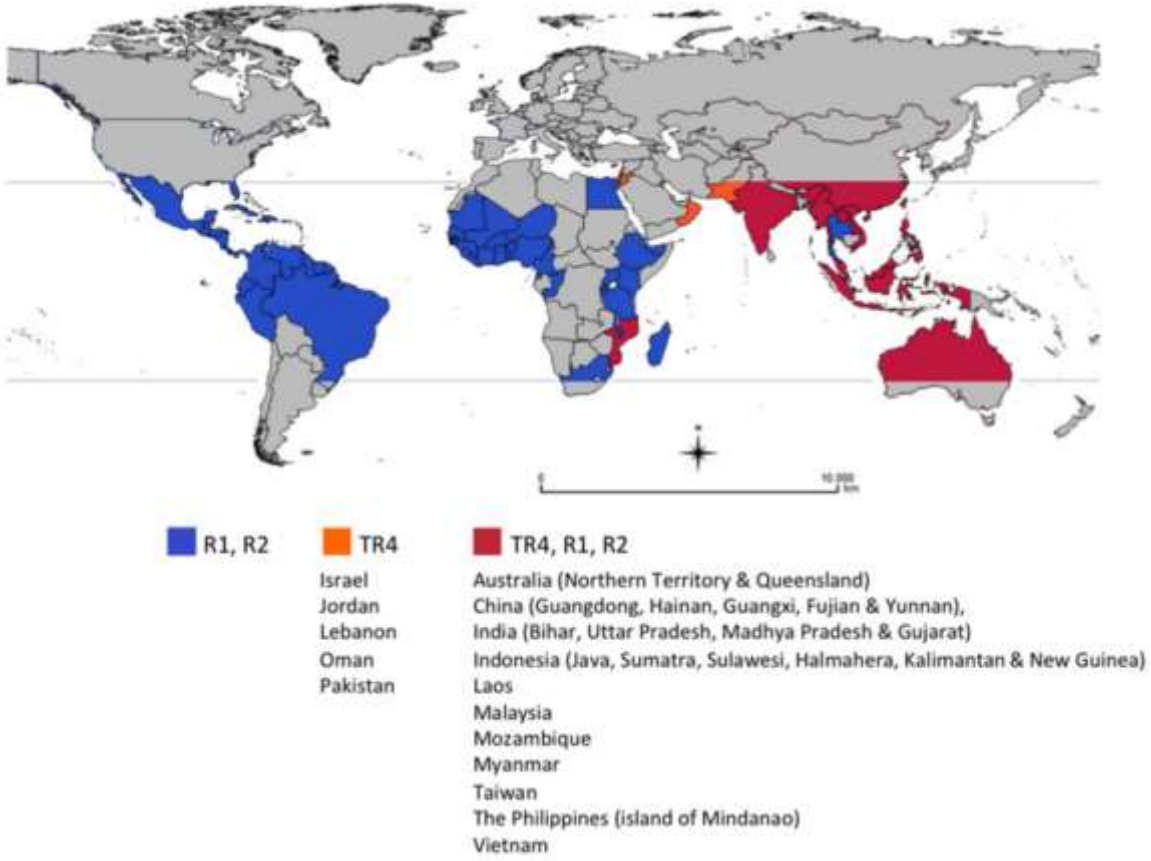

Fig 3.2. Global distribution of *Fusarium oxysporum* f. sp. *cubense* Race 1, 2 and TR4 and potential for spread around the world (Dita et al (2018)

Fusarium wilt of Cavendish banana production in the Philippines was reported as early as the 1970s, but the VCGs associated with these infections were VCG 0122, 0123, and 0126 (Magnaye, 2001), which are regarded as less aggressive populations of Foc (Pegg et al., 2019). The confirmation of epidemics TR4 in the Philippines and the subsequent spread causing abandonment of severely affected farms raised a serious threat to export Cavendish banana production (Molina et al., 2008; Molina et al., 2011). Of greatest concern for the Philippines banana industry is the dominance of TR4 on the island of Mindanao (Molina et al., 2009; Mostert et al., 2017), where 85,000 ha of bananas are grown for export (Molina *et al.*, 2016; Montiflor *et al.*, 2019). Banana production in Mindanao comprises of 80% of smallholder growers, and within Mindanao Region XI (Davao Oriental, Davao del Norte, Davao City and Compostela Valley), 51% of banana farmers own less than 2 ha, with only 14% of banana farmers owning greater than 5 ha. Furthermore, a survey of banana growing regions on Mindanao found 20% of barangays had bananas with symptoms of Fusarium wilt, with up to 80% of bananas infected in backyards, small and large scale plantations (Soguilon et al., 2015; Solpot et al., 2016). It is estimated that 34% of the banana production area in the Philippines, could be lost to Fusarium wilt TR4 in 11 years' time (Scheerer et al., 2016). Current banana production and projected loss figures indicate, the Philippines has the potential to lose US$544 million, due to the impact of Fusarium wilt TR4 on banana export revenue if Fusarium wilt is not effectively managed.

TR4 was first recorded in Australia in the Northern Territory in 1997 (Conde and Pitkethley, 2001), but was recently detected in the main banana producing area in north Queensland in the Tully valley on March 2015 (O'Neill *et al.*, 2016). In Australia it is estimated that the spread of the disease could threaten the US$450 banana industry, but lead to a losses of US$1 million per year (Cook *et al.*, 2015).

There are currently no completely TR4 resistant banana cultivars that achieve the same productivity and market acceptance as the current Cavendish cultivars (Heslop-Harrison and Schwarzacher, 2007). However, somaclonal variation was used successfully to develop replacement cultivars with improved resistance, known as Giant Cavendish Tissue Culture Variants (GCTCV) for the Taiwanese banana industry (Hwang and Ko, 2004). Furthermore, selections of GCTCVs were shown to be resistant to Fusarium wilt in different environments (Huang et al., 2005). GCTCV 218 and GCTCV 219 were introduced to the Philippines from Taiwan for evaluation as replacements to Grand Naine for the Philippine banana industry, with GCTCV 218 having better post-harvest and yield characteristics than GCTCV 219 (Molina et al., 2009; Molina et al., 2016). The introduction of new cultivars creates challenges as the post-harvest conditions may differ in the supply chain (Luyckx et al., 2018). Furthermore, these banana plantations still rely on monocultures of single clones of bananas, which can apply selection pressure on soil organisms due to a negative plant-soil feedback that promotes soil microorganisms that are deleterious to their own growth (Vukicevich et al., 2016).

The incidence of Fusarium wilt was reported to be reduced through the use of biocontrol agents, by up to 79% using *Pseudomonas* spp. strains, and up to 70% by several endophytes and *Trichoderma* spp. strains (Bubici *et al.*, 2019). However, use of biocontrols has not gained widespread acceptance for the management of Fusarium TR4, even though many biological control studies have been conducted (Ploetz, 2015). Furthermore, according to Ploetz (2015) none of the published results on biological control of Fusarium wilt indicated cost-effective, long term management in the field. Cordovez *et al.* (2019) suggested that the introduction of microorganisms into the soil were usually "washed-out" and do not persist at functionally meaningful densities The success of biological control organisms tends to have strong site-specific dependence and require extensive field experimentation to demonstrate their efficacy against Fusarium TR4 (Bubici et al., 2019; Marian and Shimizu, 2019).

An integrated management system for Fusarium wilt relies on strategies to reduce Foc inoculum from infecting plants, through increased biological suppression, while at the same time using commercially acceptable banana cultivars that have enhanced resistance to the disease (Pattison et al., 2018). Options to manage Fusarium wilt other than the use of resistant cultivars are frequently described as a challenge (Dita et al., 2018). However, management practices that improve soil health and pathogen suppression, using crop rotation, cover crops, application of organic amendments and biocontrol agents, as well as, the use of appropriate inorganic fertilizers are shown to be effective to decrease Foc inoculum, reduce disease intensity and enhance banana productivity in Australia (Pattison *et al.*, 2014b; Rames *et al.*, 2018), China (Fu et al., 2016; Huang et al., 2012) and India (Thangavelu et al., 2003; Thangavelu et al., 2004).

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). Previous investigations demonstrated that increasing microbial diversity and activity, along with management of Fusarium inoculum in the field, results in the suppression of Fusarium wilt of bananas (Huang et al., 2012; Peng et al., 1999; Sudarama and Suprapta, 2011). Both abiotic and biotic parameters were investigated in relation to soil borne disease suppression; however, no soil parameter in isolation proved to be reliable and consistent for predicting suppression (Bonanomi et al., 2010; Janvier et al., 2007). The characterisation of the soil through the use of indicators

was suggested to allow predictions of soil management impacts on soil biology and therefore disease suppression (Janvier et al., 2007).

The availability of high-throughput sequencing (or next-generation sequencing – NGS) technologies is now driving a shift that allows investigations into microbial community studies into disease suppression and microbial approaches to disease management (Massart *et al.*, 2015; Shen *et al.*, 2017). NGS allows a description of the plant microbiome, that is the microbial component comprising all of the genomes of microorganisms that interact with the plant and its various compartments, including soil, roots, stems and leaves. Often the most influential microbiome comprises the soil microbial community of the rhizosphere, which is the soil surrounding the roots of plants where complex interactions occur between the roots, soil, and microorganisms (Chaparro *et al.*, 2012). However, the composition of the plant microbiome is often dynamic controlled by multiple factors, such as in the rhizosphere by temperature, pH, chemical signals from bacteria, plants, and nematodes that provide a basis for plants and their microbiomes to selectively be associated with one another (Lakshmanan *et al.*, 2014). Investigations into how management of banana plants could influence the composition of the banana microbiome showed sensitivity to changes in location and management (Köberl *et al.*, 2015). These include attempts to drive changes in the banana microbiome associated with biocontrol of Fusarium wilt using biocontrol organisms and manipulation of the inputs into the banana cropping system (Fu *et al.*, 2017; Shen *et al.*, 2019; Xue *et al.*, 2015; Zhang *et al.*, 2018). A suggested framework, using network analysis identifies positive or negative associations with desirable or undesirable outcomes of changes in the microbial community on other soil microorganism, plant response, pathogen response and disease expression (Poudel *et al.*, 2016) and has applicability for the microbiome of bananas for suppression of Fusarium TR4.

This project aimed to develop options for an integrated system for banana production in the presence of Fusarium wilt of banana by addressing three different scenarios faced by banana growers. Firstly, banana growers that do not have Fusarium wilt require knowledge and systems to prevent the introduction of the disease onto their plantations. Secondly, when the Fusarium wilt is first detected on a plantation, banana growers need to implement practices, that limit its spread and allow the plantation to be as productive for as long as possible. Thirdly, banana growers that have widespread infection of Fusarium wilt on their plantations need to implement practices that will allow them to return to productive plantations. To answer the needs of banana growers facing the different scenarios in terms of management of Fusarium wilt, three broad objectives were addressed;

1. Developing options to limit losses of banana producers due to Fusarium wilt
2. Evaluating the effectiveness of commercial banana farm practices when Fusarium wilt is present.
3. Determining the barriers banana growers face in adopting systems to suppress Fusarium wilt.

By integrating disease management practices into banana production systems, from science-based outcomes, and understanding and overcoming the some of the barriers that banana growers face when implementing new practices improved systems for banana production can be developed that allow smallholder banana producers to continue production and maintain viable livelihoods.

4 Objectives

4.1 Specific objectives:

The goal of the project was to reduce the impacts of Fusarium wilt on bananas, and therefore, improve the livelihoods of smallholders and communities who are dependent on banana production in the Davao del Norte region of Mindanao and in Australia. The project's specific aims were to increase knowledge of effective on-farm biosecurity practices and improve farm preparedness, to minimise the risk of Fusarium wilt incursions. Furthermore, the project aimed to improve the long-term management of Fusarium wilt by slowing the spread of the disease and through the development of Fusarium wilt suppressive management practices that incorporate the use of Fusarium wilt resistant banana cultivars. The project further proposed to develop options to allow smallholder producers devastated by Fusarium wilt to return to economic banana production.

Objective 1: To develop options to limit losses of smallholder Cavendish production in Davao del Norte and Ladyfinger production in Australia due to Fusarium wilt

Activity 1.1: Establish and contrast vegetative ground cover with bare soil for the suppression of Fusarium wilt.

Activity 1.2: Determine methods of reducing soil movement from banana plants within and between plantations.

Activity 1.3: Measure suppression of Fusarium wilt in bananas due to crop residue decomposition and plant eradication.

Activity 1.4: Scope opportunities to look more deeply into the banana microbiome and its role in protecting bananas from Fusarium wilt.

Objective 2: To evaluate the effectiveness of best-bet ICM approaches in enabling commercial banana production in the presence of Fusarium wilt.

Activity 2.1: The evaluation and validation of best-bet ICM practices to enable commercial export Cavendish banana production in the Davao del Norte region of the Philippines.

Activity 2.2: The evaluation and validation of best-bet ICM practices to enable commercial banana production in Australia.

Objective 3: To determine the barriers to adoption of systems to suppress Fusarium wilt in banana production in the Philippines and Australia.

Activity 3.1: Appraisal and assessment of current practices for the management of Fusarium wilt in Cavendish banana production in Davao del Norte and to determine information needs and barriers to adoption.

Activity 3.2: Appraisal and assessment of current practices for the management of Fusarium wilt in Ladyfinger/Niche banana cultivar production in Australia and to determine information needs and barriers to adoption.

5 Methodology

5.1 Location and sites

The project was conducted in two pilot areas in the Philippines and Australia, where banana production is a large part of the local economy. In the Philippines the project centred around the Davao del Norte region that has a total planted area of 26,297 hectares making up 36.9% of the entire Cavendish banana industry. Production areas are found in Panabo (28%), Santo Tomas (25%), Tagum (13%), and Kapalong (11%). Field experiments were established initially on the border of the Panabo area to Davao City at Lasang and a second site at Kapalong at the Alberto Magpuri Soriano Fresh Fruits Cooperative (elsewhere AMSEFFCO). Grower surveys occurred primarily throughout Davao del Norte, but also included banana growers in neighbouring provinces.

In Australia the Far North Queensland (FNQ) region has 88% of Australia's banana production (Pattison *et al.*, 2008), due to favourable soils and climate. In FNQ, 76% of bananas are grown in places that range between 2,796 and 3,924 mm of average annual rainfall and with an annual maximum temperature greater than 29˚C and an annual minimum temperature greater than 19˚C (Martinez-Diaz unpublished).

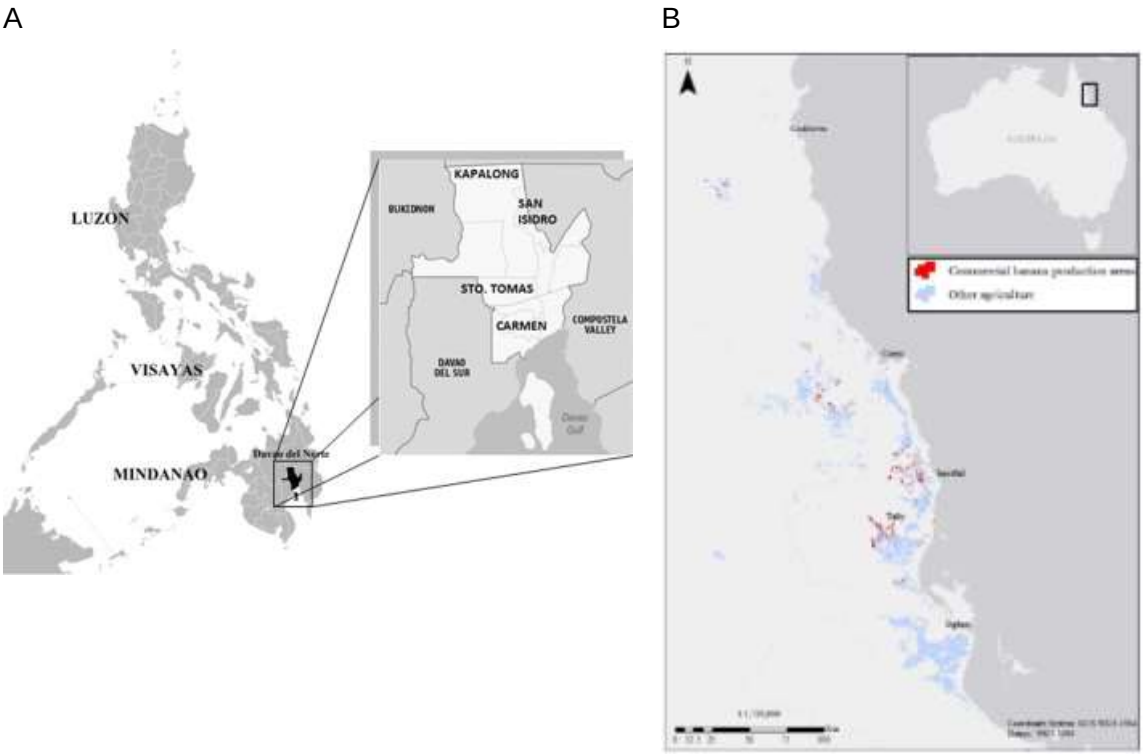

Fig 5.1: Location of study regions for integrated management of Fusarium wilt of bananas within the Philippines (A) and Australia (B)

5.2 Methods

5.2.1 Objective 1: To develop options to limit losses of smallholder Cavendish production in Davao del Norte and Ladyfinger production in Australia due to FW

Activity 1.1: Establish and contrast vegetative ground cover to current practice of bare soil to suppress Fusarium wilt

Location: *University of Southeastern Philippines, Obrero Davao, Mindanao, Philippines*

Personnel: *Ms Tamsi Gervacio, Ms Christina Ansale, Mr Marvin Tagan*

A pot experiment was established to determine the impact of vegetated groundcover species on the growth of bananas and development of disease when inoculated with Foc TR4. Four ground cover species were selected for the experiment *Arachis pintoi* (Pinto peanut), *Calopogonium mucunoide* (kudzo), *Ipomoea batatas* (ornamental sweetpotato) and *Paspalum conjugatum* (Green frog grass) and a combination of all ground cover species, compared with bare inoculated and uninoculated soil. The groundcovers were grown in 200 mm diameter pots, with 5.0 kg of soil. A banana plant cultivar Grand Naine (*Musa* AAA, Cavendish) was grown in the pots. Two resistant Cavendish cultivars GCTCV-218 and GCTCV-219 were also included in the experiment. All pots were inoculated with a 1 x 10^4 conidia/ml solution except for the uninoculated Grand Naine control.

Plant agronomic and disease assessments were conducted weekly for a 15-week period following the inoculation of the plants with Foc. At the termination of the experiment plants were destructively harvested to determine plant dry weights. The percentage of the banana rhizome displaying necrosis was determined and a rating given according to Orjeda (1998). Furthermore, at harvest biochemical analysis of soil samples included soil enzymes, fluorescein diacetate (FDA), β-glucosidase, labile C and Community Level Physiological Profiles (CLPPs) using the MicroResp™ were conducted at the University of Southeastern Philippines, Tagum Campus. More details of the soil biochemical and CLPP methodology is given in 5.2.1.

Activity 1.2: Determine methods of reducing soil movement from around banana plants within plantations and between plantations.

Location: *University of Southeastern Philippines, Obrero Davao, Mindanao, Philippines*

Personnel: *Ms Tamsi Gervacio, Ms Christina Ansale, Mr Marvin Tagan*

Experiment 1.2.1: Determining the efficacy of disinfectants used in the Philippine banana industry

Inoculum preparation

An isolate of the Foc-TR4 from the University of Southeastern Philippines was grown in ¼ strength Potato Dextrose Agar (PDA) for 2-3 days, after which conidial spores were washed from the plate using sterile distilled water and filtered through a funnel with sterile cloth gauze to obtain a microconidia spore suspension with a 1 x 10^4 conidia/ml concentration determined using a haemocytometer.

Disinfectants products used for the experiment

The products used were based on the recommendation of the Fertilizer and Pesticides Authority (FPA), and those that were commonly used by farmers in Davao City and Davao del Norte. These were Chlorox, Formo, Beloran 40 SL, Biocit, and formalin with the active ingredinets and recommended rates given in Table 5.1.

Table 5.1 List of disinfectant products tested for efficacy against Foc-TR4 spores

Commercial Name	Active Ingredient	Recommended Rate
Chlorox	7% sodium hypochlorite	4.7%*
Formo	2,2-dibromo-3-Nitrilopropionamide	9%*
Beloran	Benzoxonium chloride	2-4%***
Biocit	Benzalkonium chloride	2-4%***
Formalin	Formaldehyde	10-50%**

*Note: *manufacturer's recommended rate, **farmer's recommend rate, ***FPA's recommended rate*

Efficacy of disinfectants against Foc-TR4 in spore suspension

The five selected disinfectants were diluted based on their recommended rate plus, half or doubled the recommended rate to coincide with rates currently used on Philippine banana farms. Separately, a volume of 5 µl and 50 µl of the disinfectant solution was pipetted into a microtube. A 500 µl of 1 x 10^4 spore suspension was added and vortexed briefly for even distribution. A control made up of sterile distilled water was prepared and added with 500 µl of spore suspension. The mixture was then pipetted to a ¼ strength PDA with the use of a sterile glass spreader. Five replicates were made for each treatment. The Petri plate was placed in a dark room and incubated at 25˚C for three days, after which the number of colony forming units were determined.

Efficacy of disinfectants against Foc-TR4 in soil

A soil sample was obtained from land not previously planted with banana and passed through a 5mm sieve. A 500g sample was autoclaved for two consecutive days, after which 5ml of a 2x10^4 spores/ml Foc spore solution was added to the soil thoroughly mixed using a metal spatula for even distribution as described by Bennett *et al.* (2011). A 100 g sub-sample of the inoculated soil was placed into a 10 cm^2 section of a 'V' trough. Approximately, 10 ml of the disinfectants was evenly applied over the surface of the soil with care taken to prevent cross-contamination and desiccation of samples. The treated soils and the controls were left for 3 hours after which a 1 g sample was obtained and mixed with 9ml of sterile distilled water. A 0.5ml aliquot was taken placed onto a Petri plate with PDA, sealed in a dark room at 25˚C for 3 days. The colony forming unit was counted on the plate, with a target of 1 colony forming unit per cm^2.

Experiment 1.2.2: Determining the efficiency of soil removing from foot wear

Assessment of devices to remove soil from footwear

Five soil removal designs were tested for their efficiency in removing soil attached to soles of footwear under dry and wet soil conditions. The soil removal devices were made from easily available materials, which were also recyclable such as, bottle caps, nylon brushes, coco coir, rubber and wire mesh. Each device had equivalent dimension of 61 cm (length) x 38 cm (width). Footwear was selected to have the same tread pattern on the soles.

A banana farm was simulated by creating an improvised platform with a dimension of 13 m (length) x 3 m (width) x 0.1 m (depth). The platform was loaded with Foc-infected soil from a banana farm. Ten participants walked within the platform in a random direction for three minutes to allow soil to adhere to the boots. The soiled boots were scrubbed in a randomly selected soil removal device for 30 seconds. The soil fragments that fell in the collection tray that was placed underneath the soil removal devices were collected, placed in a re-sealable plastic bag, and weighed using an analytical balance, which allowed protocols development for further evaluation of soil removal devices combined with disinfectants.

Evaluation of disinfectants on footwear

After determining the most efficient soil removal design, three commercial disinfectants were tested for their efficacy against Foc spores in the soil left attached in boot soles after passing through the soil removal device. The disinfectants used in the experiment were

Major D (Benzalkonium chloride), Formo (2,2-Dibromo-3-nitrilopropionamide), and Chlorox (sodium hypochlorite), described previously in Table 1, and tested using the recommended rate of the manufacturer; and half and twice of the manufacturer's recommended rate.

Ten pairs of boots were soiled within the simulated platform for three minutes and scrubbed with the most efficient boot scraper design. The infested boots were dipped for two seconds in each of the prepared disinfectant concentrations with water as control. Soil adhering to the soles of footwear were scraped using a sterile spatula and placed in a re-sealable plastic bag.

Analysis for Foc remaining on footwear

One gram of the collected soil was transferred into a 0.1% water agar solution and diluted using 4-fold dilution series with sterile water. The number Fo was determined using drop plate technique, where a Petri dish with Komada's medium was divided into four quadrants and five, 10 µl drops were placed in a Petri dish from each of the dilutions (Harris and Sommers, 1968). Plates were incubated at room temperature for three days. The most probable number (MPN) of colony forming units was determined using the number of positive responses of Fo at each dilution, following the procedure of Meynell and Meynell (1970) and the colony forming units per gram fresh weight of soil (cfu/g) were then calculated, with corrections for dilution factors and sample weight (Andrews and Kenerley, 1978).

Experiment 1.2.3: Efficacy of disinfectants influenced by farm management

The experiment was conducted at two commercial farms identified with Foc, one located at Calinan, Davao City and the other at Kapalong, Davao del Norte. The experiment was conducted at the entry and exit points of the farm where foot baths were installed. At Calinan the entry/exit foot bath was loaded with 10% Biocit (Benzalkonium chloride) and at Kapalong with 9% Formo (2, 2-dibromo-3-Nitrilopropionamide), respectively. The disinfectant solutions were prepared on the concentrations used by the farmers. At Calinan the disinfectant was replaced daily before the experiment began, while in Kapalong farm replacement of disinfectant was done weekly.

The experiment ran for six and five consecutive days in Calian and Kapalong respectively. The sampling of the disinfectant solution from the foot bath was conducted at 6:00 am to 3:00 pm with four-time intervals at Calinan farm and with three-time intervals in Kapalong. Each time point was specifically chosen based on the actual time that farmer workers enter and leave the farm.

Ten farm workers participated in the experiment for each farm. After dipping in the boot bath and exiting the farm at each time point, 10 ml of disinfectant sample was collected and placed in sterile 15 ml falcon tubes with appropriate labels. The samples were immediately stored in an ice chest for transport in the laboratory. Sampling was conducted with 10 replications.

Presence of Foc on footbath solutions

The collected soil samples from both farms were analysed for Fusarium growth at the University of Southeastern Philippines, Tagum Apokon Campus, using the drop plate technique, MPN technique described for soils. The samples were shaken briefly, and one ml was pipetted into a centrifuge tube with 9 ml of the sterile distilled water, and followed a four-fold dilution series. A Petri dish with Komada's medium was divided into four quadrants, and five 10 µL drops from each dilution pipetted into each quadrant. This was repeatedly done to all samples. The Petri plates were incubated for three days at room temperature after which responses were determined.

Activity 1.3: Measure suppression of Fusarium wilt in bananas due to crop residue decomposition and plant eradication.

Location: *University of Southeastern Philippines, Obrero Davao, Mindanao, Philippines*

Personnel: *Ms Tamsi Gervacio, Mr Franz Colaja, Ms Chrinstina Ansale, Mr Jayvemar Rigor*

An experiment was established at the 'Lakatan' production area in the University of Southeastern Philippines in Apokon, Davao del Norte with a longitude of 7°25'7"N and latitude of 125°49'50"E in Mindanao Island, Philippines, to determine the impacts of burning rice hull as a practice for managing banana residue infected with Fusarium wilt (Fig 5.2). The experiment involved identification of diseased plants by inspection of external symptoms and then soil sampling and installing temperature probes prior to burning (Fig. 5.3). The experiment was designed to determine temperature changes at three soil depths 10, 20 and 30 cm below the soil surface and how the microbial ecology changed at the three depths following 48 hours of burning. Thermocouples and data loggers were set up at each depth to capture the real-time changes in soil temperature. Before and 48 hours after burning, soil was collected from 10 sites around the banana plant at three depths 0-10, 10-20 and 20-30 cm (Fig 5.3). Six plants infected with Fusarium wilt were used in the experiment. The plants were then injected with glyphosate and burned as per local practice (Fig. 5.4).

Fig. 5.2. Location of field experiments involving burning of infected banana plants

Fig 5.3. Diagram depicting the location of soil cores within the 30 cm band around the base of the infected banana plant before, soil samples collected at 0-10 cm, 10-20cm, and 20-30cm depth and placement of thermocouples.

Fig. 5.4. Burning of infect banana plants

Upon collection, soils were transferred to the laboratory and sieved using a 2 mm mesh size. Then the fresh composite sample weighing approximately 1 kg was sub-sampled to determine microbial biomass, Fluorescein diacetate test (FDA), and β-glucosidase activity as below (5.2.1). All tests were conducted in triplicate. Additionally, 150 g of sieved soil was used for a Foc TR4 bioassay, and 50g was set aside and stored at -20˚C for extraction of DNA.

Activity 1.4: Scope opportunities to look more deeply into the banana microbiome and its role in protecting bananas from Fusarium wilt.

Location: *University of Queensland, St Lucia and Department of Agriculture and Fisheries, South Johnstone Research Facility*

Personnel: *Dr Paul Dennis, Mr Henry Birt, Mr Dillon Smith and Dr Tony Pattison*

We collected bulk soil, ectorhizosphere, endorhizosphere and pseudostem samples, in triplicate, from 55 mature, field-grown plants that comprised 52 distinct Musa genotypes. In addition, triplicate leaf samples were collected from 15 plants comprising 15 distinct genotypes. This design yielded 705 samples, which were subjected to 16S rRNA gene amplicon sequencing. Only one of the genotypes was replicated in the field trial so the exercise object was to verify whether candidate core taxa from pots (Hort Innovation funded project BA14014) were key constituents of field-grown banana microbiomes.

We defined a set of abundant and prevalent field microbes for each compartment on the basis that they were present at ≥0.5% relative abundance in ≥50% of samples. We then cross-referenced the two lists (i.e. the abundance and prevalent field OTUs against the candidate core OTUs from pots) to determine the extent to which our core taxa correctly predict the composition of field grown plants.

5.2.2 Objective 2: To evaluate the effectiveness of best-bet ICM approaches in enabling commercial banana production in the presence of FW.

Activity 2.1: The evaluation and validation of best-bet ICM practices to enable commercial export Cavendish banana production in the Davao del Norte region of the Philippines.

Location: *University of Southeastern Philippines, Apokon, Tagum, Mindanao, Philippines*

Personnel: *Dr Cesar Limbaga, Mr Nelvin Villason, Mr Carlito Hindoy*

Two experiments were established at sites located in Lasang, Davao City and Kapalong, Davao del Norte which were established on July 2015 and June 2016, respectively (Table 5.2). Both sites were severely infested with Fusarium wilt and were left abandoned before the project assumed. Following the establishment of the field experiments banana plants were fertilized at fortnightly intervals for the first 10 weeks and then at 4-weekly so that each plant received equivalent to 404 kg N ha^{-1} yr^{-1}, 623 kg N ha^{-1} yr^{-1} and 184 kg N ha^{-1} yr^{-1}.

Table 5.2: Details of field experimental sites to develop an integrated management system for banana Fusarium wilt

	Site 1	Site 2
Location	Lasang, Davao City 7º 16' 16"N 125º 39' 49"E	Kapalong, Davao del Norte 7º 35' 59"N 125º 39' 15"E
Trial Establishment	July 2015	June 2016
Soil Sampling (month)	0, 6, 12,	0, 6, 12, 18, 24
Soil texture		Clay
Previous crop	Banana, GCTCV 119	Banana, Grand Naine
Banana Varieties	Cavendish, Grand Naine, GCTCV218	Cavendish, Grand Naine, GCTCV218
Plants per plot	90 Plants	31 – 90 plants
Plant density (plant ha^{-1})	1,740	1,850

Treatments and experimental design

The Integrated Crop Management (ICM) approached used three management methods to reduce losses to Fusarium wilt, the use of resistant cultivars, application of specific biological control organisms and encouraging general suppression by using vegetated ground covers in combinations (Table 2). The same treatments were used at both experimental sites in Lasang and Kapalong. In vitro plantlets of Cavendish bananas (*Musa* AAA) cultivars Grand Naine or GCTCV 218 were planted in the trial. Pre-grown *Arachis pintoi* was planted as a ground cover two months prior to planting banana seedlings in treatments 2, 3 and 5 (Table 2). An estimation of cover crop biomass was determined using a 0.1 m^2 quadrat and cutting vegetation including weeds at ground level. All soil was removed from samples, and oven-dried at 60˚C for 48 hours. The specific biological control organisms, *Trichoderma harzianum* produced by Provincial Agriculture Office – Davao del Norte (PAGRO) and a Plant Growth Promoting Bacteria (PGPB) from Biotech, Los Baños, Laguna, Philippines, were applied as outlined in Table 2. The number of applications of biocontrol organisms differed depending on the cultivar of bananas grown, with GCTCV receiving three applications whereas Grand Naine received a total of six applications (Table 5.3). The initial application of the specific biocontrol products was into the planting hole in which the in-vitro plants were placed. The subsequent application of the biocontrol products was on the soil surface, mixed with water and spread evenly around the base of the plant. The experiment was a randomized complete block design with five treatments, which were replicated four times.

Table 5.3: Treatment outline of field experimental sites to develop an integrated management system for banana Fusarium wilt

Treatment number	Banana cultivar	Ground cover	Specific biocontrol application		
			Quantity per mat	Method	Total
T1-218/B/Bio	GCTCV 218	Bare	12.5 g T. *harzianum* +12.5 g PGBP + 250 mL water	1 basal 2 monthly soil	3
T2-GN/Veg/Nil	Grand Naine	*A. pintoi*	Nil	Nil	0
T3-218/Veg/Nil	GCTCV 218	*A. pintoi*	Nil	Nil	0
T4-GN/B/Bio	Grand Naine	Bare	12.5 g T. *harzianum* +12.5 g PGBP + 250 mL Water	1 basal 5 monthly soil	6
T5-GN/Veg/Bio	Grand Naine	*A. pintoi*	12.5 g T. *harzianum* +12.5 g PGBP + 250 mL Water	1 basal 5 monthly soil	6

Disease assessments

Assessments of external plant symptoms were conducted monthly, beginning three months after planting. Plants were observed for symptom development as described by Orjeda (1998) such as yellowing, petiole collapse and splitting of the pseudostem. Furthermore, plants exhibiting external symptoms were observed to either die or produce fruits. Plants that were observed to die were examined internally to verify the presence of internal disease symptoms of vascular discoloration.

Agronomic and fruit assessments

Agronomic plant assessments were conducted at monthly intervals. The agronomic plant assessment included plant height, girth. Assessments of agronomic characters were conducted weekly to accurately determine the flowering date. Pre-harvest bunch measurements included time to flower emergence and number of hands per bunch. At harvest the total bunch weighed was recorded, along with the average hand weight and average finger length on the second hand from the top and the last hand of the bunch. The fruit was classified into three categories using export specifications developed by the Philippine Bureau of Production Standards (PNS/BAFPS 64:2008, ICS 67.080) (http://www.bps.dti.gov.ph/)

Soil assessments

Soil samples were collected to a depth of 10 cm from 20 plants in each plot at the time of crop establishment and then at 6-monthly intervals. Soil samples from each plant were combined to obtain a 2.0 kg composite soil sample per plot, thoroughly mixed, passed through an 8 mm sieve and stored at 4 C until required for analysis.

Biochemical analysis of soil samples included soil enzymes, fluorescein diacetate (FDA), β-glucosidase and labile C, which were conducted at the University of Southeastern Philippines, Tagum Campus. Labile carbon contents were determined by measuring the amount of C oxidised by 33 mM $KMnO_4$ in duplicate 5 g sub-samples using the method described by Moody and Cong (2008). Similarly, FDA hydrolysis rate was determined from duplicate 5 g sub-samples using a modified version of the method initially proposed by Schnürer and Rosswall (1982). β-glucosidase was determined with the procedure published by Eivazi and Tabatabai (1988), except the toluene was substituted with 0.1% Tween solution and the modified universal buffer was replaced with a McIlvaine buffer (pH 6.0) (Hayano, 1973).

At the Kapalong site soil chemical analysis was conducted by the Philippine Bureau of Soil and Water Management (http://bswm.da.gov.ph/Services/LaboratoryAnalysis) for pH, Olsen P, K, Ca, Mg, organic C, Zn and B using method prescribed by the Soil Science Society of America. Furthermore, at the Kapalong site, soil Community Level

Physiological Profiles (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 CO_2-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), and 5 amino acids (L-alanine, DL-aspartic acid, γ-aminobutyric acid, L-lysine hydrochloride and L-arginine). Fumaric acid was selected due to its role as a signalling molecule in banana roots (Yuan *et al.*, 2018). CO_2-trap absorbance was measured at 590nm (ThermoFisher Multiscan Spectrophotometer) immediately prior to sealing to the soil microplate and following the 6 h incubation. A respiration index (RI) was used as the difference in absorbance between zero hours and 6 h of incubation in each well containing each C substrate divided by the difference in absorbance between zero hours and 6 h recorded in wells containing deionized water (Fernández-Gómez *et al.*, 2011).

Statistics

All parameters that satisfied the assumptions of a parametric test were analysed using two-way repeated measures ANOVA, followed by paired T-test for pairwise comparison. Data from soil biochemical measurements were subject to a repeated analysis of variance to determine time differences. If no significant ($p>0.05$) time difference existed, the means from each sampling period were pooled. Residuals in all analyses were examined to ensure that the assumptions of normality and homogeneity of variance were satisfied. Kruskal Wallis H test, followed by the Mann-Whitney U test for pairwise comparison with Bonferroni correction for those that did not meet the parametric assumptions. Moreover, correlation analysis was conducted for plant biomass and soil biochemical parameters using Spearman's rho. All statistical analyses were analysed using the open source R software.

To determine the impacts of individual treatments either banana cultivar, ground cover or biocontrol application on soil properties a multivariate approach was used on the data by firstly using a stepwise discriminate analysis to determine the factors and the variability that could be explained using the terms. A Principal Components Analysis (PCA) was then performed using the variables that allowed separation of the treatments determine the individual soil variables contribution to separation of the treatments. All multivariate statistics were performed using Genstat for Windows 18th Edition (VSN International, 2017)

Activity 2.2: The evaluation and validation of best-bet ICM practices to enable commercial banana production in Australia.

Location: *Department of Agriculture and Fisheries, South Johnstone, Queensland Australia*

Personnel: *Dr Tony Pattison, Dr Hazel Gaza, Dr Anna McBeath, Mr David East, Mr Dylan Smith*

Two field sites differing in location, soil type and planting time were established on two separate banana plantations in far north Queensland, Australia (Table 5.4). Each site was laid out as a randomised block design of 24 plots (six treatments x four replications). Each plot comprised of 12 banana plants in a single row, with 2 m between plants. Each row (replication) was spaced 5 m apart, with 6 treatment plots per row. Treatments included bare and vegetated ground cover plots with the application of N fertiliser at the following rates; 350 kg N ha^{-1} $ratoon^{-1}$, 180 kg N ha^{-1} $ratoon^{-1}$ and 180 kg N ha^{-1} $ratoon^{-1}$ with a nitrification inhibitor Entec®. Vegetated ground cover plots were established using pinto peanut (*Arachis pintoi*) in combination with allowing naturalised ground cover to establish

freely and bare soil treatments were maintained free of vegetation using herbicides (glufosinate) applied 2-monthly.

Table 5.4. Details of field experimental sites to develop an integrated management system for banana Fusarium wilt

	Site 1	Site 2
Location	East Palmerston	South Johnstone
	17o 35' 33"S 145o 49' 57"E	17o 36' 19"S 145o 59' 55"E
Trial Establishment	December 2014	March 2015
Soil Sampling (month)	0, 6, 12, 20	0, 6, 12
Soil Type	Ferrosol	Dermosol
Banana Cultivars	Cavendish, Williams	Highgate, Hom Thong Mokho

Agronomy

Monthly plant measurements were conducted over the duration of the experiment. Bunch emergence times, bunch size (finger number) and weight were determined. The bunch emergence and bunch weight were used to calculate productivity, based on kg of fruit produced per ha in a year and assuming 15% loss in bunch weight due to the bunch stalk and small fruit.

Soil Sampling

Five samples from each treatment plot 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 50 mm diameter soil corer to a depth of 10cm and mixed as a composite soil sample. From each sample 200 g of fresh soil was used in the extraction of the nematode community, a 150 g sample was then air dried for 48 hours and then thoroughly homogenized and sieved through a 2 mm sieve. An additional 1.0 kg of soil was used in a disease suppression bioassay for suppression of Foc.

Soil functional biology parameters

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, in which mesh baskets, each containing a single sheet of tissue paper and 200 g soil, were placed in metal trays for 48 h (Whitehead and Hemming, 1965). All nematodes from each sample were counted at low magnification, then 100 nematodes were identified to family at higher magnification and assessed according to their tropic groups (parasites, fungivores, bacterivores, omnivores and predators) and functional guilds (Yeates and Bongers, 1999). Nematode families were classified by trophic habitat according to Yeates *et al.* (1993) and their abundance expressed as number per 100 g soil. 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) and as origins of carbon either through the detrital, predation or root channels (Pattison *et al.*, 2014a).

Soil biochemical analysis included soil enzymes, fluorescein diacetate (FDA), β-glucosidase and labile C as described previously, Similarly CLPP was conducted using the MicroResp™ systems described previously and by (Campbell et al., 2003).

Chemical analysis of soils was conducted by Nutrient Advantage Laboratories for standard nutrient analysis as well as soil particle size analysis (OC, pH, EC, NO3-N, P, PBI, K, Ca, Mg, Na, Cu, Fe, Mn, Zn, SO4, sand, silt and clay). Leaf analysis was also performed by Nutrient Advantage Laboratories for standard nutrient analysis (B, Ca, Cu, Fe, Mg, Mn, P, K, Na, S, Zn and N).

Fusarium bioassay

A bioassay of soil from each plot was conducted at the commencement and at the termination of the experiment in December 2014 and December 2016, respectively. From each plot 2.5 kg of soil was placed in a 150 mm diameter pot with a Foc Race 1 susceptible banana cultivar (cv. Ducasse, Musa sp. ABB). The soil was inoculated with 5 g of millet seed colonised with Foc Race 1 (VCG 0125) applied to the soil surface two weeks after planting bananas. The banana plants were allowed to grow for 12-weeks, with weekly assessments made on the external symptom development (1 - healthy plant; 2 – slight yellowing of lower leaves; 3 – severe yellowing of lower leaves) before being destructively harvested and the internal symptoms based on vascular discoloration determined (1 - no internal symptoms; 2 – isolated points of discoloration; 3 – discoloration of up to 1/3 of vascular tissue; 4 - discoloration of up to 1/3 -2/3 of vascular tissue; 5 - discoloration greater than 2/3 of vascular tissue; 6 – total discoloration of vascular tissue) (Orjeda et al 1998). Four transverse slices were made equally through the corm tissue from the intersection of the corm with the pseudostem, ¼, ½ and ¾ along the corm tissue.

Statistical Analysis

A two-way analysis of variance was used when comparing parameters at the same time within the experiment, with groundcover and nitrogen as the main treatment effects. The emergence of banana bunches over time for each treatment for each was could described using a Gompetz equation, where emergence time was deemed to be zero when the first bunch was visible in each ratoon crop to allow comparisons of the three crop cycles

A repeated analysis of variance was used where there were multiple time points of a measurement taken from the same area such as the soil biological measurements. Where there was a significant F test ($p<0.05$) means were separated using Fisher's 95% protected least significant difference (LSD) for pairwise comparisons. The MicroResp™ absorbance data was standardised by subtracting the water response from each substrate response and dividing by the total substrate response for each sample. A canonical discriminant analysis of principal coordinates (CAP) and a correlation between the individual substrates and the CAP axes were also calculated. A stepwise discriminant analysis was conducted to determine the error rate in separating treatments using the leave-one-out error validation.

Additionally, soil biological parameters that showed significant treatment differences ($p<0.05$) were analysed using partial redundancy analysis (RDA) (Legendre & Legendre 1998) to examine the relationship between soil carbon content and banana production with fertiliser or groundcover treatments. The replicate treatments were fitted as conditional variables, and an ordination biplot was prepared to represent the relationship between the soil C and banana production and the soil biological parameters.

All statistical analyses were performed using GenStat for Windows 18th Edition (VSN International, 2015).

5.2.3 Objective 3: To determine the barriers to adoption of systems to suppress FW in banana production in the Philippines and Australia.

Activity 3.1: Appraisal and assessment of current practices for the management of Fusarium wilt in Cavendish banana production in Davao del Norte and to determine information needs and barriers to adoption.

Location: *Provincial Agriculturist Office, Tagum, Davao del Norte, Mindanao, Philippines*

Personnel: *Dr Anastacia Notarte, Dr Merlina Jurhena,*

A survey of 504 men and women banana growers in Davao del Norte was conducted between January to June 2015 and repeated between January and June 2018. The study used criteria-based approach to determine the current level of education, ownership, importance of banana farming to the household, the current state of the disease on their properties, along with the level of knowledge that banana growers had on management practices to overcome Fusarium wilt.

Primary data was both qualitative and quantitative with banana growers answering demographic information, information about the farm and environment where they were located, knowledge of banana management practices and then more specifically about banana diseases and Fusarium wilt management practices. Banana growers were surveyed in all the Barangays within Davao del Norte.

The second survey conducted between January and July 2018 dealt more in detailed questionnaires on the reasons banana growers were implementing various approaches in managing Fusarium wilt. This aimed to determine the respondents' perception on the nature of the disease that would also provide insights on the manner of the disease spread in their farms. 362 respondents (284 males, 78 females) were surveyed out of the targeted 500 respondents.

Activity 3.2: Appraisal and assessment of current practices for the management of Fusarium wilt in Australia and determine information needs and barriers to adoption.

Location: *Department of Agriculture and Fisheries, South Johnstone, Queensland Australia*

Personnel: *Mr Stewart Lindsay*

Following the initial Fusarium wilt TR4 disease outbreak at Tully in March 2015, a project was contracted to the Australian Banana Growers' Council (ABGC) to extend knowledge about TR4 and biosecurity strategies to the banana industry. This was part of a sector-wide strategy and action plan involving and meant that most banana growers in the study region to raise awareness, develop and implement strategies to delay the spread of the disease, increase capacity to identify and manage the disease. Priority for the project team was banana growers, then their suppliers who visit farms.

During the project banana growers received training in;

- disease characteristics,
- disease identification and
- preventative biosecurity practices;

The activities from the ABGC program meant that most banana growers in the Far North Queensland region had acquired some knowledge about Fusarium wilt. Furthermore, most growers had implemented biosecurity practices. Responses from the grower survey indicated 2 main barriers to implementing biosecurity practices were cost (38%) and time (31%), with only 4% of respondents felt knowledge of good biosecurity practices was the limiting factor to adoption.

Therefore, activities were modified to determine economic costs of the implementation of biosecurity practices and changing banana cultivars based on research results of variety trials conducted in Far North Queensland.

Economics of on-farm biosecurity.

Scenarios were developed for a model farm to implement biosecurity practices in either a contiguous scenario or non-contiguous and the costs that would be involved to implement biosecurity practices to the level determined by the ABGC workshops.

Economics of changing banana cultivars

The current method of banana production using Cavendish (Williams) in a continuous ratooning, was compared with GCTCV 218 (Formosana), GCTCV 219 (improved selection of GCTCV 119) grown in a suppressive farming system with a standard 1-year fallow, and Williams grown in 1-year 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

6 Achievements against activities and outputs/milestones

Objective 1: To develop options to limit losses of smallholder Cavendish production in Davao del Norte and Ladyfinger production in Australia due to FW

No.	Activity	Outputs/ milestones	Completion date	Comments
1.1	Establish and contrast vegetative ground cover to current practice of bare soil to suppress FW	Yearly field trial results for soil parameters under 4 contrasting vegetative management systems at 4 sites in Davao del Norte determined with student updates (PC).	Completed October 2018	Completed the greenhouse pot experiment for testing cover crops Completed the analysis of soil samples in field experiment at Puyod Farm. Soil analysis of the samples from AMSEFPCO farm has been finalised.
		Yearly results for soil parameters under contrasting vegetative management systems and suppression of FW at 2 sites in Australia determined (A).	June, 2019	A field experiment on a commercial banana farm has been completed, The final results on disease suppression have been completed and are currently being analysed with manuscript preparation underway.
1.2	Determine methods of reducing soil movement from around banana plants within plantations and between plantations	Measurement of on-farm soil movement using different soil removal techniques at 6 sites in Davao del Norte completed (PC).	Completed October 2018	All aspects have been completed including, use of boot scrapers, types of disinfectants and their longevity in field conditions, soil movement in soil kept bare or with vegetation and how this can impact movement of the diseases. Draft reports testing on-farm biosecurity practices have been received and are being collated into a manuscript for publication.
1.3	Measure of suppression of FW in bananas due to crop residue decomposition and plant eradication.	Completed field trial on chemical treatment to eradicate infected plants (PC).	Dec 2016	Use of urea as an alternative to burning has been tested in proof of concept experiments Experiments using the burning protocol of infected plants have been undertaken in Feb 2019 to determine soil temperatures and changes in soil biology
		Completed field trial on comparison of systems of plant eradication and decomposition of plant material with student update (PC)	Dec 2017	The use of urea to reduce the return of inoculum to the soil has been implemented in the field trial at AMSEFFPCO, Kapalong and is continuing to be evaluated. Experiments using the burning protocol of infected plants have been undertaken in Feb 2019 to determine soil temperatures and changes in soil biology

No.	Activity	Outputs/ milestones	Completion date	Comments
1.4	Scope contributions of banana microbiome to suppression of Fusarium wilt	Laboratory capital and disposable equipment purchased and functioning at USeP (PC)	Sep 2018	Inspection of laboratory facilities for microbiome studies
		Initiate investigation of microbiome of cultivated and wild type bananas using DAF germplasm collection (A)	Oct 2018	An initial investigation using the DAF collection has been completed and data is being analysed.
		Training on techniques for measuring soil biological activities and Foc completed (PC)	Dec 2018	Ms. Tamsi Gervacio underwent training on the techniques to extract soil DNA at the University of Queensland under the supervision of Dr Paul Dennis
		Extract DNA for soil microbiome analysis using ICM field site in Davao del Norte (PC).	Mar 2019	Due to the completion of the ICM field trial, DNA was extracted from an experiment in Davao del Norte investigating the rice hull burning destruction protocol
		Finalise investigation of microbiome of cultivated and wild type bananas using DAF germplasm collection (A)	May 2019	A manuscript has been prepared which has identified the core microbiome of bananas across 55 different variety accessions, which included wild type bananas.
		Finalise DNA sequencing of banana microbiome from ICM field site in Davao del Norte (PC).	Jun 2019	The ICM field trial was terminated in October 2018, therefore, the DNA has been extracted from rice hull burning experiment and is currently undergoing analysis.

PC = partner country, A = Australia

Objective 2: To evaluate the effectiveness of best-bet ICM approaches in enabling commercial banana production in the presence of FW.

No.	Activity	Outputs/ milestones	Completion date	Comments
2.1	The evaluation and validation of best-bet ICM practices to enable commercial export Cavendish banana production in the Davao del Norte region of the Philippines	Completed measurements of plant agronomic, disease incidence, soil biological parameters and crop yields. (PC)	Completed October 2018	The trial was terminated in October 2018, due to high disease pressure and changes in crop ownership. Further data analysis, including treatment impacts on the measured soil parameters have been conducted and are currently being incorporated into a draft manuscript of the trial, together with soil measurements made from activity 1.1.

No.	Activity	Outputs/ milestones	Completion date	Comments
		Trial reference group meeting and direction setting with student updates (PC)	Feb 2018	Several meetings were conducted with the farmer co-operators to ensure a consistent approach to the trial from both parties.
2.2	The evaluation and validation of best-bet ICM practices to enable commercial Ladyfinger/Niche banana production in Australia.	Completed measurements of plant agronomic, disease incidence, soil biological parameters and crop yields. (A)	Completed June 2019	All field trials have recently been completed and the final measurements of soil parameters and disease bioassays are currently underway. Manuscript preparation is also underway using the completed results.
		Trial reference group meeting and direction setting (A)	July 2018	Dissemination of results with the banana industry through industry roadshows and grower articles have occurred with discussions of the strategic research taken place in consultation with the Australian Banana Grower's Council reference group.

PC = partner country, A = Australia

Objective 3: To determine the barriers to adoption of systems to suppress FW in banana production in the Philippines and Australia.

No.	Activity	Outputs/ milestones	Completion date	Comments
3.1	Appraisal and assessment of current practices for the management of FW in Cavendish banana production in Davao del Norte and to determine information needs and barriers to adoption.	Conducted regional agriculturalist project update meeting. (PC)	October 2018	Regional Agriculturists meetings were conducted with over 500 participants and Mindanao-wide symposium on Fusarium wilt management in banana was attended by 140 participants. The current status of Foc TR4 and growers' experiences in Australia were shared by four (4) Australian team members, including members of the ABGC.
		Deliver grower information material on FW	Dec 2015 – Mar 2017	IEC materials have been prepared; • leaflets, 1 poster in Visayan • 2,100 pieces reproduced and distributed • 1,300 banana growers served • 7 LGU's served
		Re-conduct grower questionnaire	Jan – Jun 2017	All questionnaires have been completed

No.	Activity	Outputs/ milestones	Completion date	Comments
3.2	Appraisal and assessment of current practices for the management of FW in Ladyfinger/Niche banana cultivar production in Australia and to determine information needs and barriers to adoption.	Deliver grower information material on FW (A)	July 2018	National Banana Industry Roadshows at 6 locations with 120 industry personnel.
		Re-conduct grower questionnaire	Feb 2016	Grower knowledge of the key aspects of Foc TR4 is now high across the industry since the incursion, with only 4% of respondents identifying lack of knowledge/information as a barrier.
		Economic assessment of case study farms to a determine the economic impacts of different FW management practices at the individual farm level	Nov 2016	Activities have been undertaken to examine costs associated with the adoption of biosecurity practices which ranged from approximately $3,000 to $8,500/ha depending on whether the production area was contiguous or non-contiguous respectively.

PC = partner country, A = Australia

7 Key results and discussion

7.1 Objective 1: To develop options to limit losses of smallholder Cavendish production in Davao del Norte and Ladyfinger production in Australia due to FW

7.1.1 Activity 1.1: Establish and contrast vegetative ground cover to current practice of bare soil to suppress Fusarium wilt.

The pot experiment established to determine the impact of vegetated ground cover on the growth of bananas and development of Fusarium wilt when inoculated with Foc TR4 demonstrated that certain groundcover species could delay the onset of symptom expression (Fig 7.1). Plants that were uninoculated had less disease, however, cross contamination during the experiment meant that some plants demonstrated disease symptoms after about 4-weeks. Half of the plants inoculated with FocTR4 and grown in bare soil were expressing diseases symptoms after 6-weeks and 100% of plants had disease symptoms by the end of the experiment (Fig 7.1) The addition of kudzo as a ground cover, did little to reduce symptom expression and the trend was very similar to the inoculated, bare soil. The bananas grown with pinto peanut, sweetpotato and green frog grass all had reduced disease symptom expression, over the course of the experiments, but still had high incidence of plants infected with TR4. The treatment with a mixture of all four ground cover species, also delayed the expression of Fusarium wilt symptoms (Fig 7.1).

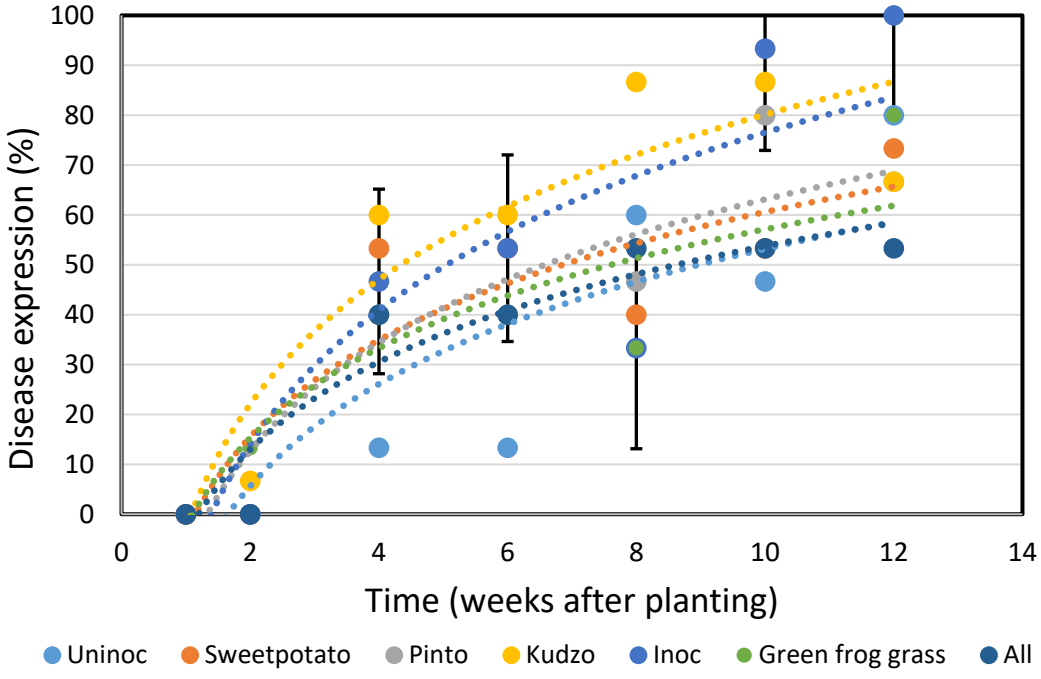

Fig 7.1. Banana plants expressing symptoms of Fusarium wilt in a pot experiment with four ground cover species ornamental sweet potato, Pinto peanut, Kudzo, green frog grass and a combination of all four relative to an inoculated and uninoculated bare soil as controls over a 12-weeks period.

The severity of necrosis in the banana rhizome at the termination of the experiment was not significantly different between the different treatments, indicating that ground covers

had little impact on preventing the pathogen entering the plant, but could influence the external expression of Fusarium wilt symptoms. Similarly, the growth of the plants over the 12 weeks period determined by measuring changes in plant height and girth of the bananas did not show any significant differences due to ground cover treatments (data not shown).

Indicators of soil biological activity and the resources available for microbial growth showed differences between ground cover treatments but were not able to explain the variation seen in disease expression. The labile C measured in the soil of pots ranged from 0.33 to 0.58 g kg^{-1}soil. Similarly, differences were observed in FDA as a measure of general microbial activity ranging from 9.1 to 17.9 mg of FDA hydrolysed kg $soil^{-1}$ hr^{-1} and β-glucosidase, a measurement of cellulose degradation ranging from 13.3 to 47.5 µg pNP g^{-1} soil hr^{-1}. There was a tendency for lower β-glucosidase activity in bare soil control relative to the soils that contained vegetated ground cover treatments, however, this could not explain the differences in the observed expression of symptoms in banana plants.

The MicroResp™ test data was analysed using Principal Component Analysis (PCA) to see the patterns of carbon source utilization in the data. In general, the observations from the bare soil treatments and soil planted with kudzu had greater than the average utilization of oxalic acid, citric acid and DL-malic acid and less utilization of γ-aminobutyric acid, L-alanine, L-arabinose, NAD, and D-glucose. Also, the soil from treatments with ornamental sweet potato, green frog grass utilised greater than average γ-aminobutyric acid, L-alanine, L-arabinose, N-acetyl-D-glucosamine, and D-galactose. The treatments with the presence of pinto peanut tended to have greater than the average utilization of D-glucose, D-fructose, and fumaric acid (data not shown). Faba beans when inoculated with *Fusarium oxysporum* f.sp. *fabae* were shown to increase root exudates including organic acids, amino acids and sugars, but intercropping decreased the root exudates reducing the incidence of Fusarium wilt (Lv *et al.*, 2020). Further analysis of the biochemical changes in the soil are required to understand pathogen-plant-microbial interactions in the development of Fusarium wilt of bananas. However, uncontrolled variability of disease in this experiment may prevent further elucidation of the results

7.1.2 Activity 1.2: Determine methods of reducing soil movement from around banana plants within plantations and between plantations.

Experiment 1.2.1: Determining the efficacy of disinfectants used in the Philippine banana industry

All disinfectants were able to reduce the germination of *Foc* microconidia in comparison to the sterile distilled water (Table 7.1). There was no *Foc* germination or growth in all concentrations used for Beloran 40SL and Biocit (Table 7.2). There was *Foc* germination in the recommended and half the prescribed rate of Chlorox, and in the lowest concentration and amount of Formo and *Foc* growth were observed in all concentrations of formalin (Table 7.1). The highest colony forming unit in the laboratory set-up was observed in sterile distilled water with 10.13 cfu/ml and 10.71 cfu/ml when 5 µl and 50 µl volume were respectively added to 500 µl of *Foc*-TR4 suspension.

The selected disinfectants were further tested against *Foc* spores inoculated in the soil. The largest reduction of spore growth was observed in 18% Formo and 50% Formalin with 0.1 *Foc*-TR4 cfu g^{-1} of soil. The sodium hypochlorite solution that performed well in the laboratory setting, failed to reduce the *Foc* cfu in the soil. Unlike in the test against conidial spore suspension, the disinfectants were not able to eliminate *Foc* in the soil. The least reduction of *Foc*-TR4 in soil was observed in sterile distilled water with 34 cfu g^{-1} of soil (Table 7.1).

Table 7.1. Average *Fusarium oxysporum* f. sp *cubense*-TR 4 colony forming unit (cfu) after three days incubation at 25ºC treated with different disinfectants

Disinfectants	Concentration (%)	Foc-TR4 cfu/ml in vitro experiment		Foc-TR4 cfu/g of soil
		5µl Disinfectant	50µl Disinfectant	
Chlorox	2.35	0.91	0	23.5
	4.7	0.47	0	21.3
	9.4	0	0	13.5
Formo	4.5	1.03	0	9.3
	9	0	0	0.4
	18	0	0	0.1
Beloran 40 SL	2	0	0	15.3
	3	0	0	9.7
	4	0	0	15.7
Biocit 40	2	0	0	18.8
	3	0	0	16.3
	4	0	0	14.6
Formalin	10	0.23	0.14	2.0
	50	0.19	0	0.1
Sterile Distilled Water	-	10.13	10.71	34.0

Results from the *in vitro* experiment of disinfectants showed that the largest reduction of *Foc*-TR4 colony forming unit (cfu) was observed with the products Beloran and Biocit at 10% and 1% of the recommended rates, when 50 µL and 5 µL of disinfectant were used respectively (Table 7.1). Both disinfectants belong to Quaternary Ammonium Compounds (QACs), reported to have wide germicidal range that disrupts the protective cell membrane of microorganisms resulting in cell structural disintegration (McDonell and Russell, 1999). In the study of Nel et al., (2007), the surface sterilants with QACs as active ingredients, showed efficacy against *Foc* spores with exposure times of 30 seconds, 5, 15 and 30 minutes. Moreover, Bennett et al. (2011) also documented that the commercial cleansers and detergents with quaternary ammonium compounds were able to inhibit spores of *Fusarium oxysporum* f. sp. *vasinfectum (Fov),* the causal pathogen of *Fusarium* wilt of cotton. Similarly, Nguyen *et al.* (2018) found that quaternary ammonium compounds containing ≥10% active ingredient were the most effective against two races of Foc when exposed for ≤30 s, 5 min, 30 min, and 24 h.

When soil was inoculated with *Foc*-TR4 to simulate a field setting, Formo and Formalin had the lowest number of *Foc*-TR4 cfu/g of soil. The presence of soil influenced the effectiveness of the disinfectants, which indicates that removal of all soil material prior to disinfection is essential, as soil reduces the activity of the products reducing their effectiveness to kill microorganisms (Bek et al., 2000; Dovrak, 2005)(Nguyen et al., 2018).

Formalin with formaldehyde at 10% and 1% concentrations showed a large reduction in cfu but did not completely kill *Foc*-TR4 spores in both the laboratory and greenhouse settings. This is an important observation as Formalin is used by farmers because it is relatively cheap, despite the serious health hazards upon using the chemical. It is considered to be carcinogenic and highly toxic to humans and animals (Bek et al., 2000). It is also classified by EPA and WHO as a human carcinogen and highly corrosive. Formaldehyde interacts with DNA, RNA, and protein *in vitro*. Basically, the mode of action of formaldehyde is through protein denaturation and alkylation of nucleic acids (McDonell and Russell, 1999). It is strongly recommended that the use of Formalin as a disinfectant

in bananas be strongly discouraged, due to its potential toxicity to human health and its lack of efficacy at eliminating Foc TR4 in soil and laboratory tests.

Experiment 1.2.2: Determining the efficiency of soil removing from foot wear

Among the five boot scrub designs tested, wire mesh had the highest amount of soil removed in both dry and wet conditions, 0.351 g ($p<0.05$) and 10.8 g ($p<0.05$) respectively (Table 7.2). In wet soil condition, the most efficient boot scraper design was wire mesh followed by bottle caps, rubber, brush, and coco coir. The least amount of soil removed occurred using coco coir with wet soil (1.0 g), but coco coir (0.36 g) was not significantly worse at removing soil than other designs, except the wire mesh in dry soil conditions. In the dry soil condition, the most efficient boot scraper design was wire mesh followed by brush, bottle caps, rubber, and coco coir. (Table 7.2).

Table 7.2. Mean soil weight removed by boot scrapers in dry and wet conditions

Boot Scraper Design	Soil Weight (g)	
	Dry Condition	Wet Condition
Mesh Wire	0.351 [a]	10.8 [a]
Brush	0.173 [b]	4.2 [a]
Botttle Caps	0.121 [b]	8.4 [a]
Rubber	0.079 [b]	7.5 [a]
Coco Coir	0.036 [b]	1.0 [b]

Means within columns with different letters are significantly different from each other at p=0.05 using Tukey's HSD test

Following the removal of soil from footwear using the boot scrapers, the soil adhering to the footwear was treated with different concentration of disinfectants in a foot bath (Table 7.3). After treatment with the disinfectants the soil was removed from the footwear and the number of Fusarium colony forming units determined. All disinfectants at the different concentrations reduced to recovery of Fusarium from the footwear relative to the water control. However, no disinfectants could eliminate the recovery of Fusarium from the soil adhered to foot wear in both dry and wet conditions (Table 7.3). Furthermore, there was no significant difference in the number of colony forming units of Fusarium between the different disinfectants with the different concentrations used (Table 7.3). However, Formo at 18% concentration had the greatest reduction of cfu in both dry and wet conditions with 951 cfu g soil^{-1} and 126 cfu g soil^{-1} respectively. Relative to the water control only 6% and 0.7% of the Fusarium was recovered from the soil remaining after foot wear was treated with an 18% Formo solution. Generally, for all the products tested Formo, Major D and Chlorox, increasing concentration tended to reduce Fusarium recovery, but large numbers of Fusarium could be recovered at the highest concentrations used of both Major D and Chlorox in dry and wet soils, which was approximately 10% of the abundance of the water controls (Table 7.3).

Formo (2,2-Dibromo-3-nitrilopropionamide) at 9% concentration is a commonly used disinfectant on small-holder banana farms. This experiment evaluated the efficacy of the disinfectant at half and double its recommended rate. Formo is reported as an algicide, bactericide, and fungicide, noted for their effectiveness in preventing the growth of microorganisms (Dvorak, 2008). However, the results from using boot scrapers and treating with disinfectants indicate that without a

precautionary measure against the movement of the pathogen, infected soil can be easily transferred from one area to another. Evaluation of disinfectants indicates that the three commercially available disinfectants significantly reduced the number of spores at their recommended rates. Additionally, the number of spores recovered emphasizes the importance of complete removal of contaminated soil from footwear or exchanging dirty foot wear for clean foot wear at farm entry points.

Table 7.3. Mean *Foc* colony forming unit per gram of soil in each soil condition

Disinfectant (Active Ingredient)	Concentration (%)	Dry Conditions (cfu g soil^{-1})	Wet Conditions (cfu g soil^{-1})
Major D (Benzalkonium chloride)	2%	6,249 ^{b}	3,265 ^{b}
	3%	6,028 ^{b}	3,135 ^{b}
	4%	5,517 ^{b}	1,402 ^{b}
Formo (2-2 Dibromo 3-nitrilopropionamide)	4.5%	1,515 ^{b}	321 ^{b}
	9%	1,938 ^{b}	595 ^{b}
	18%	951 ^{b}	126 ^{b}
Chlorox (Sodium hypochlorite)	4.7%	5,631 ^{b}	5,457 ^{b}
	9.4%	3,360 ^{b}	3,072 ^{b}
Water (control)	-	15,930 ^{a}	17,820 ^{a}

Means within columns with different letters are significantly different from each other at p=0.05 using Tukey's HSD test

Experiment 1.2.3: Efficacy of disinfectants influenced by farm management

The recovery of *Foc* cfu from the foot baths at two commercial banana farms tended to increase with time. At the Mauro Farm, where the footbath solution was replaced daily, no *Fusarium* cfu was recovered at 6:00 am. However, by 9:00 am Fusarium could be detected in the foot bath solution, with the greatest abundance of Fusarium recovered at 3:00 pm on all 6 days of the evaluation (Tables 7.4).

However, Fusarium could already be detected in freshly made disinfectant solution in AMSEFFPCO farm, which ranged in abundance from 316 to 1421 cfu ml^{-1} (Table 7.5). The recovery of Fusarium from the foot bath solution at AMSEFFPCO at the beginning of each day indicates that the foot bath solution was replaced weekly and is already ineffective by the end of the first day of evaluation (Table 7.5) Furthermore, as the evaluation progressed the Fusarium cfu continuously increased. The *Fusarium* cfu in the foot bath in AMSEFFPCO over the duration of the evaluation ranged from 316 to 1720 cfu ml^{-1}, steadily increasing from the first to the last day (Table 7.5).

Table 7.4. *Foc* colony forming unit (cfu) recovered from foot bath solutions over five days at Mauro Farm, Calinan, Davao City

Date	Time collected and *Foc* cfu/mL recovered			
	6:00AM	9:00AM	12:00PM	3:00PM
1st day	0	18	200	290
2nd day	0	18	200	312
3rd day	0	36	206	262
4rd day	0	20	202	266
5th day	0	22	198	246
6th day	0	22	202	276

Table 7.5. *Foc* colony forming unit (cfu) recovered from foot bath solutions over five days at AMSEFFPCO farm, Kapalong Davao del Norte

Date	Time collected and *Foc* cfu/mL recovered		
	6:00AM	10:00AM	3:00PM
1st day	316	480	673
2nd day	582	828	853
3rd day	870	910	940
4th day	1005	1130	1245
5th day	1421	1525	1720

The evaluation of the foot baths on commercial banana farms demonstrated a decrease in efficacy of the two commercial disinfectants within only a few hours. The greatest abundance of Fusarium cfu was consistently recorded at 3:00 pm, at the end of the day when most farm workers had passed through the foot baths at least twice, entering and leaving the farm. The decline in the efficacy of the footbath solutions is due to the increase in soil and organic matter that accumulates as workers pass through the foot baths many times during the day. A study by Meldrum et al., (2013), found the exposure of the disinfectants to sunlight and the changing temperature over six months in field settings resulted in a decrease in disinfectant efficacy and led to an increase in the percentage of *Foc* germination.

In contrast with the AMSEFFPCO farm, there was no *Foc* cfu in the fresh disinfectant solution collected at 6:00 am at the Mauro farm. This could be because, the foot bath at the Mauro farm was made from a recycled plastic material, which was movable and could be easily cleaned daily, removing any soil or organic material before refilling with fresh disinfectant. Conversely, at the AMSEFFPCO farm, the foot bath was a more permanent structure, which was constructed of wire mesh, fixed at the farm entrance. This was cleaned and replaced at weekly intervals. The accumulation of organic material and soil material has been demonstrated to reduce the efficacy of disinfectants, particularly quaternary ammonia compounds. Therefore, regular inspection and cleaning is required if the foot bath solutions are to retain their efficacy in preventing the transmission of TR4 on foot wear (Nguyen et al., 2018; Stringfellow *et al.*, 2009) (Dovrak, 2005). The contrasting results in Mauro and AMSEFFPCO demonstrates the importance of regular cleaning of the foot bath for any organic material and knowing the limitations of the disinfectant solutions. The number of users and exposure of disinfectants to weather conditions also probably influenced the efficacy of the disinfectant solutions (Meldrum *et al.*, 2013; Stringfellow et al., 2009).

7.1.3 Activity 1.3: Measure suppression of Fusarium wilt in bananas due to crop residue decomposition and plant eradication.

Data logger recordings from thermocouples buried at 10, 20 and 30 cm below plants demonstrated differences between the maximum temperatures and the interval when maximum temperature was achieved between the six different plants used in the experiment (Fig 7.2). For plant 1, the temperature at 10 cm below the soil increased rapidly to 75^{o}C and remained above 60^{o}C for 48 hours (Fig 7.2). However, for the other five plant (plants 2-6) there was a more gradual increase in temperature at 10 cm below the soil surface, reaching a maximum after approximately 48 hours (Fig 7.2). Soil temperature at 10 cm below the burnt plant was greater than at all other depths. However, under plants 3, 4 and 5 the temperature at 30 cm depth remained greater than soil at 20 cm depth and temperatures did not to exceed 60^{o}C for long periods of time (Fig. 7.2)

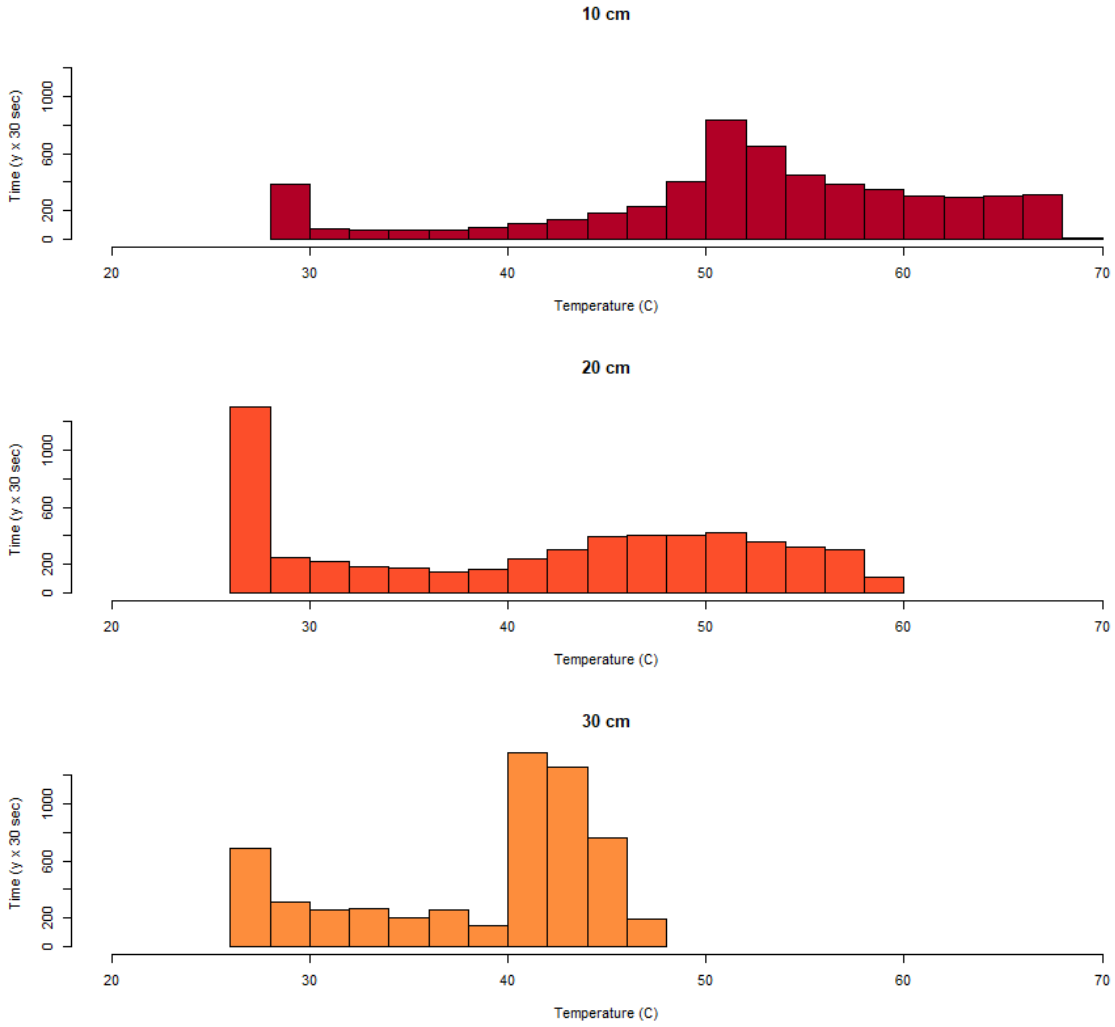

Fig.7.2. The average amount of time soil was at different temperatures during burning at different three soil depths 10, 20, 30 cm (n=6).

Microbial activity determined through substrate induced respiration (SIR)

In this experiment, where soils at different depths were subjected to high temperature for a prolonged time, it was expected that the respiration of soil organisms before burning would be greater than that after burning, particularly at 10 cm depth due to high temperatures. However, the results were inconsistent, indicating no difference in SIR before and after burning or due to soil depth using MicroResp™ as a method of determining substrate induced respiration (Fig 7.3).

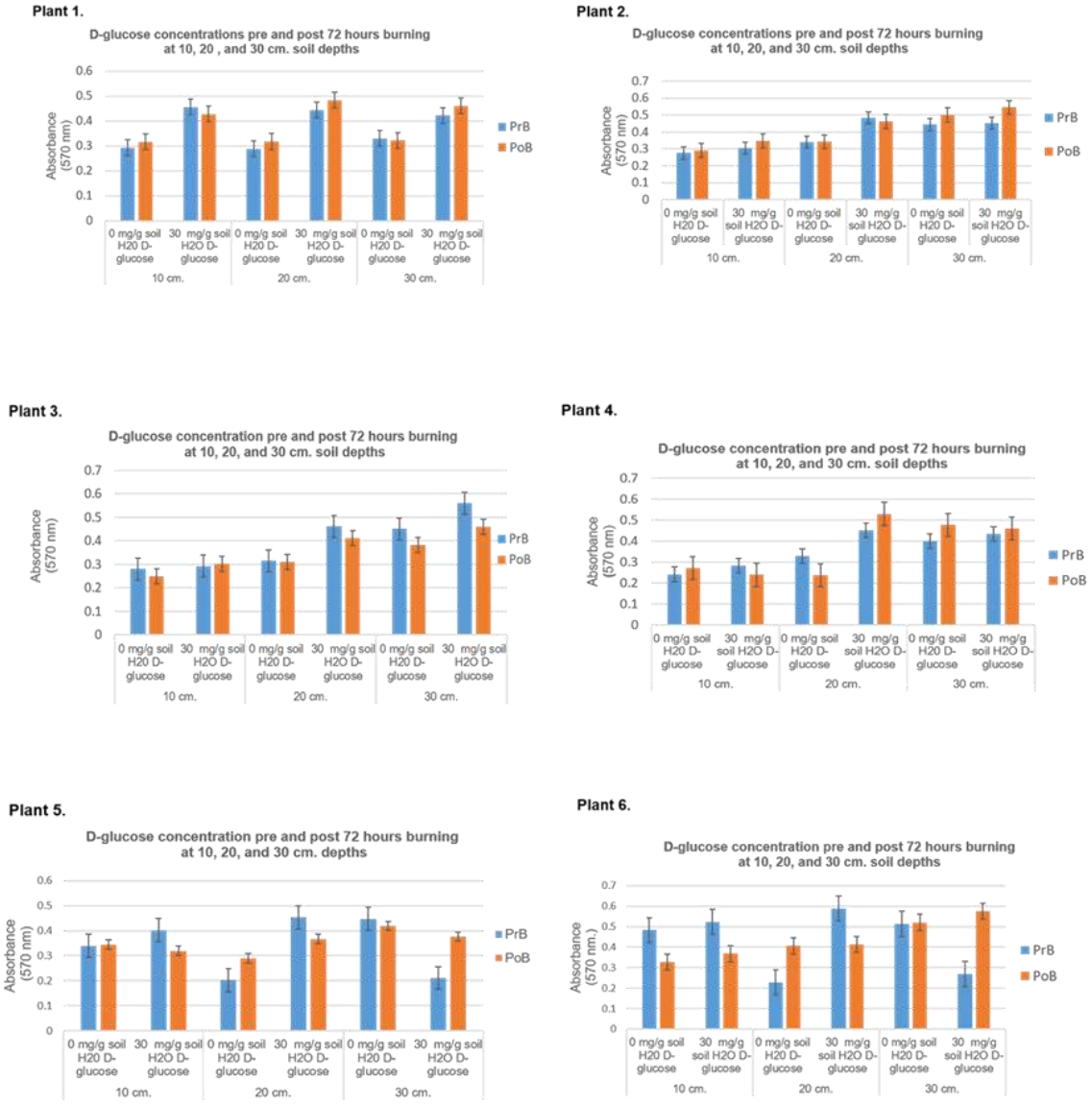

Fig 7.3. Change in absorbance using D-Glucose of *Fusarium* wilt infected 'Lakatan' banana pre and post 48 hours burning at 10, 20, and 30 cm. depths

Microbial activity determined through fluorescein diacetate (FDA)

Likewise, it was expected that activity of soil organisms determined by FDA would be greater before burning than after burning, particularly at 10 cm depth due to high temperatures. FDA activity was consistently greater before burning than after burning, particularly in the top 10 cm of soil for all 6 plants (Fig 7.4). At 20 and 30 cm soil depths the impact of burning on FDA activity was less consistent (Fig 7.4). The results from burning banana plants and determining soil microbial activity using FDA indicated that soil microbial activity was compromised by the burning in the top 10 cm. Furthermore, there was a reduced impact of burning at greater depths below the soil, but it was still possible to reduce the microbial activity at 20 cm depending on the efficacy of the burning treatment (Fig 7.4).

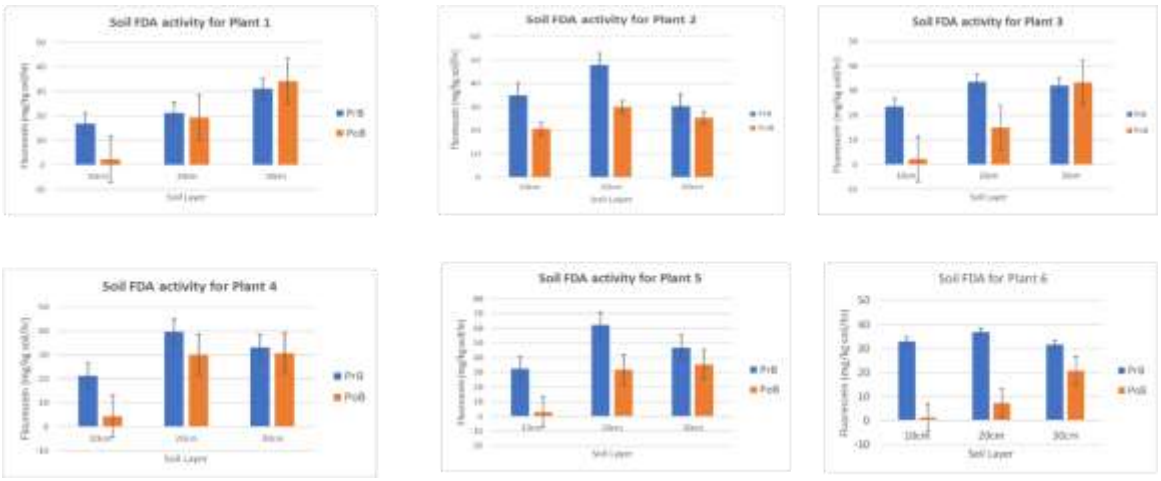

Fig 7.4. Microbial enzyme activity as represented by FDA activity pre (blue) and post (orange) burning for six plants at 10, 20 and 30 cm depth.

The practice of burning rice hulls to eliminate banana material infected with Foc and disinfest the soil under the plant showed considerable variation between individual plants, resulting in differences in temperatures and efficiency achieved. Preliminary data measuring microbial enzyme activity before and after burning indicates that the burns are sufficient to compromise microbial activity (Fig. 7.4). However, to be effective at reducing spores it was suggested that greater than 50-55 ^{o}C, be achieved at a depth of 30 cm (Walduck and Daly, 2007). While exceeding 55 ^{o}C was possible in the top 10 cm of soil it was difficult to achieve at greater depths. However, burning not only reduces Foc, it also impacts on the general soil microbial community activity. The method of measuring soil microbial activity was an important factor to determine the impacts that the practice of burning rice hulls of Fusarium wilt infected banana material had. The use of the MicroResp™ technique to measure substrate induced respiration failed to discriminate the microbial activity between pre and post burning or soil depths and therefore would not be recommended. However, the FDA technique proved to be sensitive to changes in microbial activity due to heat transfer from burning infected plants. The microbial activity in the top 10 cm of soil was consistently reduced after burning and occasionally down to 20 cm. This would indicate a loss of soil microbial activity and therefore, the loss of potential antagonists to Foc. Further work is required to determine if the loss in microbial activity due to heat has a greater impact on Foc or general microbial activity and at what temperature the balance changes to favour the other organisms.

7.1.4 Activity 1.4: Scope opportunities to look more deeply into the banana microbiome and its role in protecting bananas from Fusarium wilt.

Of the 47 candidate core bacterial taxa of banana identified from our previous research, 79% (37/47) of the taxa (OTUs) were represented within all genotypes of field grown plants across the 52 genotypes (Fig. 7.5). More specifically, our core taxa represented 71% of bacteria found in the ectorhizosphere, 95% of bacteria in the endosphere, 67% of bacteria in the pseudostems, and 78% of bacteria in the leaves of a wide range of banana genotypes grown in the field.

The 52 varieties screened harboured highly similar bacterial communities, with a high representation of core taxa throughout. This was particularly the case in the endorhizosphere of the banana root, which is a key environment through which Foc infects banana plants (Fig. 7.6). These results are currently being compiled with data from previous banana microbiomes investigations for publication in the journal Microbiome (impact factor = 9).

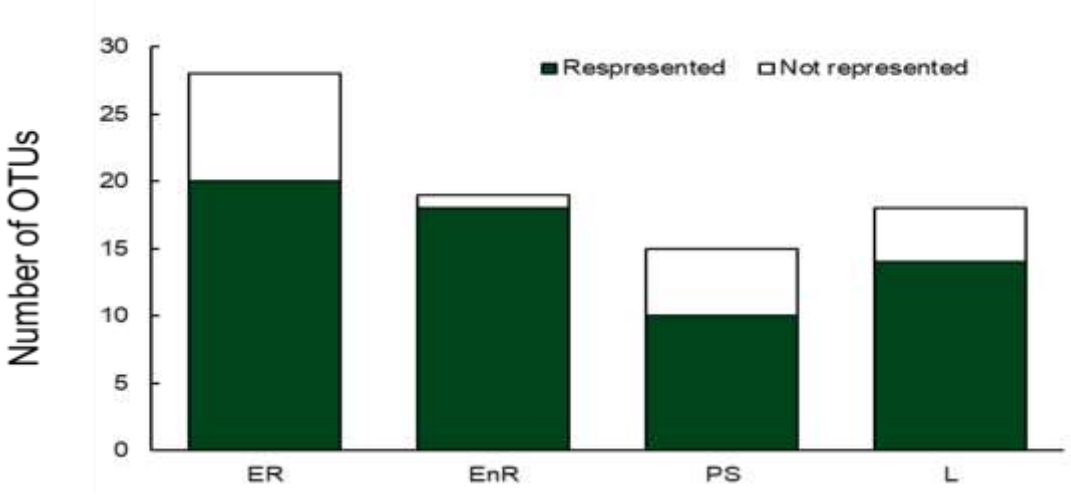

Fig. 7.5 The number of candidate core taxa that were represented in the important OTUs (i.e. present at ≥0.5% relative abundance in ≥50% of samples) of field-grown plants by compartments: ectorhizosphere (ER), endorhizosphere (EnR), pseudostem (PS) and leaves (L).

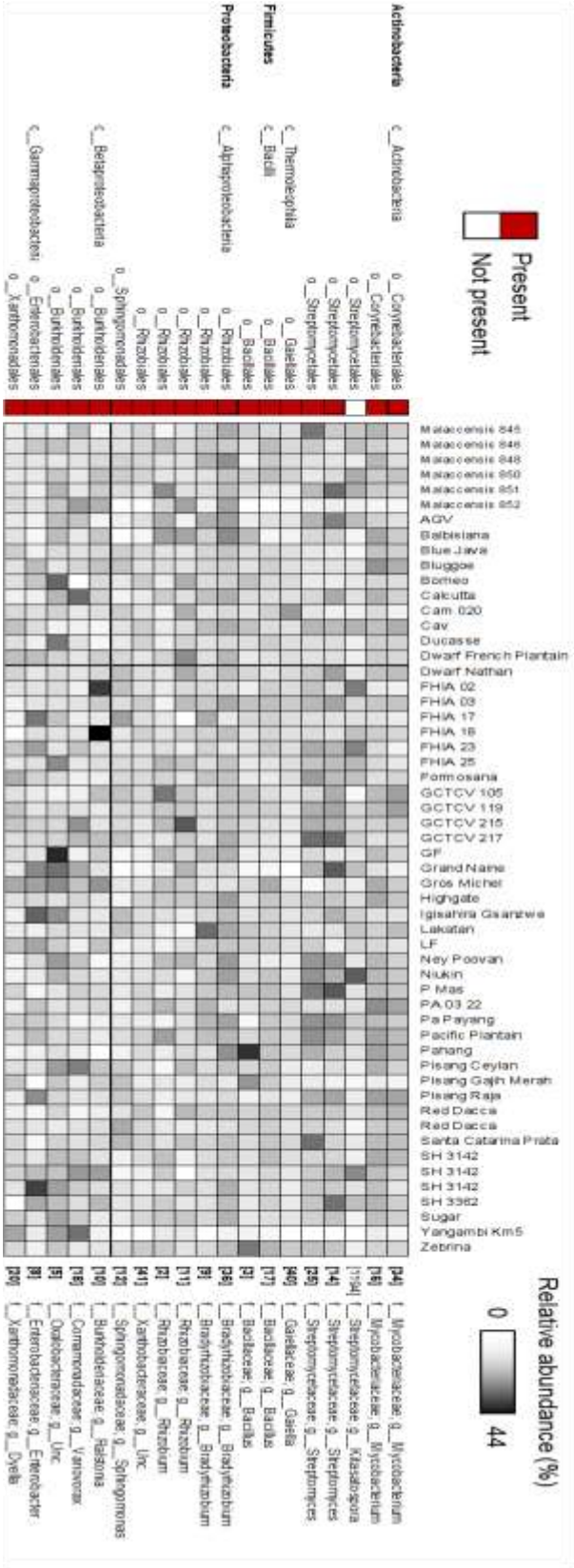

Fig. 7.6 Heatmap representing the relative abundances of important OTUs in the endorhizosphere of field-grown plants (i.e. present at ≥0.5% relative abundance in ≥50% of samples). The figure is landscape to fit and has taxa in rows and varieties in columns. The red squares indicate taxa that are members of the core microbiome. The figure highlights that all but one of the important endorhizosphere taxa in field grown plants are encompassed by our core taxa.

7.2 Objective 2: To evaluate the effectiveness of best-bet ICM approaches in enabling commercial banana production in the presence of FW.

7.2.1 Activity 2.1: The evaluation and validation of best-bet ICM practices to enable commercial export Cavendish banana production in the Davao del Norte region of the Philippines.

Disease assessment

Fusarium wilt was evident in banana at both experimental sites, Lasang and Kapalong and causing plant death. The cultivar Grand Naine showed a significantly ($p<0.05$) greater incidence of Fusarium wilt relative to GCTCV 218 at both experimental sites regardless of biocontrol or ground cover treatments (Table 7.6). At the Lasang site, less than 12% of GCTCV 218 showed symptoms of Fusarium wilt (Table 7.6). However, incidence of Fusarium wilt on Grand Naine plants at the Lasang site appeared lower than the incidence of Fusarium wilt on the Grand Naine plants at the Kapalong site. At the Lasang site approximately 40% of Grand Naine plants showed symptoms of Fusrium wilt, whereas approximately 85% of Grand Naine plants showed symptoms at the Kapalong site (Table 7.6). Furthermore, at the Kapalong site there was an apparent recovery with regrowth of the primary pseudostem of infected GCTCV 218 plants, which was significantly greater ($p<0.05$) relative to Grand Naine (Table 7.6). Less than 3.1% of Grand Naine plants showed recovery from the initial infection, whereas approximately 19.3% and 13.2% of GCTCV 218 in treatments T1 and T3 respectively, showed signs of recovery of the primary pseudostem (Table 7.6). In the secondary suckers, the incidence of Fusarium wilt was significantly greater ($p<0.05$) in the Grand Naine, where approximately 90% or more of the plants showed symptoms of Fusarium wilt (Table 7.6). In the GCTCV 218 treatments 38.6% and 55.7% of plants showed symptoms of Fusarium wilt, although there was no significant difference ($p>0.05$) between T1 and T3 (Table 7.6).

Table 7.6. Incidence of Fusarium wilt of the primary pseudostem of banana at two experimental sites, and the secondary shoots along with apparent recovery of primary plant at Kapalong

Treatment	Site 1: Lasang	Site 2: Kapalong		
	Disease incidence (primary) (%)	Disease incidence (primary) (%)	Recovery (primary) (%)	Disease incidence (secondary) (%)
T1-218/B/Bio	12.0 b	35.2 b	13.2 a	38.6 b
T2-GN/Veg/Nil	40.8 a	94.5 a	0.7 b	95.7 a
T3-218/Veg/Nil	9.4 b	59.2 b	19.3 a	55.7 b
T4-GN/B/Bio	39.8 a	86.3 a	0.0 b	89.9 a
T5-GN/Veg/Bio	38.5 a	96.0 a	3.1 b	90.8 a

Where 218 = GCTCV 218, GN = Grand Naine, B = bare soil, Veg = vegetated ground cover, Bio = Biocontrol applied and Nil = No biocontrol applied. Means in columns followed by different letters are significantly different from one another ($p<0.05$).

Agronomic and fruit assessments

There were significant ($p<0.05$) agronomic differences in bunch emergence time, plant height, circumference and proportion of plants producing a bunch between treatments at the Lasang site, but only significant differences in the proportion of plants producing bunches at the Kapalong site (Table 7.7). Grand Naine in bare soil with biocontrol application produced bunches in the fastest time, 286 days on average, which was significantly faster than Grand Naine grown with vegetated ground covers, either with or without biocontrol application, which took 313 and 303 days respectively (Table 7.7). The

GCTCV 218 plants took at least another 53 days for bunches to emerge relative to the Grand Naine plants, with GCTCV 218 grown with ground covers taking a further two weeks on average for bunches to emerge (Table 7.7).

The GCTCV 218 plants were all significantly ($p<0.05$) taller at bunch emergence than the Grand Naine, by at least 28 cm (Table 7.7). Furthermore, the circumference of the GCTCV plants was significantly ($p<0.05$) greater than Grand Naine. (Table 7.7). The vegetated ground cover significantly ($p<0.05$) reduced the girth of the GCTCV 218 and Grand Naine by 2.0 and 2.5 cm respectively (Table 7.7). Only 29-37% of plants produced a bunch at the Lasang site, with no differences due to treatments. The low proportion of plants producing a bunch at Lasang was due to a high incidence of plants being infected with Banana Bunchy Top Virus (BBTV), with between 37 to 62% showing symptoms of BBTV (data not shown). The Lasang site was terminated following harvested of the bunch from the initial primary pseudostem.

Plant agronomic data collected from the Kapalong site was unreliable for Grand Naine due to the high incidence of plant mortality and severe Fusarium wilt incidence observed at the experimental site, resulting in only 19 to 25% of Grand Naine producing a bunch (Table 7.7). This was compared to GCTCV 218 where on average 76% and 74% of plants produced a bunch for T1 and T3 respectively (Table 7.7).

Table 7.7: Agronomic plant measurements of bananas at bunch emergence at two experimental sites to develop an integrated management system for banana Fusarium wilt

Treatment	Site 1: Lasang				Site 2: Kapalong			
	Bunch emergence (days)	Height (cm)	Girth (cm)	Proportion (%)	Bunch emergence (days)	Height (cm)	Girth (cm)	Proportion (%)
T1-218/B/Bio	366[b]	252[a]	60.3[a]	30	242	246	56.5	76[a]
T2-GN/Veg/Nil	303[c]	214[b]	52.8[d]	32	182	237	54.6	19[b]
T3-218/Veg/Nil	382[a]	249[a]	58.3[b]	37	240	258	58.8	74[a]
T4-GN/B/Bio	286[d]	221[b]	55.3[c]	29	206	226	51.5	26[b]
T5-GN/Veg/Bio	313[c]	213[b]	52.5[d]	33	241	231	53.2	19[b]

Where 218 = GCTCV 218, GN = Grand Naine, B = bare soil, Veg = vegetated ground cover, Bio = Biocontrol applied and Nil = No biocontrol applied. Means in columns followed by different letters are significantly different from one another ($p<0.05$).

There were significant ($p<0.05$) differences in banana bunch and fruit characteristics, number of hands, bunch weight, length of the second finger, and proportion of class A fruit between treatments at the Lasang site, but no significant differences ($p>0.05$) at the Kapalong site (Table 7.8). Bananas grown in bare plots, without vegetated ground covers, had significantly more hands per bunch than plants grown with vegetated ground cover. Grand Naine grown with vegetated ground cover and without biocontrol application had an intermediate number of hands per bunch relative to Grand Naine grown in bare soil and Grand Naine grown with vegetated ground cover with biocontrol application (Table 7.8). The weight of bunches reflected the same result as the number of hands per bunch, tending to be greater in bare soil treatments regardless of banana cultivar and ranging between 15.4 to 19.1 kg (Table 7.8). The finger length on the second hand of GCTCV 218 bunches tended to be 0.6 to 1.3 cm longer than Grand Naine bunches, with no significant influences due to ground cover or biocontrol application treatments (Table 7.8). However, the weight of fruit regarded as class A was significantly less ($p<0.05$) in GCTCV 218, by 3.2 to 7.0 kg per bunch, compared with Grand Naine (Table 7.8).

Table 7.8. Banana bunch and fruit characteristics at two experimental sites to develop an integrated management system for banana Fusarium wilt

Treatment	Site 1: Lasang				Site 2: Kapalong			
	Number of hands	Bunch weight (kg)	2nd finger length (cm)	Class A (kg)	Number of Hands	Bunch Weight (kg)	2nd finger length (cm)	Class A (kg)
T1-218/B/Bio	7.1[ab]	17.3[b]	20.5[a]	7.5[b]	7.4	20.2	24.2	14.1
T2-GN/Veg/Nil	6.7[bc]	16.4[bc]	19.4[b]	10.9[a]	8.2	19.0	23.5	14.8
T3-218/Veg/Nil	6.6[c]	15.8[c]	20.2[a]	6.5[b]	7.4	21.6	23.2	16.2
T4-GN/B/Bio	7.3[a]	19.1[a]	19.2[b]	13.5[a]	7.3	22.6	22.8	17.8
T5-GN/Veg/Bio	6.6[c]	15.4[c]	19.6[b]	10.7[a]	7.6	24.4	24.7	18.6

Where 218 = GCTCV 218, GN = Grand Naine, B = bare soil, Veg = vegetated ground cover, Bio = Biocontrol applied and Nil = No biocontrol applied. Means in columns followed by different letters are significantly different from one another ($p<0.05$).

Soil biological assessments

A repeated analysis of variance of soil biochemical properties failed to find any time differences, so the means from the different sampling times were pooled (Table 7.9). However, no significant treatment differences were observed for labile C, FDA or β-glucosidase between treatments at both experimental sites (Table 7.9). Similarly, plant biomass on the soil surface was not significantly different at the assessment times between treatments (Table 7.9).

Table 7.9. Surface plant biomass and soil biochemical parameters at two experimental sites to develop an integrated management system for banana Fusarium wilt

Treatment	Plant biomass ($g\ m^{-2}$)	Labile C ($g\ kg^{-1}$)	Fluorescein diacetate ($mg\ kg^{-1}\ hr^{-1}$)	β-glucosidase ($PNG\ g\ soil^{-1}\ hr^{-1}$)
Site 1: Lasang				
T1-218/B/Bio	136.41 ± 62.96	0.71 ± 0.05	11.1 ± 1.56	39.7 ± 8.80
T2-GN/Veg/Nil	65.15 ± 21.84	0.68 ± 0.01	8.5 ± 1.63	44.7 ± 3.03
T3-218/Veg/Nil	98.23 ± 27.49	0.71 ± 0.03	10.6 ± 1.24	46.7 ± 5.94
T4-GN/B/Bio	69.57 ± 24.64	0.63 ± 0.02	6.9 ± 1.77	39.6 ± 5.87
T5-GN/Veg/Bio	122.72 ± 36.05	0.67 ± 0.04	8.7 ± 1.70	42.7 ± 8.61
Site 2: Kapalong				
T1-218/B/Bio	18.8 ± 0.68	0.44 ± 0.02	11.4 ± 0.85	18.1 ± 2.52
T2-GN/Veg/Nil	53.1 ± 1.22	0.49 ± 0.04	15.3 ± 1.76	18.2 ± 1.96
T3-218/Veg/Nil	71.2 ± 1.75	0.49 ± 0.03	12.0 ± 1.56	20.9 ± 2.42
T4-GN/B/Bio	60.2 ± 1.55	0.45 ± 0.03	12.3 ± 1.11	16.0 ± 1.86
T5-GN/Veg/Bio	75.2 ± 1.60	0.46 ± 0.03	15.6 ± 1.40	20.7 ± 2.36

Where 218 = GCTCV 218, GN = Grand Naine, B = bare soil, Veg = vegetated ground cover, Bio = Biocontrol applied and Nil = No biocontrol applied. Means in columns followed by different letters are significantly different from one another ($p<0.05$).

At the Kapalong site no significant differences ($p>0.05$) in soil chemical properties were detected between treatments at the four different sampling times (data not shown). The soil had a mean pH 6.29 ± 0.03, P 55.2 ± 1.62 ppm, K 1660 ±64.1 ppm, Ca 12.6 ±0.39 meq/100g, Mg 18.7 ± 0.30 meq/100g, organic matter 2.27 ± 0.10 %, Zn 15.6 ± 1.05 ppm and B 1.08 ± 0.11 ppm across the experimental site.

A repeated analysis of variance indicated there was a significant time difference ($p<0.05$) in the CLPP values between sampling periods (data not shown). The first sampling period taken at the time the experiment was established was significantly different from the subsequent three sampling times. Therefore, the data from the initial sampling time were excluded from further analysis and data from the subsequent sampling time points were pooled, with no significant differences ($p>0.05$) observed in the CLPP between the five different treatments.

A multivariate approach using soil biochemical, chemical and CLPP values was used to determine the treatment factors, cultivar, ground cover or biocontrol, altered soil properties. A minimum set of discriminating variables was selected using a stepwise discriminate analysis for each treatment factor, with an error rate of 12.6, 9.4 and 5.3% for discrimination between the cultivar, ground cover and biocontrol treatments respectively. The minimum set of discriminating variables were used in a Principal Component Analysis (PCA) for each treatment factor (Fig. 7.7). Six variables FDA, K, labile C, Ca, Mg and organic matter accounted for 70.6% of the variation between data points representing each experimental plot, but failed to group individual plots according to the banana cultivar grown, either Grand Naine or GCTCV 218 (Fig 7.7 A). Similarly, six variables, FDA, plant biomass, B, K, organic matter and oxalic acid utilisation accounted for 56.5% of the variation between the data points but failed to adequately group individual plots according to ground cover treatment either bare or with *A. pintoi* (Fig 7.7 B). Furthermore, five variables, Ca, Mg, γ-aminobutyric acid, D-glucose and oxalic acid utilisation could explain 77.9% of the variation between experimental data points but did not adequately group points according to whether they received a biocontrol treatment or not (Fig 7.7 C).

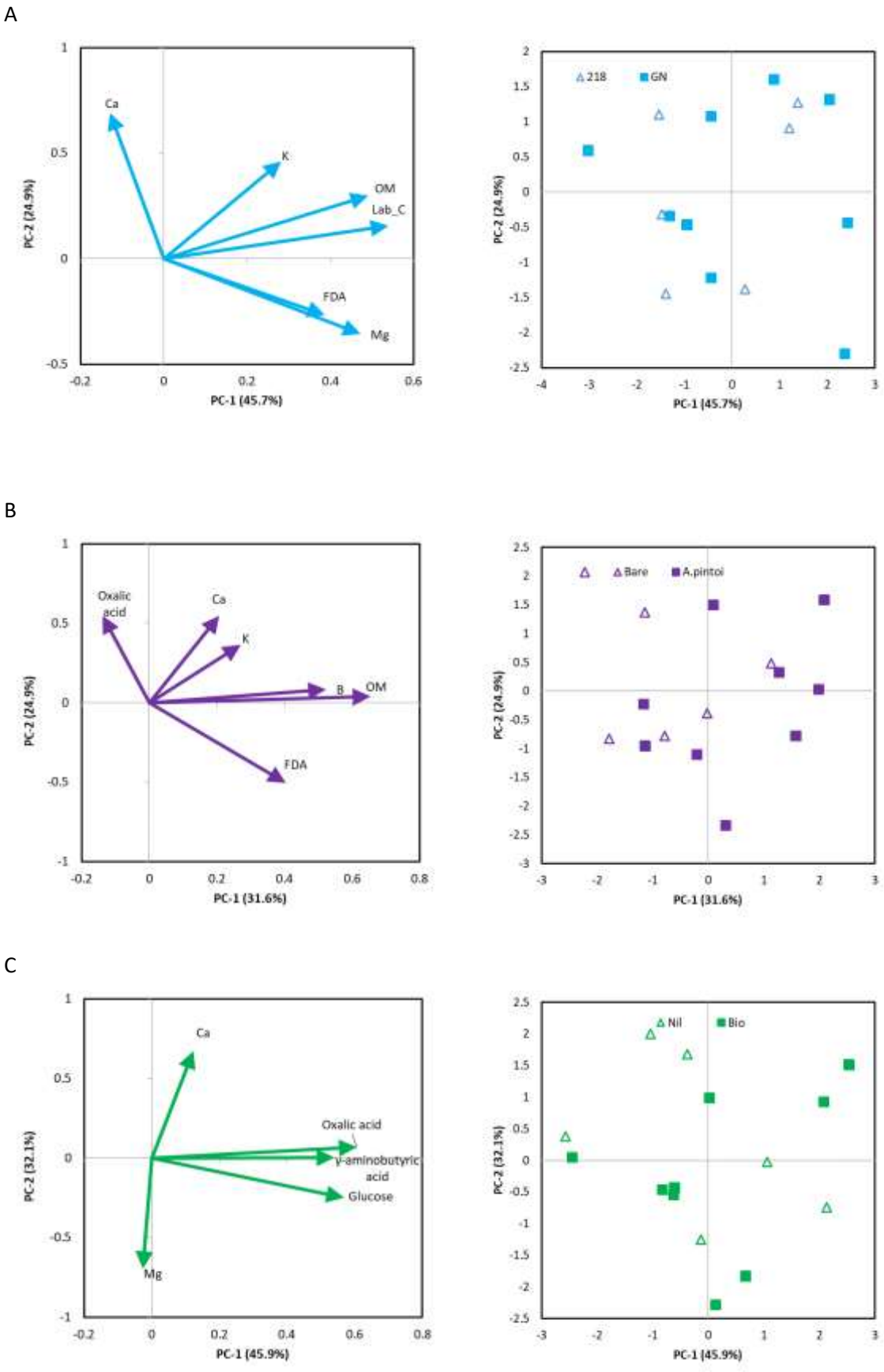

Fig. 7.7. Principal Component Analysis biplots of vectors and data points for discrimination between cultivar treatments, Grand Naine (GN) and GCTCV218 (A), bare soil and *A. pintoi* ground covers (B) and with (Bio) or without (Nil) biocontrol treatments (C).

7.2.2 Activity 2.2: The evaluation and validation of best-bet ICM practices to enable commercial banana production in Australia.

Site 1. Agronomy

The growth of banana plants in a newly established banana crop was similar between treatments up until the emergence of the banana bunches. The rate of bunch development and the uniformity of bunch development could be determined by calculating when each treatment achieved 50% or 85% of plants producing bunches. In the initial crop there was a delay of two weeks to achieve 50% between the low N and 350 kg N treatments (Table 7.10). However, to achieve 85% of plants in the treatments with bunches required 12.4 weeks in the 350 kg N treatment and 17.3 weeks, a further 7.8 weeks in the low N treatment (Table 7.10). The low N with Entec® was intermediate between the high and low N treatments. In the subsequent ratoon crops the time taken to achieve 50% of plants producing a bunch increased to 11.0 and 19.1 weeks for the 350 kg N treatment in the first and second ratoon respectively and 14.2 and 22.6 in the low N treatments in the first and second ratoon respectively (Table 7.10). To achieve 85% of plants with bunches required 14.7 and 27.1 weeks respectively for the first and second ratoons in the 350 kg N treatment and 23.8 and 30.6 weeks respectively for the first and second ratoons in the low N treatments (Table 7.10). Again, the low N with Entec® was intermediate between the other two treatments. There was less than a 2-week difference in the bunch emergence rates between bare soil and vegetated groundcover for the three crop cycles, except to achieve 50% of plants bunched in the second ratoon crop, where there was a 3.2-week difference between bare soil and vegetated groundcover (Table 7.10).

The time taken to harvest each crop was not significantly different between treatments, due largely to the variability within treatments (Table 7.10). Furthermore, not all bunches were harvested if they fell outside the commercial harvest window period. This was evident in plant crop and first ratoon crops with only 77% of plants being harvested in the low N treatment compared to 92% in the 350 kg N and low N with Entec® treatments (Table 7.10).

The bunch weight was significantly reduced in the low N with Entec® treatment in plant crop compared with the 350 kg N and low N treatments (Table 7.10) However, in the subsequent ratoon crops there was no significant ($p>0.05$) difference in bunch weights between the treatments (Table 7.10). In the first two crops, there were similar bunch weights between bananas grown in bare soil and those grown with vegetated groundcovers (Table 7.10). However, in the second ratoon crop, bunches harvested from bare soil treatments were significantly ($p<0.05$) heavier (43.3 kg/bunch) than bunches harvested from bananas grown with vegetated ground covers (41.6 kg/bunch) (Table 7.10). The annual production of bananas in the plant crop, less than 20 t/ha/yr, was nearly half that of the following two ratoon crops, which produced greater than 40 t/ha/yr. Furthermore, bananas grown in the low N treatment had lower production than the other two nitrogen treatments, although this was not significant ($p>0.05$) (Table 7.10). The average annual production determined over the life of the experiment indicated significantly lower production in the low N treatment relative to the 350 kg N treatments, with the low N Entec® treatment being intermediate (Table 7.10). The effect of the ground cover treatment was only apparent in the second ratoon crop, where the vegetated treatment produced a calculated 6 t/ha/yr less fruit than bananas grown in bare soil (Table 7.10). Over the two-year life of the experiment the average production in bare soil treatment was greater than the vegetated soil but this was not significant (Table 7.10).

Table 7.10. Agronomic measurements of bananas grown with different groundcover management and nitrogen treatments over three consecutive crops.

Treatment	Bunch emergence time (weeks)		Harvest generation (weeks from planting)	Finger number per bunch	Bunches harvested (%)	Bunch weight (kg)	Production (t/ha/yr)	Average annual production (t/ha/yr)
	50%	85%						
Fertiliser effects								
Plant crop								
Low N	9.5	17.3	48		77	19.2 a	16.0	-
Low Entec N	8.8	14.5	48		92	17.8 b	15.5	-
350 kg N	7.6	12.4	48		92	19.7 a	19.0	-
1st ratoon crop								
Low N	14.2	23.8	85		77	34.0	42.7	-
Low Entec N	12.7	17.1	83		92	35.4	45.4	-
350 kg N	11.0	14.7	81		92	39.1	50.6	-
2nd ratoon crop								
Low N	22.6	30.6	119		77	41.1	53.6	26.2 b
Low Entec N	21.3	29.2	119		92	42.7	52.8	29.4 ab
350 kg N	19.1	27.1	115		83	43.3	53.5	30.5 a
Groundcover effects								
Plant crop								
Bare	8.8	15.6	48		85	19.7 a	17.6	-
Vegetated	8.3	13.6	48		83	17.8 b	16.1	-
1st ratoon crop								
Bare	11.9	17.8	82		85	37.4	48.7	-
Vegetated	13.0	18.1	84		92	34.9	43.8	-
2nd ratoon crop								
Bare	19.4	28.0	116		77	43.3 a	56.0 a	29.5
Vegetated	22.6	30.2	119		83	41.6 b	50.7 b	27.9

Soil biology

Changes in the soil biological parameters measured were determined over time, due to nitrogen treatment effects or ground cover effects and their interactions. All soil parameters were significantly different over time as the experiment progressed. The changes in soil biological parameters could be in demonstrated using the canonical correlation, which could separate the different sampling times using nematode functional groups and CLPP of carbon substrates. The nematode functional groups could be separated based on times from planting. Similarly, the CLPP canonical correlation could distinguish between sampling times which resulted in a 60% of the variation explainable, with a 30% validation error.

No soil biological parameters indicated a significant ($p>0.05$) nitrogen treatment effect. However, there were significant effects due to the vegetated ground cover treatment with increased abundance of soil nematode taxa Pangrolaimidae, Rhaditidae, Cephalobidae, Tylenchidae, Aphelenchidae and Helicotylenchus dihystera (data not shown). The increased abundance of these taxonomic groups of nematodes resulted increased abundance of nematode functional groups Ba1, Ba2 and Fu2 under bananas grown in soil with vegetated ground covers. The vegetated ground cover reduced the number of *Criconema* sp compared with the bare soil. The vegetated ground cover also increased β-glucosidase, FDA activity and the labile C, as well as the catabolism of fumaric acid and D-fructose.

There was a significant time interaction for five nematode parameters and two soil enzyme parameters (Fig 7.8). The abundance of the plant-parasitic nematode *Criconema* sp. tended to increase linearly faster in the bare soil relative to soil with vegetated ground cover, with significantly more nematodes later in the experiment compared to the beginning of the experiment (Fig 7.8 A). Similarly, there was a significantly ($p<0.05$)

different rate of increase in the herbivorous nematode *H. dihystera*, but the abundance of the nematode increased faster in the vegetated ground cover treatment relative to the bare soil (Fig 7.8 B). Following the planting of banana, there was an exponential decline in the abundance of nematodes belonging to Fu2 guild, with the decline significantly more rapid in the bare soil treatment relative to the vegetated soil treatments (Fig 7.8 C). The decline in fungivorous nematodes contributed to a similar exponential decline in the nematodes associated with the detrital cycle, fungivores and bacterivores (Fig 7.8 D). There was a significant ($p<0.05$) interaction between ground cover treatments over time from planting bananas and the nematodes that were associated with the roots of plants, although the ground cover effect was not as evident (Fig 7.8 E). There was a significant variation in the β-glucosidase activity, which tended to vary with seasonal changes, but greater β-glucosidase activity was evident in the vegetated treatment compared with the bare soil after 12-months (Fig 7.8 F). The activity of FDA increased linearly over the duration of the experiment with a greater increase in FDA activity in the vegetated soil, resulting in greater FDA activity at the termination of the experiment in the vegetated ground cover treatment relative to the bare soil (Fig 7.8 G).

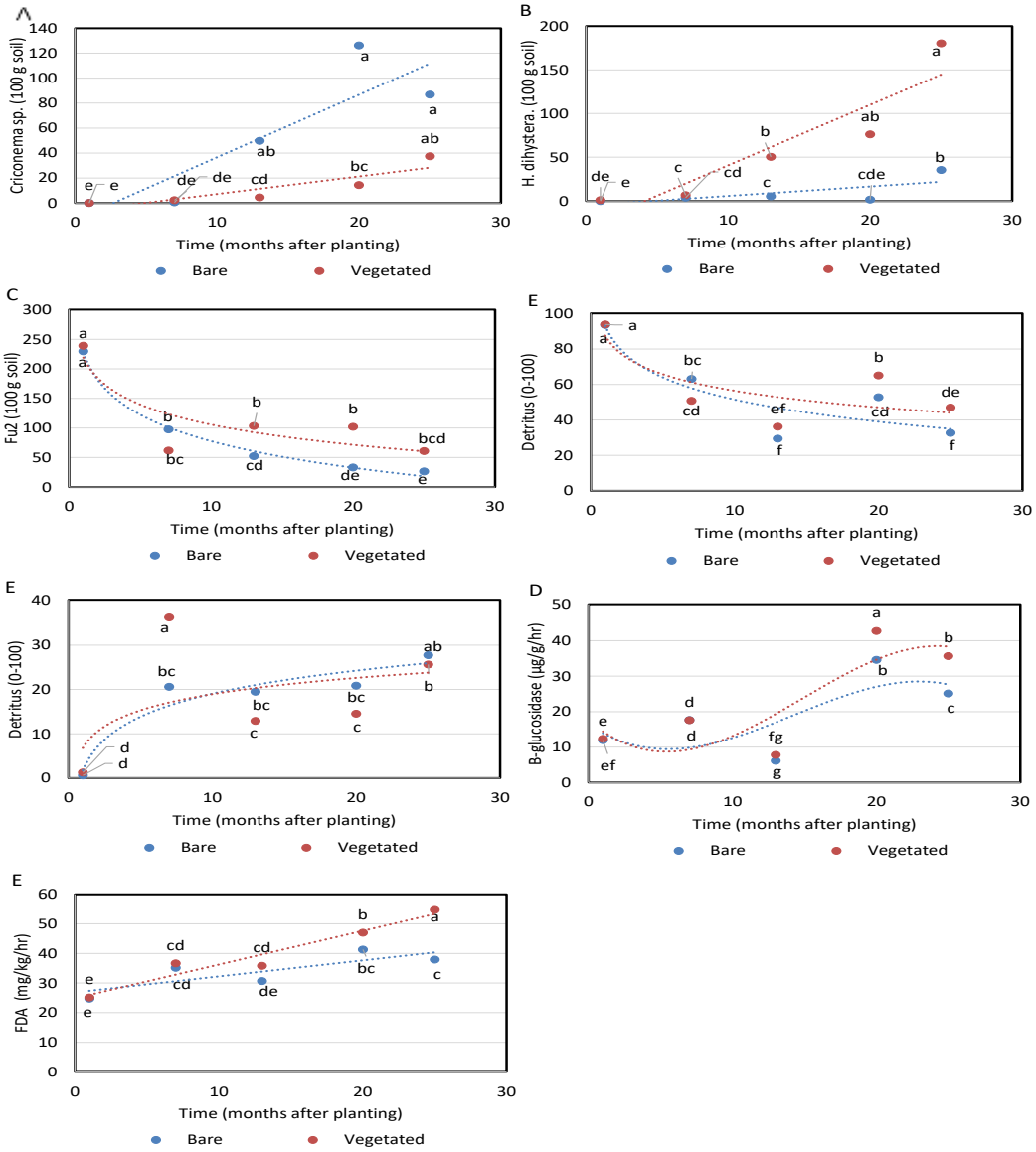

Fig 7.8. Time trends (A) Criconema sp (B) Helictoylenchus dihystera (C) Fu2 nematodes (D) nematodes associated with detritus activity (E) nematodes associated with plant roots (F) β-glucosidase and (G) FDA enzyme activity under vegetated groundcover compared with bare soil. Time points with different letters are significantly different according to Fisher's 95% protected least significant difference.

Foc suppression assay

As Fusarium wilt was not present in the soil at the experimental site a bioassay was used to determine if there was a difference in the expression of the disease in soils from the different nitrogen and groundcover treatments. A susceptible banana cultivar was grown in soil from the field experiment and inoculated with Foc Race 1 demonstrated less internal disease symptoms when plants were grown in soil that had been treated the low N Entec® regime (Table 7.11). There were no significant differences in the external symptoms initially due to ground cover treatments, both internally and externally (Table 7.11). However, when the soil was tested again using the same bioassay technique two years later there was significantly ($p<0.05$) greater external and internal disease symptom development in the bioassay plants grown in the bare soil treatment, relative to the bioassay plants grown in the vegetated ground cover treatments (Table7.11). No significant ($p>0.05$) differences were recorded between the nitrogen treatments in the second bioassay.

Table 7.11. Plant bioassay of soil from nitrogen and groundcover treatments for the suppression of Foc Race 1, based on external and internal disease development at the commencement and termination of the experiment.

Treatment	Initial plant bioassay (Dec 2014)		Final plant bioassay (Dec 2016)	
	Leaf (1-3)	Corm (1-6)	Leaf (1-3)	Corm (1-6)
Fertiliser effects				
Low N	2.4	3.0 ab	1.9	2.62
Low Entec N	2.4	2.5 b	1.5	2.62
350 kg N	2.6	3.5 a	1.7	2.97
Groundcover effects				
Bare	2.5	2.9	2.0 a	3.08 a
Vegetated	2.5	3.1	1.4 b	2.40 b

Values in columns with the same letter are not significantly ($p>0.05$) different from one another for the main treatment effects determined according to Fisher's 95% protected least significant difference.

Soil functions; disease suppression and crop production

The two soil functions that are pertinent to banana production are production of fruit and disease suppression. A redundancy analysis was used to determine the contribution of the different soil parameters that demonstrated significant changes over the duration of the experiment, with production of bananas and suppression of Foc from the final plant bioassay results. The redundancy analysis ordination plot indicated that four parameters β-glucosidase, FDA, and the proportional indices indicating if soil nematodes obtain carbon from detritus or roots, could separate plots based on production and disease suppression (Fig 7.9). The biplot showed that increased β-glucosidase and FDA activity were opposed to an increase in the internal rating of the bioassay plants, indicating that increased soil enzyme activity increased suppression of Foc internal symptoms in bioassay plants (Fig 7.9). Increased banana productivity was associated with an increase in the nematode detritus and root indices (Fig 7.9).

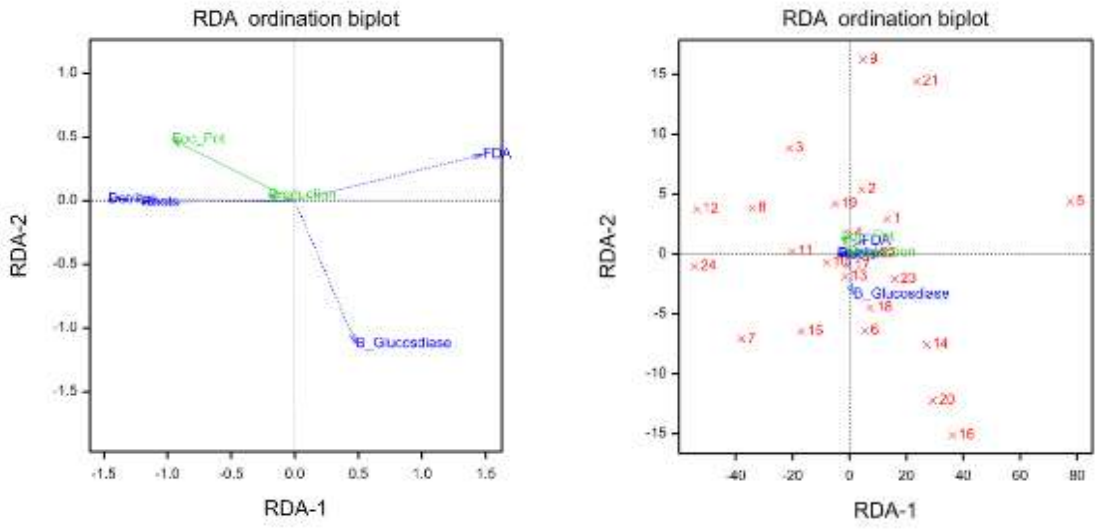

Fig 7.9. Redundancy analysis ordination biplots for the soil functions (green lines) disease suppression and banana productions and soil biological parameters (blue lines) β-glucosidase, FDA, detritus and root nematode indices (A) and separation of treatments based on plots (B).

A linear regression analysis of the internal disease symptoms with β-glucosidase activity showed no relationship with the initial plant bioassay in December 2014, but a significant negative linear relationship between increasing β-glucosidase activity and internal disease rating (Fig 7.10). In the final plant bioassay 97% of the variation in internal disease rating could be explained by the β-glucosidase activity in the field soil (Fig 7.10).

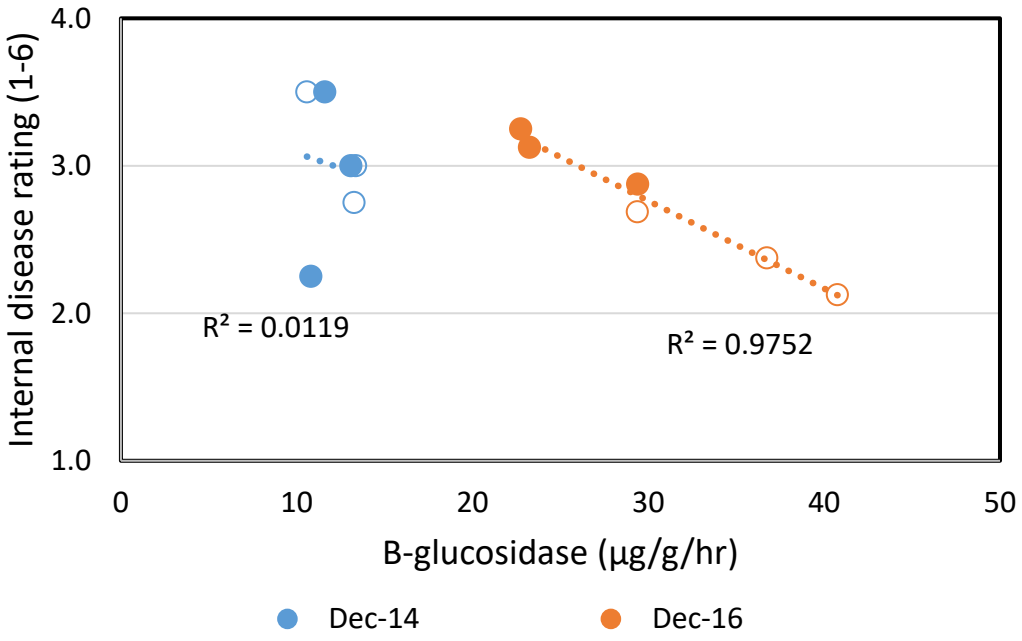

Fig 7.10. Linear regression of internal disease rating of susceptible bioassay banana plants inoculated Foc Race 1 and grown in soil without vegetated ground cover (solid markers) or with vegetated ground cover (open markers) at the commencement of the experiment in December 2014 (blue markers) and again two years later in December 2016 (orange markers).

Vegetated ground cover changed the soil microbial environment over a two-year period as evidenced by changes in nematode community structure, MicroResp and soil enzyme activity. Differences in nitrogen management did not have a significant impact on microbial functions at this site. The measurement of suppression of Fusarium wilt could only be done using a bioassay, using FocR1 inoculum. After two-years Fusarium wilt symptoms were reduced where ground covers had been used relative to soil that was kept bare. However, due to the constraints of the bioassay system definitive changes in disease suppression could not be assigned to each treatment, but the results indicate that the inclusion of vegetated ground covers can increase suppression to Foc, but require time for microbial changes.

The use of vegetated ground covers increased the β-glucosidase activity by up to four-fold relative to bare soil when growing bananas. β-glucosidase has a strong correlation with a reduction in the appearance of internal Fusarium symptoms, indicating that it could be used as a surrogate measure for suppression of Fusarium wilt (Pattison et al., 2018; Rames et al., 2018).

Banana production over two years was determined by the weight of fruit produced and the time taken to produce a bunch. The results from these filed experiments indicate that high production of bananas and disease suppression, are opposed to one another. Therefore, in order to obtain high banana production soils become conducive to Foc, and vice versa to obtain Fusarium wilt suppressive soil, some production may need to be sacrificed.

There was some indication from the field experiments that high nitrogen applications can mask biological changes due to vegetated ground covers. High nitrogen applied to bare soil had the highest production but also the highest disease. Conversely, Low N with ground cover had the lowest production and least disease. The use of Entec with the nitrification inhibitor as a nitrogen source can offset some of the lost production and possibly retain disease suppression.

The underlying principle of community level profiling is that functionally diverse microbial communities have the capability to break-down a broad range of carbon substrates that vary in decomposition complexity than less functionally diverse communities. The application of the MicroResp™ method was used to assess community level profiling of microbial activity across a range of soil management practices on 2 banana plantations in a 24-month soil sampling period. Distinct groupings based on the 15 substrates were evident between soil sampling times indicating a change in functional diversity of microorganism with time. There was a progression from utilization of simple carbon substrates like carboxylic acids at the commencement of the experiment to complex compounds like amino acids at the end of the experiment, with indications that microbial homeostasis was obtained after 18 to 24 month of banana production.

7.3 Objective 3: To determine the barriers to adoption of systems to suppress FW in banana production in the Philippines and Australia.

7.3.1 Appraisal & assessment of current practices for the management of Fusarium Wilt in Cavendish banana production in Davao del Norte and to determine information needs and barriers to adoption

Appraisal of current banana growers practices

The survey for the assessment on the adoption of approaches for the management of Fusarium wilt in Cavendish banana was conducted in major Cavendish banana producing municipalities of Davao del Norte (Fig 7.11).

The first survey completed in June 2015 and included:

- 504 survey participants from Davao del Norte,
- 74% of respondents farmed 3 ha or less
- 19% of respondents reported no incidence of Foc TR4,
- 47% reported Foc TR4 incidence of greater than 5%
- 60% of the respondents did not know the causal organism of Fusarium wilt
- 54% of the respondents don't practice the recommended eradication practices by burning the infected plants and about 48% of the respondents did not cordon off the affected areas

The result of the survey indicated the need to provide continuous education to the growers to disseminate basic information on the biology of the causal organism and the proper management approaches of the disease through trainings (Fig 7.11).

Figure 7.11. Farmers' Information forum on the management practices for Foc TR4 in banana farms conducted from October to November 2014 in Davao del Norte

Communication and extension activities including development and distribution of information, extension and communication materials to banana growers

Information, extension and communication materials in the form of leaflets and posters containing information on basic biology of *Foc,* epidemiology of the disease, quarantine and proper management protocols were developed in Visayan dialect (Fig 7.12) and disseminated to banana growers during training activities, consultations and the Mindanao-wide symposium. They also served as a template for municipal agriculture extension materials and were distributed to banana growers.

Fig 7.12. Examples of information, extension and communication material produced in Visayan as part of HORT/2012/097 project activities.

Furthermore, the initial results from project activities, by identifying options to limit losses in banana production due to *Foc* and evaluating the development of an integrated approach to managing *Foc* were disseminated during the Mindanao-wide Symposium on Fusarium wilt management, March 29-30, 2017 (Fig 7.13). At this event the status of *Foc* TR4 and growers' experiences in Australia were shared by Mr. Stewart Lindsay and 3 members of the Australian banana industry – Australian Banana Grower's Council (ABGC) Chairman and grower Mr Stephen Lowe, grower Mr. Patrick Leahy and ABGC CEO Mr. Jim Pekin. The symposium was attended by 140 banana growers and extension workers from the major producing regions and provinces in Mindanao.

Fig 7.13. Images of speakers and participants from the Mindanao-wide Symposium on Banana Fusarium wilt management held on March 29-30, 2017 at Pinnacle Hotel, Davao City

Re-conduct grower questionnaire

The final survey for the assessment on current practices for managing banana Fusarium wilt was conducted January to June 2017 and included 368 participants growing Cavendish banana.

There was an increasing level of Fusarium wilt incidence reported by banana growers in the second survey, with fewer growers having no disease, declining from 26% of growers with no Fusarium wilt in 2015 to only 8% of growers reporting they did not have the disease two years later (Fig 7.14). About 38% of the growers surveyed had less than 5% incidence and 54% reported a disease incidence of greater than 10% of plants affected by Fusarium wilt. Furthermore, 10% of the respondents had totally abandoned their farms and/or shifted to other crops due to severe infection.

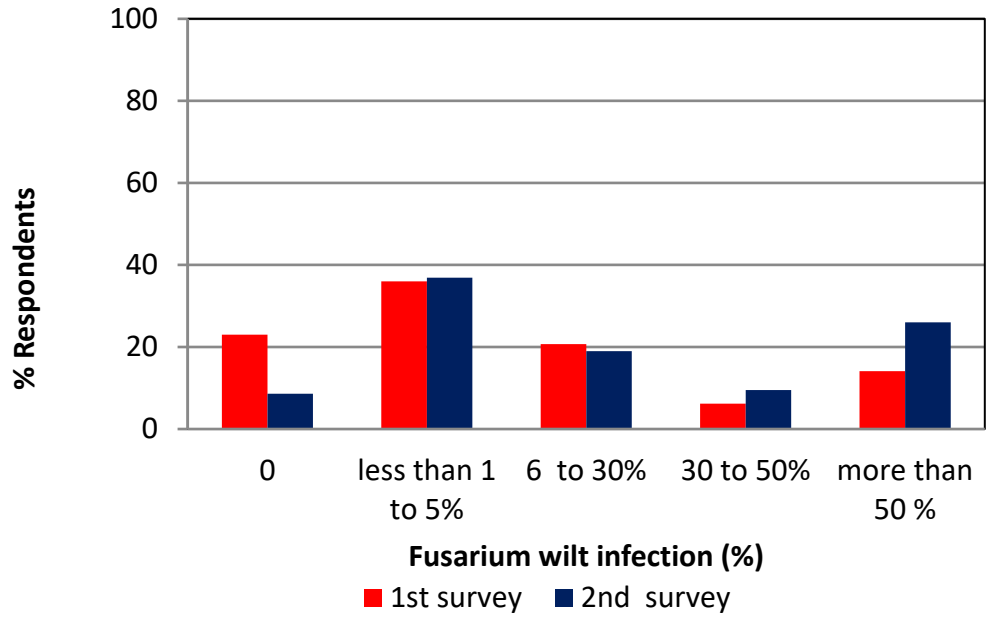

Fig 7.14. The reported incidence of Fusarium wilt on Cavendish banana farms in Davao del Norte from grower surveys in 2015 and 2017.

There was little change in the eradication methods used by banana growers between the two surveys (Fig 7.15). However, there was a decrease in the number of growers killing infected plants with herbicides in the second grower survey relative to the first survey conducted in 2015 (Fig 7.15). Furthermore, 18% of the growers left infected plants without any form of eradication, stating financial constraints limited the purchase of materials for burning.

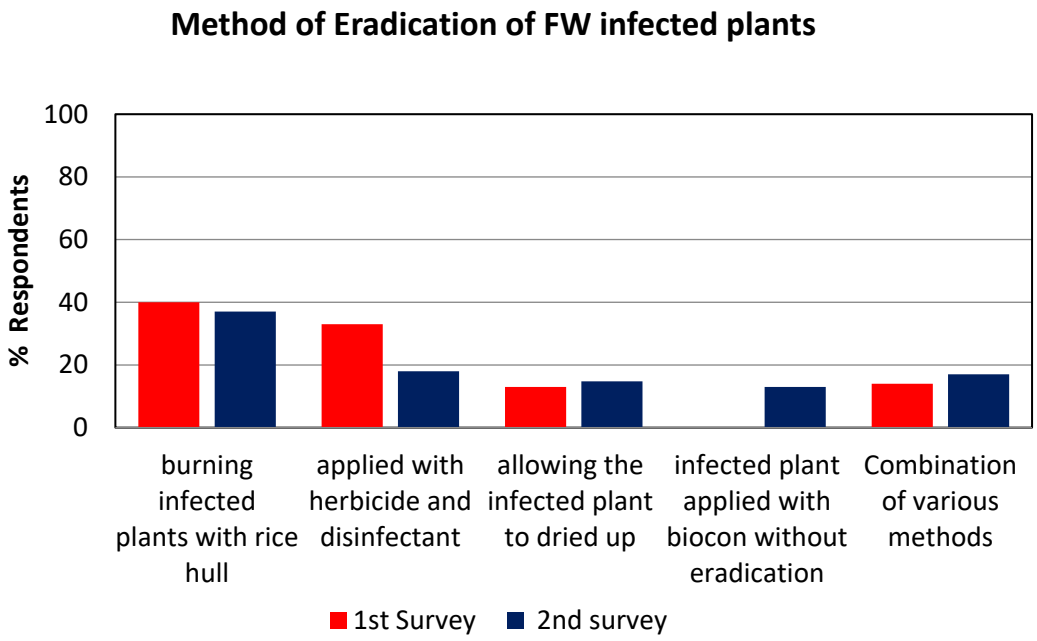

Fig 7.15. Methods of eradication of Fusarium wilt affected banana plants by banana grower in Davao del Norte during two surveys in 2015 and 2017

The perception of the method implemented to manage Fusarium wilt by banana growers was determined. Among the respondents practicing burning of infected plants with rice hull, 28% believed that this method killed the fungus in the infected plant, 28.7% observed this method was effective in controlling the disease and 19.6% noted the spread of the disease was reduced. Of the respondents, 23.4% said they practiced this method because it was recommended by the government, their cooperative and/or fruit buyers.

When banana growers were questioned about why they had used the different practices for managing infected plants either rice hull burning or herbicide, most believed it would control the disease and limit the spread of the disease (Table 7.12). There was very little change in the attitudes of banana growers to the different disease control measures, although in 2017 a policy of payment for banana growers to burn infected plants meant there was a proportion of banana growers (15%) who were using crop destruction techniques due to government incentives and recommendations (Table 7.12).

Although the number of growers that practiced herbicide injection for infected plants reduced by 15% in the recent survey, the reason behind those who continued to implement the use of herbicides (65%) believed that hastening drying and killing the infected plant immediately could help in reducing the disease (Table 7.12).

Table 7.12. Reasons given by banana growers to why they had adopted either rice hull burning, or herbicide treatment of banana plant infected with Fusarium wilt

Reason	Burning infected plants with rice hulls (%)		Herbicide treatment of infected plant (%)	
	2015	2017	2015	2017
To control disease	27.3	28.7	17.6	15.1
Limit disease spread	25.7	19.6	13.2	19.2
Kill pathogen	28.8	28.0		
Kill plant	18.2		69.1	39.7
Hasten drying	-	-		26.0
Recommended by government	-	15.4	-	-
Recommended by buyer	-	8.4	-	-
No. respondents	66	143	68	73

Leaving the infected plants to dry up in place without any treatment was still practiced among 15% of banana growers in Davao del Norte. The majority of banana growers (46.5%) practiced this method because they believed that avoiding contact of the infected plants would limit the spread of Fusarium wilt (Table 7.13). The lack of resources to finance eradication of infected plants was another reason by 16% of the respondents who practiced this method.

Table 7.13. Reasons given by banana growers to why they had adopted not treating banana plants infected with Fusarium wilt

Reason	No treatment of infected plants (%)	
	2015	2017
To control disease	80.0	
Limit disease spread	20.0	46.5
Follow other grower practices		9.5
Lack of finances		16.1
Too many cases		27.9
No. respondents	15	30

Respondents were asked about their knowledge on the role of vegetated ground cover for Fusarium wilt management. There was a 4% increase in awareness among respondents about using vegetated ground covers for Fusarium wilt management in bananas (Fig 7.16).

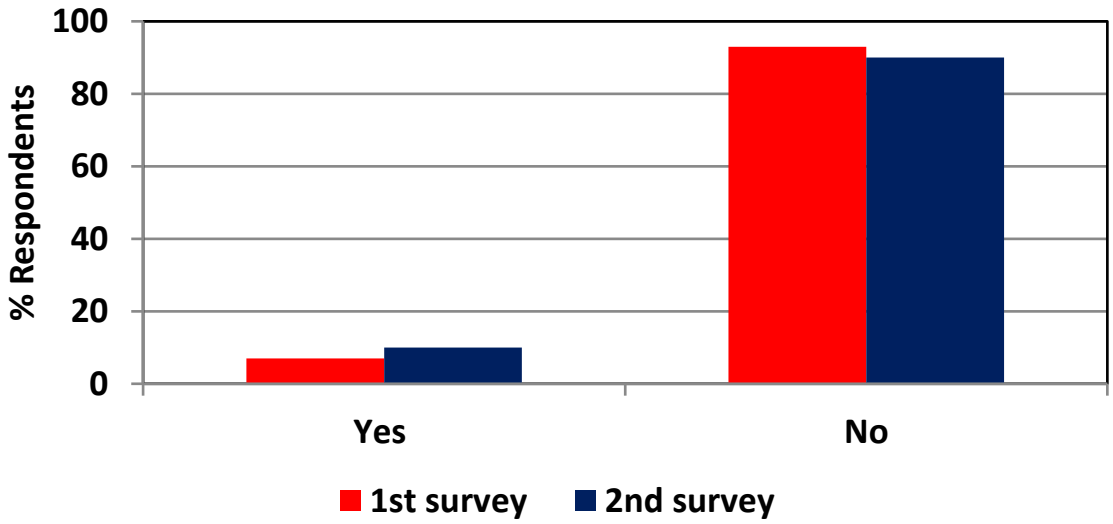

Fig 7.16. Awareness of banana growers about the use of vegetated ground covers for the management of Fusarium wilt in Davao del Norte during two surveys in 2015 and 2017

There was a significant increase in the number of respondents using a resistant variety to manage Fusarium wilt in the two years between the two surveys (Fig 7.17). About 38% of the respondents were now aware and practice replanting their Fusarium wilt affected areas with GCTCV 218 compared to only 10% at the first survey in 2015 (Fig 7.17).

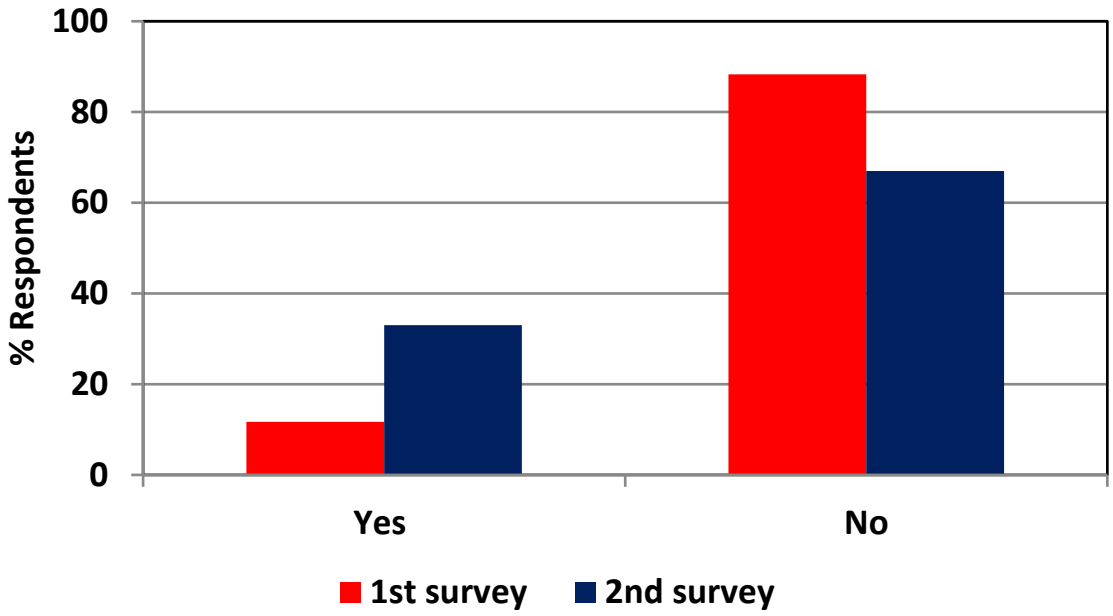

Fig 7.17. Adoption by banana growers to re-establish plantations with resistant banana cultivars for the management of Fusarium wilt in Davao del Norte during two surveys in 2015 and 2017

From the two surveys the following conclusions have been drawn:

1. Most of the smallholder banana growers in Davao del Norte still lacked sufficient knowledge about the disease to implement the proper management approaches.
2. Although banana growers were knowledgeable about the recommended practices, many growers lacked sufficient capital to consistently support effective Fusarium wilt management practices.
3. The misconception that there was no effective method to manage the Fusarium wilt was popularized, which led to some growers lacking technical knowledge or capability, not to adopt any management practices and therefore suffer the most from the impact of the disease.
4. The adoption of the resistant variety GCTCV 218 had increased over the life of the project, but was still low due to limitations around access to a reliable sources of planting material.

The information derived from the survey on the adoption of approaches to manage Fusarium wilt among male and female banana growers has provided insights on the status of disease in Davao del Norte and the identification of the support required by the banana industry. Priority interventions to mitigate the impact of Fusarium wilt are:

1. Support for the availability of Fusarium wilt resistant variety GCTCV 218 - aside from current distribution of 800,000 plants of GCTCV 218, the Department of Agriculture and Province of Davao del Norte have provided funding for the establishment of a banana tissue laboratory and explant seed garden area.
2. Intensify capability building among independent growers through training and on farm consultation about Fusarium wilt.
3. Develop and reproduce information, extension and communication material for information dissemination to banana growers
4. Provide more research outcomes to identify measures to manage Fusarium wilt of bananas.

7.3.2 Appraisal & assessment of current practices for the management of Fusarium Wilt in Ladyfinger/Niche banana cultivar production in Australia and to determine information needs and barriers to adoption

Economic assessment of case study farms to a determine the economic impacts of different Fusarium wilt management practices

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. Based on the application of identified effective practices the cost of capital investment ranged from $3,070 to $8,500 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 weight, crop cycle times and population mortality to identify the key requirements and productivity drivers that any potential production system must achieve.

Assessments of adoption of biosecurity practices and alternative production systems using cultivar resistance were 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 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 economics of banana biosecurity

Activities have been undertaken to examine costs associated with the adoption of identified biosecurity practices but 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 heavily on the attributes of each individual property.

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 north Queensland. 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 e.g. 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 with 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 7.14.

Table 7.14. 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 - $35,000 Farm vehicle - $30,000	Lime spreader - $35,000 Farm vehicle - $30,000 Bagging machine - $100,000 Tractor - $45,000 Quad bike - $15,000 Picking trailer modifications - $1,200
Fencing	Boundary fencing undertaken by contractor, 3510m at $8/m - $28,080	Boundary fencing undertaken by contractor, 5820m at $8/m - $46,560
Washing/boot change facilities	Compliant wash-down - $50,000 Earthworks/ballast/concrete - $17,000 Footbaths – 3 at $1,100 - $3,300 Shuttle and electric pump - $700 Boot room (shipping container) - $6,000 Boot change points (internal) - $500	Compliant wash-down X 3 - $15,0000 Earthworks/ballast/concrete X 3 - $35,000 Footbaths – $1,100 X 6 - $6,600 Shuttle and electric pump X 3 - $2,100 Boot room (shipping container) - $6,000 Boot change points (internal) X 3 - $1,500
Provision of footwear	1.75 sets of rubber boots at $25/pair for 30 staff - $1,320	4.3 sets of rubber boots at $25/pair for 30 staff - $2,700
Disinfectant product	Spray application – 6,000L/yr at $0.14/L of 1% mixture of DDAC - $840 Footbath sanitiser replacement – 3 footbaths at 100L replaced weekly at $0.14/L - $2,184	Spray application – 12,000L/yr at $0.14/L of 1% mixture of DDAC - $1,680 Footbath sanitiser replacement – 6 footbaths at 100L replaced weekly at $0.14/L - $4,368
Labour inputs	Wash down, 4 hours per week at $21.45/hr - $4,462	Wash down, 22 hours per week at $21.45/hr - $24,540
Capital costs	$172,000 $1,230/acre; $3,070/ha	$477,000 $3,405/acre; $8,512/ha
Operating costs	$7,500 $53/acre; $134/ha	$30,590 $218/acre; $546/ha

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 north Queensland industry is presented below (Table 7.15).

Table 7.15. Costs of implementing biosecurity for three different scenarios for the north Queensland banana industry

Scenario	Cost across industry
NQ industry (11,150 ha) – contiguous scenario	$34,230,500 capital $1,500,000 operating annually
NQ industry (11,150 ha) – separated scenario	$94,909,000 capital $6,090,000 operating annually
NQ 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 north Queensland industry is crude due to the inherent inaccuracies built into this assessment that have been outlined, there are several 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 were 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 commercially acceptable resistant variety, then large investments of up to $10M could still be regarded as providing excellent value for money if it removed the need to implement full on-farm biosecurity.

The economics of alternative banana production systems

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 a Northern Territory (NT) banana 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 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 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

The productivity outputs from the 4 modelled scenarios are presented in Table 7.16.

Table 7.16. Comparisons of modelled productivity for alternative Cavendish varieties in suppressive production systems with disease-free Williams Cavendish

Williams Cavendish (*Foc* TR4 free)	**GCTCV 218 (suppressive production system)**
• 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	• 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
GCTCV 219 (suppressive production system)	**Williams Cavendish (NT production system)**
• 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	• 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

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 there 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’-based 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 weight, crop cycle times and 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 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.

8 Impacts

8.1 Scientific impacts – now and in 5 years

There has been an exponential increase in the publication of scientific outcomes on research on Fusarium TR4, with 25 papers published on the disease in 2019. The scientific interest in Fusarium TR4 is in recognition of the devastating effect the disease has on banana production as it continues to spread globally. HORT/2012/097 contributed to the scientific knowledge on the epidemiology, management and social impact of Fusarium TR4.

The interaction of the soil microbial community with pathogens and host crops to develop disease suppressive systems has gained widespread credibility as an area of science. The initial scoping studies on microbiomes of banana cultivars is at the forefront of this research area and is likely to have greater impact within 5-years with publication of outcomes. The results from microbiome research are currently being written up for publication in the journal Microbiome (impact factor = 9). Meanwhile, methodology used to determine soil microbial functions used in this project, such as assessment of soil enzymes and community level physiological profiling, are being used in other crops, like sugarcane and avocado, for soil health assessments in Australia.

Management of banana plantations to increase soil biodiversity using vegetated ground covers for suppression to Fusarium wilt is being examined in other banana locations, such as Africa, Latin America and Asia. There is also increasing scientific interest in how farm soil management practices can be used to slow the spread of soilborne diseases. Furthermore, the integration of disease suppressive soil management practices with other management strategies has increased interest within agricultural science to reduce the impacts of soilborne diseases on crop production. The testing of integrated production systems are currently being developed in a new research project to overcome Fusarium TR4.

The impacts of extension and theory of change in banana grower's knowledge and attitudes to Fusarium TR4 is being written up for publication. The results from this project will add to current social science publications to understand how to alleviate the stress and improve the well-being of banana growers facing Fusarium TR4.

Scientific articles published from the outcomes of HORT/2018/097 have so far have been read 388 times and cited 15 times in other scientific research articles. Further scientific impacts from all research areas are anticipated in 5-years as research outcomes are published.

8.2 Capacity impacts – now and in 5 years

The project had a large emphasis on building the capacity of project participants. Four research assistants have been employed on the project and completed their Master of Science at the University of Southeastern Philippines;

- Ms Christine Ansale – soil biology
- Mr Marvin Tagan – soil biology
- Mr Nelvin Villason – agronomy
- Mr Carlito Hindoy -agronomy

Furthermore, 30 students participated in field research activities with seven students (2 male and 5 female) presenting outcomes at scientific forums.

The project HORT/2012/097 was successful in facilitating two scholarship opportunities for post graduate studies.

- Ms Tamsi Gervacio was successful in gaining a John Allwright Fellowship to complete a PhD at the University of Queensland, which commenced in June 2018. Her thesis outline is given in Appendix 1.
- Ms Alphabet Gulanes commenced an Australian Award scholarship in July 2019 to complete a Master of Science at the University of Queensland. Her thesis outline is given in Appendix 1.

Five specific activities have taken place in the Philippines to increase banana grower and their service provider knowledge about Fusarium TR4;

- 556 banana growers participated in 11 focus group activities in 2015 in Davao del Norte.
- 385 banana growers participated in Good Agricultural Practice (GAP) training in 2016 in Davao del Norte.
- 368 banana growers participated in focus group activities in 2017 in Davao del Norte
- 500 professional agriculturists attended the Philippine Agriculturist Summit held in Davao City November 2016.
- 150 banana growers and service providers attended the Mindanao Wide Symposium in February 2018.

In Australia an increase in the knowledge of Fusarium TR4, its impacts and management were presented at banana industry forums;

- 50 banana growers at the Cassowary Coast Banana Growers' Association, meeting in Silkwood 2015
- 100 banana growers at the Panama disease field day, South Johnstone, May 2017.
- 30 agribusiness and policy makers at Horticulture and Forestry Science Stakeholder engagement day, April 2017
- 200 banana growers and service personnel at the Australian Banana Industry Congress, Sydney, June 2017

8.3 Community impacts – now and in 5 years

8.3.1 Economic impacts

The adoption of practices to suppress Fusarium wilt of banana can lead to a short-term reduction in profitability. The implementation of on-farm biosecurity results in additional capital expenses of $3,070 - $8,512/ha, with annual operating costs of $134 - $546/ha depending on the farm configuration. Furthermore, if a change in cultivar is required, no alternative production system was as profitable as the current disease-free system over a 7-year cropping life cycle. Implementing a semi-resistant cultivar, such as GCTCV 218 and cropping for 4 years, with 3 years of fallow, was 37% as productive as the current system without Fusarium TR4. Use of a more resistant cultivar, like GCTCV 219 producing 6 crops in 7-years was only 50% as productive as the current banana production system free of TR4. Lastly, an annual cropping system using a susceptible cultivar, such as Williams, and requiring a fallow period was only 37% as productive as the current disease-free production system. The economic modelling was confirmed by field trials in the Philippines that demonstrated that there were penalties in the adoption of more resistant banana cultivars like GCTCV 218 in low disease situations. However, in situations where Fusarium TR4 was prevalent, no production would be obtained from susceptible cultivars like Grand Naine, whereas semi-resistant cultivars were able to produce marketable fruit.

The implementation of practices to improve suppression of Fusarium wilt on commercial banana plantations, such as reduced nitrogen and use of vegetative ground covers throughout the plantation, were shown to be less productive than practices that focused

solely on productivity with high input use. Over the five-year trial in Australia the combination of vegetative groundcover and reduced nitrogen rates led to a decrease in returns of approximately $10,000/ha. However, in Australia the collaborating banana grower implemented the 100% ground cover strategy to increase microbial suppression of Fusarium wilt and cited a 10% increase in productivity, with an 80% reduction in the use of herbicide and further plans to decrease the use of soil applied pesticides.

The treatment of infected plants with toxic rates of urea applied to bagged plant material, rather than rice hull burning, offers a more cost-effective treatment. Initial indications are that this will reduce the cost of treating individual infected plants by 50-60% compared to rice hull burning. The demonstration of this approach is now being driven by PAGRO staff in Davao del Norte.

8.3.2 Social impacts

Changes in equity

The demand for resistant banana cultivars like GCTCV 218 outstripped supply from established nurseries, meaning that smallholder growers were unable to access the cultivar to replant fields. Through this project banana growers at the AMSEFFPCO cooperative farm were able to access GCTCV 218, allowing them to continue farming. However, some cooperative members elected to lease their land to Dole Philippines rather than risk financial loss caused by Fusarium TR4.

The limited access that smallholder growers had to resistant cultivars was recognised through project activities resulting in tissue culture laboratories being established at USeP Tagum and the PAGRO office in Davao del Norte. The experience at USeP that access to GCTCV 218 has improved confidence for the members to continue production of bananas in the presence of the disease, thus providing hope for on-going employment and investment.

Cultural impacts

On-farm biosecurity practices have greatly altered the culture surrounding banana farms. To reduce the movement of Fusarium TR4, on-farm biosecurity practices are required to restrict the movement of people, requiring cultural changes in the attitudes of farm workers if biosecurity practices are to be effectively implemented. There is increasing awareness that if banana farms are to be maintained as important contributors to employment and the economy, free movement across the farm is no longer viable.

Grower surveys in the Philippines and Australia, found the feeling of hopelessness being expressed by growers in the face of Fusarium TR4. The impacts of the disease on the social wellbeing of people in north Queensland was documented by Maclean *et al.* (2018), but similar feelings were also expressed by banana growers in the Philippines. The development of biophysical strategies to manage Fusarium TR4, which can be implemented on-farm, empowers banana growers to do something positive to prevent the impacts of the disease, to reduce anxiety that the farm will become unproductive and to promote optimism in the future of banana production. The changes in attitudes were observed in communications with farm staff at the AMSEFFPCO site, having increased optimism with greater knowledge about practices that can reduce the impacts of the Fusarium TR4.

Health impacts

The outcomes from HORT/2012/097 should see positive health impacts in the banana growing communities in the Philippines. The use of formalin as a foot bath product was shown to be ineffective, and a campaign has commenced for it to be discontinued. The removal of formalin from the farm environment will reduce the exposure of banana workers to known carcinogenic chemicals. Furthermore, the adoption of soil health practices like vegetated ground covers, will result in reduced chemical exposure to farmers, farm workers, families, communities and consumers.

Gender impacts

This project encouraged gender equality and has practiced gender inclusion, which was demonstrated by the significant engagement by female participants (43% female) and recent graduates (70% female) within the banana industry. By actively encouraging a new generation of agricultural scientists with innovative research, new ideas and skills have entered the banana industry to better manage Fusarium wilt. Furthermore, a large proportion of the project staff engaged in the decision-making process are female, with greater prospects arising from project opportunities to develop greater female leadership within the agricultural sectors of Australia and the Philippines. Within the project there was a shift to greater inclusion of females in decision making position, but there was no obvious change in the roles of men and women in banana farming.

Political impacts

Biosecurity research outcomes from this project have influenced Davao del Norte policy makers on the implementation of biosecurity protocols for Fusarium TR4. The project provided an intensified information campaign on the biology, disease epidemiology and implementation of Fusarium wilt management, to support the implementation of regulations at the municipal and barangay (suburb/village) level.

HORT/2012/097 contributed to the development of research projects funded through Horticulture Innovations Australia following the incursion of Fusarium TR4. The implementation of biosecurity policies and protocols for the Australian banana industry were directly and indirectly influenced by activities undertaken within the project.

The story of Mr Stewart Lindsay and his role in ACIAR projects was used as a case study for the 2017 Foreign Policy White Paper https://www.fpwhitepaper.gov.au/foreign-policy-in-action/helping-others/stewart-horticulture

8.3.3 Environmental impacts

Use of 100% vegetated ground covers has had a positive environmental impact in banana plantations, reducing soil erosion and sediment movement. Research work undertaken using a rainfall simulator, identified a twentyfold reduction in sediment movement with ground cover, compared with bare soil. Furthermore, there has been a reduction in the use of soil applied agrichemicals, such as a 75% reduction in herbicide use and a reduction in soil applied insecticides. This has led to an improvement in the quality of water leaving the plantation of a collaborating grower, who seeing the benefits converted his 200 ha banana plantation to having full ground cover with no exposed soil surface.

Vegetative ground covers are also contributing to an increase in soil organic matter. Coupled with the increase in organic matter has been an increase in soil biological activity and diversity, determined through nematode community, microbial function and microbiome analysis, which has led to increased suppression of soil borne diseases of bananas, such as Fusarium wilt and plant-parasitic nematodes.

In the Philippines, the practice of burning Fusarium TR4 infected banana plants has the potential to decrease air quality. By implementing practices to reduce the infection of Fusarium TR4 in banana plantations, using resistant cultivars and better soil management has the potential to reduce contributions to greenhouse gas emissions.

Similarly, reduction in nitrogen application used in the Australian banana research has the potential to reduce nitrous oxide emissions from the soil. Furthermore, knowledge of the results that high nitrogen rates can make soils conducive to Fusarium wilt are currently being incorporated into a nutrient management plan for bananas. The banana industry in north Queensland is required to meet nutrient application targets in order to improve runoff water quality to protect the Great Barrier Reef. Therefore, additional information that supports the case for better nutrient management, such as increased disease suppression with reduced nitrogen inputs, will aid in the adoption of better environmental management outcomes for the banana industry.

8.4 Communication and dissemination activities

Scientific publications:

Pattison AB, Molina AB, Chao CP, Viljoen A, Lindsay SJ (2018) Integrating management practices to support banana production in the presence of Fusarium wilt. 1196 edn. International Society for Horticultural Science (ISHS), Leuven, Belgium.

McBeath AV, East DJ, Wright CL, Pattison AB (2018) Monitoring microbial functional and structural diversity for management of disease-suppressive soils. 1196 edn. International Society for Horticultural Science (ISHS), Leuven, Belgium.

Pattison AB, East D, Ferro K, Dickinson G (2018) Agronomic consequences of vegetative groundcovers and reduced nitrogen applications for banana production systems. 1196 edn. International Society for Horticultural Science (ISHS), Leuven, Belgium.

Rames EK, Pattison AB, Czislowski E, Smith MK (2018) Soil microbial community changes associated with ground cover management in cultivation of Ducasse banana (Musa sp. ABB, Pisang Awak subgroup) and suppression of Fusarium oxysporum. Australasian Plant Pathology 47: 449-462. doi: 10.1007/s13313-018-0578-4.

Scientific presentations:

Philippine Agriculturalist Summit, November 2016, Davao with over 500 participants

An oral presentation was made at the International Society of Horticultural Science ProMusa Symposium, 'Agro-ecological approaches to promote innovative banana production systems', 10-14 October, 2016, Montpellier, France titled 'Integrating management practices to support banana production in the presence of Fusarium wilt' by Pattison et al.

An oral presentation was also made at the Australasian Soilborne Disease Symposium, 14-17 November 2016 at Lincoln University New Zealand on the 'Development of an integrated management system to suppress Fusarium wilt of bananas' by Pattison et al.

An oral presentation was made at Science Protecting Plant Health Conference, titled *Engineering banana cropping systems to suppress soil borne diseases*, Brisbane, 26-28 September 2018, by Pattison et al.

Two poster presentation were at Science Protecting Plant Health Conference, titled Soil quality indicators to detect suppression of Fusarium wilt in banana in the Philippines, by Gervacio et al and Improving on-farm biosecurity in the Philippine banana farms by reducing movement on footwear by Gervacio et al, Brisbane, 26-28 September 2018,

An oral presentation was made at the 22nd ACORBAT International Banana Congress titled The Australian Experiences in the management of Fusarium wilt Tropical Race 4, Miami, USA 2-4 May, 2018 by Pattison et al.

An oral presentation was made at the 11th International Congress of Plant Pathology titled Manipulation of the soil microbial community to suppress soil-borne diseases of banana through soil management, July 29-August 3, 2018, Boston, USA by Pattison et al.

Oral presentation and poster displays on sanitiser and boot scrubber R&D at the International Symposium on Tropical Fruits, August-September 2016, Grand Regal Hotel, Davao City by Gervacio et al.

Soil health papers

- "Labile carbon in soil planted with Arachis pintoi in a Fusarium wilt infested Cavendish banana plantation at Kapalong, Davao del Norte" was presented by Ms. Erl Mejean P. Friales.

- "Fluorescein diacetate (FDA) activity in soil infested with Fusarium oxysporum f. sp. cubense at AMSEFFPCO Banana Plantation, Davao del Norte" was presented by Ms. Vanessa M. Olivar.
- "β-glucosidase activity in Fusarium wilt infested Cavendish plantation planted with Arachis pintoi at Barangay Sampao, Kapalong, Davao del Norte" was presented by Ms. Dominique N. Clarabal.

Soil movement

- "Inoculum load distribution of Fusarium oxysporum f. sp. cubense through surface run-off" presented by Mr. Joanny Jaspher R. Raz, which combined the results from several researchers.
- "Inoculum load distribution of Fusarium oxysporum f. sp. cubense (Foc) through surface run-off with pinto peanut (Arachis pintoi) in Calinan, Davao City by the research team together Ms. Shaira S. Pairat, Ms. Queenie M. Mandalunes and Ms. Trisha Dianne F. Coronel.

On-farm biosecurity

- "Longevity of Benzalkonium chloride against Fusarium oxysporum f. sp. cubense (Foc) presented by Ms. Rosemarie G. Badillo
- "Response of Fusarium oxysporum f. sp. cubense to Benzalkonium chloride in footbaths with an established boot scrub in Barangay Lacson, Calinan, Davao City" was presented by Ms. Keren Hapu A. Ga and research team.

Grower presentations:

There have been five major campaigns with banana growers to increase their knowledge about the project and outcomes from research efforts.

Mindanao-wide symposium on Fusarium wilt, March 2017, Davao with 150 participants from 11 provinces, including banana growers, provincial agriculturalists and the industry representative bodies

Panama disease field day, May 12, 2017 South Johnstone with 100 banana growers and agribusiness represented

Australian Banana Industry Congress, June 22, 2017, Sydney with 200 banana growers and agribusiness represented.

Australian Banana Industry Roadshows (6), July 24-August 30, 120 banana industry personnel

Grower articles:

Campbell, S (2014) Philippine projects gives our industry TR4 insights, Australian Bananas Magazine, Spring 2014, 18

Pattison, T (2015) TR4 Why Queensland's different. Australian Bananas Magazine, Autumn-Winter 2015, 14.

Pattison, T (2018) Lessons from the Philippines on TR4 management Australian Bananas Magazine, Summer 2018, 15.

Pattison, T, Gaza, H, Dennis, P and Birt, H (2018) Unravelling the mystery of the banana microbiome. Australian Bananas Magazine, Summer 2018.

Gaza, H and Pattison, T (2018) The banana nitrogen dilemma: Balancing production and soil health? Australian Bananas Magazine, Summer 2018.

Videos and multimedia:

ABC Landline program May 2019 https://www.abc.net.au/landline/red-tips:-the-story-behind-the-bananas-with-the/11126862

ABC Landline program November 2019 https://www.abc.net.au/landline/banana-resilience:-banana-industry-stronger-than/11733372

ACIAR web site https://reachout.aciar.gov.au/stopping-panama-diseasethe-fight-to-save-australias-bananas

ACIAR Stopping Panama Disease – the fight to save Australia's bananas https://www.youtube.com/playlist?list=PL5-wYcyMyLz9TX8BWHmOsS9AndCAofhRg

Importance of bananas https://www.youtube.com/watch?v=g9ntupzMC3s&list=PL5-wYcyMyLz9TX8BWHmOsS9AndCAofhRg&index=2&t=0s (202 views)

History of Panama disease https://www.youtube.com/watch?v=8mymlLmB8oM&list=PL5-wYcyMyLz9TX8BWHmOsS9AndCAofhRg&index=3&t=0s (773 views)

The science of stopping Panama disease https://www.youtube.com/watch?v=GAZGa1v7u8A&list=PL5-wYcyMyLz9TX8BWHmOsS9AndCAofhRg&index=4&t=0s (3,836 views)

The benefits to Australian bananas https://www.youtube.com/watch?v=GAZGa1v7u8A&list=PL5-wYcyMyLz9TX8BWHmOsS9AndCAofhRg&index=4&t=0s (111 views)

9 Conclusions and recommendations

9.1 Conclusions

Fusarium wilt of bananas caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) is considered one of the most destructive banana diseases. The Tropical Race 4 (TR4) strain of the pathogen is particularly virulent on commercial Cavendish banana production, which supplies bananas to consumers around the world. Currently, there are 15 countries that have reported to have TR4, mostly in South-East Asia. Predictions indicate that within 10 years there will be a loss of 160,000 ha of bananas, resulting in a 2% decrease in banana production, and a loss of 240,000 jobs, with South-East Asian countries likely to suffer the greatest losses.

To reduce the impacts of Fusarium wilt and improve the livelihoods of banana growers in the Davao del Norte region of the Philippines and the north Queensland region of Australia, HORT/2012/097 was undertaken. The project aimed to;

- increase knowledge of effective on-farm biosecurity practices and improve farm preparedness to minimise the risk of Fusarium wilt incursions,
- develop long-term management strategies to slow the spread of the disease and
- develop options to allow smallholder producers to return to economic banana production.

To achieve the overall aims, three objectives were undertaken;

Firstly, was to develop options to limit losses in banana production by improving knowledge of on-farm biosecurity. On-farm biosecurity protocols were very effective in slowing the spread of Fusarium TR4 in Australia, following the outbreak of the TR4 near Tully in March 2015. Project work conducted in the Philippines demonstrated deficiencies in the implementation of on-farm biosecurity, due to lack of effective chemicals, such as formalin, and not understanding the limitations of the foot baths, particularly amongst smallholder banana producers. Furthermore, the project found that foot baths at farm and field entrances were ineffective, either due to the chemical used, the concentration of chemical used, or the amount of soil that was transported into the footbaths. However, this project identified how local solutions, using low cost boot scrapers constructed with wire mesh and effective disinfectant chemicals, could be implemented to improve farm biosecurity practices.

The second objective was to evaluate integrated crop management approaches to enable commercial banana production in the presence of Fusarium wilt. In Australia, there was strong evidence that increasing soil microbial activity was related to suppression of Fusarium wilt. In the Australian context, changes in soil microbial activity took approximately 18-24 months to stabilise, following changes in farm management practices. The use of vegetated ground cover was not an unfamiliar concept for Australian banana growers, with partial and full adoption of the practice over an estimated 1,500 ha (10%) within the north Queensland banana industry during the project. The implementation of vegetated ground covers by a collaborating banana grower was cited to increase productivity by 10%, reduce herbicide use by 80%, reduce soil erosion and improve water quality leaving the farm.

In the Philippines, the use of a resistant cultivar, GCTCV218, was the only option to continue banana production for growers who had Fusarium TR4 on their farms. The differences between Australia and Philippine banana production systems, and availability of resources to growers, meant that implementation of vegetated ground covers on smallholder commercial banana farms in the Philippines was problematic. Both glasshouse and field experiments, in the Philippines, found there were no changes in soil microbial activity from ground covers, and as a result there were no changes in Fusarium wilt incidence or severity. In the field experiments, the application of biocontrol products

did not alter microbial activity in the soil or reduce Fusarium wilt incidence. A synopsis from the field experiments, indicated that any integrated crop management system for banana where Fusarium TR4 was present, requires cultivars with at least partial resistance as a basis.

The third objective was to determine the barriers to adoption of Fusarium wilt management practices. In Australia, banana growers tended to have greater knowledge of Fusarium wilt and how it was spread. However, the greatest barrier was the financial capacity to implement all suggested biosecurity practices. The establishment of on-farm biosecurity practices amounted to capital costs around 13% and annual running costs of 0.8% of the value of the banana enterprise.

In the Philippines, Fusarium wilt continued to spread within Davao del Norte, with more growers indicating they have the disease and a greater number of smallholder banana growers going out of production over the life of the project. The main barriers to adopting Fusarium wilt management practices in the Philippines for smallholder banana growers, were around knowledge of what to do, the widespread extent of the problem and the availability of resources. Furthermore, there was a lack of credible information on which management practices could reduce Fusarium wilt, making it difficult for banana growers to know what to do.

The project HORT/2012/097 focused heavily on increasing the capacity of Philippine project partners to develop solutions to Fusarium TR4, with training activities to increase the capacity to quantify soil biology and disease suppression. Four graduate students (1 female and 3 male) received post graduate qualifications, 30 students participated in field research activities with seven students (5 female and 2 male) presenting outcomes at scientific forums, and two project personnel (both female) received scholarships (John Allwright Fellowship and Australia Award) for post-graduate study in Australia over the life of the project. The involvement of banana growers as project partners allowed additional communication of project results and added value to the research outcomes. HORT/2012/097 was also used to demonstrate how international collaborative projects could benefit both partner countries and contributed to a case study for the 2017 Foreign Policy White Paper.

9.2 Recommendations

Fusarium TR4 continues to spread throughout South-East Asia and within the Philippines. There is a need to develop more resilient banana production systems that can slow the spread of disease and reduce plant losses on farm. Therefore, banana growers require;

1. Improved knowledge of how farm management practices interact with Fusarium TR4 and the soil environment, with the development of more grower inclusive information about Fusarium wilt, its cause, spread and management options.
2. Specifically, more information is required on;
    a. how to reduce Fusarium TR4 inoculum before replanting bananas,
    b. how to enhance the plant tolerance to Fusarium wilt and
    c. how to prevent Fusarium TR4 inoculum from building up within the banana crop.
3. Cavendish banana cultivar resistance to Fusarium TR4 with improved agronomic characteristics are required for the commercial banana industry. The use of GCTCV218 allows banana production to continue, but there are financial penalties when it is cultivated, compared to the current Cavendish cultivars William or Grand Naine, due to the longer vegetative cycle and increased fruit rejection.
4. A greater understanding of how the Fusarium TR4 pathogen interacts with other soil and plant organisms, is an important component in developing resilient systems to suppress Fusarium wilt and prolong crop production, even with the use of partially resistant banana cultivars.

5. Outside of the scope of the current project effective Fusarium TR4 management systems can be found amongst smallholder banana producers that use a poly-cropping system. These systems need to be better understood for the characteristics that allow the growers to continue to supply local markets with Fusarium TR4 susceptible banana cultivars, even in the presence of the disease. Furthermore, there is a need to determine if the characteristics that make the smallholder growers systems suppressive to Fusarium TR4, can be built into high yielding monoculture banana systems.
6. How banana growers make decisions and who informs these decisions is required when developing a change in production systems to overcome Fusarium TR4. In the Philippines, smallholder banana producers are largely influenced by “standard operating procedures” developed by the exporting companies.
7. A better understanding of the information networks and decision-making processes will allow targeted information dissemination, so that key influencers on farm decision making can have up to date information about Fusarium TR4 that can be passed onto smallholder banana producers.
8. Decision-making aids are required that can inform growers of their options to manage Fusarium TR4 with changes in the scenarios they face with the disease;
    a. Not having Fusarium TR4 and management options on how to keep the farm disease free.
    b. First detection of Fusarium TR4 and management options to slow the spread of the disease to reduce its impact.
    c. Fusarium TR4 is widespread and management options to return to a productive banana system in the presence of the disease.
9. The transboundary spread of Fusarium TR4 throughout South-East Asia requires coordination of research, development and extension efforts from all banana producing countries, to slow the spread of the disease and to reduce its impact on smallholder banana producers.

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10.2 List of publications produced by project

Scientific publications:

Pattison AB, Molina AB, Chao CP, Viljoen A, Lindsay SJ (2018) Integrating management practices to support banana production in the presence of Fusarium wilt. 1196 edn. International Society for Horticultural Science (ISHS), Leuven, Belgium.

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11 Appendixes

11.1 Appendix 1: Capacity Building of Project Staff

Two capacity building activities were conducted to train the staff involved with the project. (1) Training on the Assessment of Soil Biological Characteristics - was conducted last September 1-3, 2014 at the University of Southeastern Philippines-Obrero Campus with Dr. Tony Pattison and Ms. Tegan Kukulies of QDAFF. (2) Training on *Fusarium oxysporum* f.sp. *cubense* (Foc) Laboratory Techniques last November 14-15, 2014 at the Polymerase Chain Reaction (PCR) Laboratory of USeP-Tagum Apokon Campus with Mr. Wayne O' Neill and Stewart Lindsay.

The trainings were attended by participants from USeP-Obrero Campus, USeP Tagum-Apokon Campus, USeP Mampising Campus, and the Provincial Agriculturist's Office (PAGRO) of Davao del Norte. The two trainings also served as an opportunity to discuss the procedures in the protocol given and the equipment that will be used on the course of the project.

A training on the use of biocontrol agents that will be used for the management of Foc TR4 on farmer's field was suggested.

Figure 1. Training on the Assessment of Soil Biological Characteristics conducted last September 1-3, 2014 at USEP Obrero Campus.

Figure 2. Training on *Fusarium oxysporum* f.sp. *cubense* Laboratory Techniques conducted last November 14-15, 2014 at the Polymerase Chain Reaction (PCR) Laboratory of USeP-Tagum Apokon Campus

11.1.1 Ms. Tamsi Gervacio (PhD through John Allwright Fellowship

Ms Gervacio's work focusses on TR4 inoculum management – specifically on the impacts of burning infected plant material on Foc activity and the soil microbiome. She visited USeP Jan-Mar 2019 to conduct a series of experiments to measure soil temperatures during burning of infected plants and determine the impact of these burns on soil microbial activity. During this time, she benefited from logistical and technical support from two USeP students employed in part through the ACIAR project.

Her work plan is as follows:

Title: Managing Inoculum Load in Fusarium Wilt infected Banana Plants

A. Chapter 1: Literature review
B. Chapter 2: Burning of Foc infected plants
    a. How effective is the burn in removing plant biomass?
        i. Weight shoot biomass before and after burn
    b. What temperatures are reached at different soil depths and for how long?
        i. Temperature probes at 10 – 30 cm
    c. Is the average burn hot enough to inactivate Foc?
        i. Field isolate Foc from soil and plant before and after burning
        ii. Lab study temperature time using SR4
    d. Is the average burn hot enough to reduce microbial biomass and activity?

i. Field study – CFE, enzymes
ii. Lab study – soil in jars exposed to different temperatures for different amounts of time then measure respiration rate by GC, enzyme activity and then biomass using CFE

C. Chapter 3: The influence of microbial biomass and *Trichoderma* on Foc colonisation
   a. Quantify the change in Foc over a 21 day period in soils before and after burning with and without *Trichoderma*
   b. Quantify the change in Foc over a 21 day period in a gradient of biomass created by mixing non-treated and gamma irradiated soil with and without *Trichoderma*.
D. Chapter 4: Composting as an alternative inoculum control measure
   a. Identify suitable composting mixtures to degrade banana plant biomass at temperatures that inactivate Foc
   b. Determine whether composting effectively inactivates Foc
   c. Determine whether composted material can be used as substrate to increase biomass of *Trichoderma* and grow disease-free plants
E. Chapter 5: General Discussion

11.1.2 Ms Alphabet Gulanes (MPhil through an Australian Award)

Her work plan is as follows:

Title: Defining the fundamental niche of Fusarium oxysporum f. sp. cubense and its relatives

General Objective:
This study aims to: 1) characterise the growth optima of fungal isolates under a range of environmental conditions, including pH, nitrogen status and temperature; and 2) determine whether these fungal isolates exhibit phylogenetic signal with respect to their environmental preferences.

Specific Objectives:

A. Obtain a collection of fungal isolates including *Fusarium oxysporum* f. sp. cubense and its close and more distant relatives.

B. Characterise growth rates of isolates exposed to different environmental conditions, including pH, nutrient status and temperature.

C. Generate a high-resolution phylogeny of the isolates using long-read sequencing

D. Determine whether the isolates exhibit phylogenetic signal with respect to their environmental preferences

E. Predict the environmental preferences of isolates that have not been characterised using ancestral state reconstruction and then empirically test the accuracy of predictions

11.2 Appendix 2: Field trial sites in the Philippines

First trial site (Puyod Farm, Lasang, Davao City)







Second trial site (AMSEFFPCO, Sampao, Kapalong, Davao del Norte)

Establishment

Management and assessment


T3R1


T3R2


T1R1

11.3 Appendix 3 Panama R&D Field day, 12 May 2017, South Johnstone

Image 1. Pre-field day seminar outlining biosecurity practices to access the research station

Image 2. Temporary footwear exchange and footbaths for entry to the field for over 100 attendees

Image 3. The field day displays worked as concurrent presentations to 3 separate groups

Image 4. Dr Tony Pattison and Dr Anna McBeath presenting the ACIAR project Australian trial results

11.4 Appendix 4: Newspaper and magazine articles

SATURDAY FEBRUARY 20 2016 FACEBOOK.COM/INNADVOCATE

Grower joins team to study TR4 hot spots

BANANA researchers hope to harness the lessons learned in two of the world's Panama disease hot spots to better protect the Australian industry from the scourge of the soil-borne fungus.

Australian Banana Growers' Council research and development manager Rosie Godwin, Queensland Department of Agriculture and Fisheries principal nematologist Tony Pattison and Tully banana grower Patrick Leahy have travelled to the Philippines and Taiwan, where Panama disease Tropical Race 4 has destroyed thousands of hectares of bananas.

Over two weeks, the trio will inspect field trials, visit growers, and discuss a range of issues with banana scientists, including biosecurity best-management practice and suppressive cropping systems that can allow areas infested with the disease to return to export production.

Their trip is part of an Australian Centre for International Agricultural Research project looking into the integrated management of fusarium wilt of bananas in the Philippines and Australia.

Tully banana grower Patrick Leahy has travelled to the Philippines and Taiwan.

Fusarium oxysporum f. sp. cubense is the form of fusarium that causes TR4 in bananas.

"Both Taiwan and the Philippines have for many years been managing the fusarium wilt incursions in their banana production areas that have a detrimental effect on their smallholder producers and export industries," Ms Godwin said.

"It's extremely useful for members of the Australian banana industry to be able to view field trials and speak with scientists about the progress that's being made with ways to suppress the spread of the disease and develop disease-tolerant banana varieties.

"The visit will assist with the Australian industry's continuing response to Panama disease tropical race 4."

The trio will also spend time in one of the Philippines' major banana production regions, Tagum, near Davao, as well as the University of the Philippines Los Banos and the Taiwan Banana Research Institute.

'The Tully Times' Thursday, February 18, 2016. Page 13

Industry team heads to Panama disease tropical race 4 hotspots

An Australian banana industry team is travelling to two of the world's Panama disease hotspots for an update on an international project investigating controls for the disease's most serious strain, Panama disease tropical race 4.

Australian Banana Growers' Council Research and Development Manager Rosie Godwin, Queensland Department of Agriculture and Fisheries Principal Nematologist Tony Pattison, and Tully banana grower Patrick Leahy will travel to the Philippines and Taiwan over two weeks, having left on February 13.

The trio will inspect field trials, visit growers, and speak with banana scientists about biosecurity best management practice, the development of disease-tolerant cultivars and suppressive cropping systems that can allow areas infested with the disease to return to export production and ways to increase awareness and knowledge of the disease.

Ms Godwin said the trip was part of an Australian Centre for International Agricultural Research (ACIAR) project on The Integrated Management of Fusarium wilt of bananas in the Philippines and Australia.

Fusarium oxysporum f. sp. cubense is the form of Fusarium which causes Panama disease tropical race 4 in bananas.

"Both Taiwan and the Philippines have for many years been managing the Fusarium wilt incursions in their banana production areas that have a detrimental effect on their small holder producers and export industries," Ms Godwin said.

"It's extremely useful for members of the Australian banana industry to be able to view field trials and speak with scientists about the progress that's being made with ways to suppress the spread of the disease and develop disease-tolerant banana varieties. The visit will assist with the Australian industry's continuing response to Panama disease tropical race 4."

Ms Godwin, Mr Pattison and Mr Leahy will visit one of the Philippines' major banana production regions, Tagum, near Davao, as well as the University of the Philippines Los Banos near Manila.

They will also visit one of the world's major banana plant breeding groups, the Taiwan Banana Research Institute, to discuss varieties exhibiting tolerance to Panama disease tropical race 4.

The ACIAR project began in 2014 and is being led by DAF Queensland in collaboration with the ABGC and Philippine institutes including Bioversity International, Provincial Agricultural Office Davao del Norte and Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development.

TR4... Why Queensland's different

The recent discovery of Panama disease Tropical Race 4 in North Queensland has led to doomsayers predicting the end of the banana industry in Australia. Story by Tony Pattison.

While not discounting the seriousness of the outbreak, there are several factors weighing in favour of the survival of the Australian banana industry, including lessons learnt from outbreaks overseas and the development of international partnerships to help tackle the problem.

The greatest enemy in an outbreak is complacency and denial. This complacency, lack of diagnostic tools, reluctance to admit the presence of the disease and inability to change farming practices is why the disease has spread and continues to spread around the world.

Early detection

There are three main factors in favour of the Australian banana industry being able to manage and survive the outbreak of Tropical Race 4.

The first is early detection and recognition of the disease.

While there is more work to be done determining the extent of the outbreak, the fact that we recognise the disease is present, and have acted, puts us ahead of most international outbreaks of the Tropical Race 4.

The fungal pathogen is easily moved in soil and in planting material, which means that early detection and quarantining can slow the spread of the disease to other areas.

It is still too early to determine if the Tully site where the first detection was made was the original site of infection, but this detection is now contained.

In other outbreaks of TR4, the recognition of the disease has often taken years, either through denial or through lack of diagnostic capabilities, which has meant the disease had spread from the initial site.

On-farm biosecurity

The second factor working in the favour of the Australian banana industry is the knowledge of on-farm biosecurity.

While not every farm may implement on-farm biosecurity, the concept was not new for banana growers.

In outbreaks of TR4 overseas, the concept of restricting the movement of people though farms was difficult or impossible to implement.

Many of the on-farm biosecurity arrangements in place in overseas outbreaks occurred after the disease had reached the farm, with little knowledge of what to do to disinfect machinery and footwear.

The implementation of on-farm biosecurity is now up to individual farms, but there are resources available for Australian banana farmers including checklists, product recommendations, guides and signage. Overseas outbreaks did not have these resources.

Farm practices

The third factor in the favour of the survival of the north Queensland banana industry is the way bananas are grown.

Over the past 20 years there has been a concerted effort to improve the farming practices to reduce inputs on-farm and protect the environment off-farm.

The subtle practices of grassed interrows, reduced soil pesticide applications, lower nitrogen application and soil pH management all contribute to improve the biodiversity in the soil.

In the soil, the Fusarium fungus that causes the Panama disease requires carbon to grow.

The fungus obtains its carbon by infecting the plant, surviving on alternate host plants or undecomposed organic matter in the soil.

pH and organic matter

By increasing the biological diversity in the soil there is greater competition between other soil organisms and the Fusarium fungus. As only one to 10 per cent of soil organisms can be cultured from the soil, the best tactic to improve soil biological diversity is to encourage the organisms naturally occurring in the soil.

By having a neutral soil pH, greater recycling of organic matter (such as banana trash) and an increase in the diversity of different plant species on the soil, each with their own complement of microorgansims around their roots, the competition in the soil for carbon increases.

Soil organisms that compete with pathogens for carbon are usually slower growing, but commonly produce anti-microbial compounds to protect their spot in the soil.

The anti-microbial compounds produced come at an energy cost to the organisms and therefore they tend to be slower growing, requiring stable environments to prosper. This is where the improvements in farming practices in north Queensland become important.

Not the end

Groundcovers in the row, the continual breakdown of banana trash, frequent but smaller fertiliser applications, reduced pesticide applications, good irrigation systems and neutral soil pH, all create a more stable soil environment to promote soil biodiversity.

By contrast, outbreaks of TR4 in commercial plantations overseas have typically occurred where the soil is kept clean of vegetation, farm inputs tend to be higher of both fertilisers and pesticides and soils tend to be more acidic. Increased soil biodiversity will not stop the disease on infected sites, but it tips the balance away from the fungus.

The identification of TR4 in north Queensland is a serious threat to banana production in the region.

However, it will not be the death of the industry as predicted by some, as long as the industry continues to act swiftly to recognise infected plants, isolate infected areas, implement on farm biosecurity practices to reduce the movement of the fungus and improve soil management, with a focus on creating a stable soil environment for a biologically diverse microbial community.

Tony Pattison is Principal Nematologist with the Queensland Department of Agriculture and Fisheries at South Johnstone.

LESSONS FROM THE PHILIPPINES ON TR4 MANAGEMENT

By Tony Pattison

Filipino banana growers differ greatly in how effectively they manage Fusarium Wilt (Panama Disease) of banana.

In a nutshell, the farms that were most effective in managing the disease were typically those with more resources to allocate to the problem. These stark contrasts in disease severity between farms became apparent during a recent visit to Mindanao as part of an Australian Centre for International Agricultural Research (ACIAR) project focussing on integrated management of Panama Disease.

The Philippine banana industry is the second largest banana exporting nation and includes large multinational and national farms that supply around 70% of export bananas from approximately 80,000 ha, with small-holder banana growers or cooperatives, supplying the remaining 30%. The Philippines banana industry was first affected by Tropical Race 4 (TR4) in 2005, but since 2013, the disease has spread rapidly. An estimated 15,000 ha of land is now believed to be infested with TR4 on Mindanao, the country's main production area.

In response to TR4, Philippine growers are now starting to plant GCTCV218 (Formosana), which is a partially resistant cultivar from Taiwan. Critically, our visit revealed stark differences in the success of GCTCV218 on farms with and without effective biosecurity and inoculum management.

Things I learnt about TR4 while in the Philippines

Tony Pattison, Researcher, DAF
"Management of disease inoculum is essential to producing bananas in TR4 infected areas, but we still don't know how to do this effectively"

Stewart Lindsay, Researcher, DAF
"Seeing the different performance for the same resistant Cavendish variety on different farms has reinforced for me that any working solution for growers wanting to keep growing Cavendish is more than just changing the variety"

Paul Dennis, Researcher, University of Queensland
"Growers need more information about how to reduce pathogen load and avoid it building up in the first place"

Jim Pekin, CEO ABGC
"It is essential to keep TR4 fungal levels low in order to profitably grow any currently available and market acceptable banana variety"

Leon Collins, Banana Grower, Tully
"If you are 1000 km away from this disease you are too close"

Irene Kernot, Research Program Manager ACIAR
"Farmers can't afford to follow advice not grounded in good science and that science must provide answers relevant to the whole banana farming system. In some ways the key thing the industry needs is hope that a solution is within reach"