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Final report

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prepared by	Agustin B. Molina
co-authors/ contributors/ collaborators	Catur Hermanto, Tony Pattison, Siti Subandiyah, Winarno and Sukarman
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2 Acronyms

ACIAR	Australian Centre for International Agricultural Research
BAPNET	Banana Asia-Pacific network
BBD	Banana blood disease
BBTD	Banana bunchy top disease
BBTV	Banana bunchy top virus
BMP	best management practice
BPTP	Balai Pengkajian Teknologi Pertanian
CDSI	Corm Disease Severity Index
CTAB	cetyltrimethyl ammonium bromide
CWTA	Centre for Wet Tropical Agriculture
DAFF	Queensland Department of Agriculture, Fisheries and Forestry
DI	Disease incidence
Dinas	Dinas Pertanian (Agriculture Office)
EAHB	East African highland bananas
FDA	fluorescin diacetate
Foc	*Fusarium oxysporum cubense*
Fol	*Fusarium oxysporum* f. sp. *lycopersici*
FW	Fusarium Wilt
GGPC	Great Giant Pineapple Company
ICHORD	Indonesian Centre for Horticultural Research and Development
ICM	integrated crop management
IPM	integrated pest management
ITFRI	Indonesian Tropical Fruit Research Institute
LDSI	Leaf Disease Severity Index
LSD	Least significant difference
LSI	Leaf Symptom Index
NTF	Nusantara Tropical Fruits
PDA	Potato Dextrose Agar
POT	package of technologies
TR4	Tropical Race 4
UGM	University of Gadjah Mada
VCG	Vegetative compatibility group
WSA	water stable aggregates

3 Executive summary

This project aimed at developing science-based disease management approaches of banana wilts, with emphasis on Fusarium wilt caused by *Fusarium oxysporum f. sp. cubense* (*Foc*), to improve productivity and profitability of bananas thereby improving livelihoods of banana growers in Indonesia and Australia. The research activities focused on two major areas. One focused on identifying and adapting known Integrated Pest and Disease Management practices (IPM) through integration with cropping systems approaches. The second focus area was to carry out strategic studies to further build knowledge and capacity that would improve abilities to formulate and implement sound IPM tactics. These included basic and applied studies to better understand the underlying basis of host-pathogen interactions, as in the case of soil-supression in Foc epidemics; and studies on host-pathogen interactions as influenced by virulence of various strains of the pathogen against different *Musa* cultivars. These research activities aimed to fill in knowledge gaps for developing more efficient disease management strategies.

Piloting of ICM/IPM practices

In Indonesia, best disease and crop management practices developed both in previous ACIAR-funded projects[1] were reviewed, identified and subsequently validated through pilot studies. This also including integrating results of other national and regional studies in managing banana wilt diseases and improving productivity Through expert brainstorming/workshop and farmer-participatory appraisal workshops, 15 disease management options were identified and adopted in various combinations by farmer-collaborators in pilot sites in Lampung, Sumatra, and Cianjur, West Java in Indonesia. *Fusarium wilt* Tropical Race 4 (TR4) and banana blood disease (BBD), are the major constraints to banana production in these two areas. Being both soil-borne pathogens, crop and disease management approaches are similarly relevant for both diseases, so the work on Foc will be also applied to controlling BBD. Banana bunchy top disease (BBTD) has also been observed to be an important emerging disease problem. The packages of technologies (POTs) recommended to farmers included the use of healthy planting materials, appropriate land preparation, plant density and planting pattern management, soil fertility and nutrient management, identification and eradication of infected plants, water and drainage management, weed management, and the use of diversified cropping system to reduce disease epidemics.

Farmers adopted different combinations of the above technologies according to their needs and capacities. Results of a rapid participatory appraisal study showed that farmer-collaborators in Lampung had lower knowledge of IPM and ICM technologies and initially adopted more diverse practices compared to farmers from Cianjur, who were more informed and adopting better farming practices. Cluster analyses comparing farmers in Lampung and Cianjur showed that towards the end of the project, farmers in Lampung became more closely clustered in adopting IPM/ICM technology as a result of capacity building along the implementation of the project. It should be noted that capacity building was embedded in the implementation of the pilot studies thus abilities of farmers became dynamic throughout the study. In Lampung, results showed that farmers who use healthy planting materials produced as tissue culture or corm-bits, coupled with fertilization and population management, generated as much as three-fold income increase over non-adopters . These practices were more widely adopted in Lampung because bananas are grown as a major livelihood crop. In Cianjur where average landholdings are larger, and

[1] *"Diagnosis and management of wilt diseases of banana in Indonesia" (HORT/2004/034)*

Mitigating the threat of banana Fusarium wilt: Understanding the agroecological distribution of pathogenic forms and developing disease management strategies (HORT/2005/136)

farmers were growing a wider range of high value cash crops together with bananas results showed that mixed cropping yielded higher income. The level of contribution of bananas to their income is generally lower compared to Lampung farmers but overall household income from farming is higher.The intercropping and use of cultivar diversity in both areas resulted in generally low levels of diseases. This demonstrated the value of genetic and cropping diversity in disease management, and their contribution to a more profitable and sustainable production system.

Conducting such pilot studies proved challenging in terms of methodologies and implementation, but have neverthless resulted in relevant outputs worth validating and outscaling. The generally low levels of disease incidence of both Fusarium wilt and blood disease in the pilot sites is clearly a result of the diversity of both cropping systems and cultivar diversity used by farmers. Increases in yield and income although may have been attributed to lower disease levels as much as to improved production practices such as healthy seedlings, population management, fertilization and mixed cropping systems. The stability of such mixed systems contrasts with less resilient monoculture of Cavendish farms of Nasuntara company in Lampung, where severe epidemics of Foc TR4 continued to destroy their plantations during the 1990s, until they changed to Foc-resistant somaclones.

Research on Foc-soil suppression

In Australia, the project addressed the need to manage *Foc* Race 1 on the popular lady finger variety, based on the hypothesis that increasing certain biological activities of the soil can reduce the incidence of *Foc*. Pot studies have shown that some soil characteristics such as activities of fluorescin diacetate (FDA) and β-glucosidase increased significantly in organic soil, but failed to demonstrate reduction of infection of Foc. Field demo-trials were carried out with lady finger bananas in Timberland, South Johnstone, where the effect of ground covers in modifying both soil-biological-chemical characteristics and *Foc* infection was compared with bare-soil around the base of the plants. Providing plant ground cover around the base of banana plants resulted in lower incidence of *Foc* Race 1, compared with the traditional practice of bare soil. It was found that ground-covered soil suppressiveness to *Foc* infection is related to more biologically active soil, as indicated by the soil nematode enrichment index and β-glucosidase levels.The nematode enrichment index is a measure of the resource available to the soil food web, which is generally considered to be a response of the nematode community to bacteria that can quickly multiply with the availability of nutrients.

The project has generated further evidence to suggest that *Fusarium* wilt can be suppressed by manipulating the soil microbial community and stimulating antagonistic organisms by redesigning the cropping systems, such as the inclusion of vegetative groundcover. The increase in microbial activity under vegetative groundcover confirms the glasshouse experiment results. Increased microbial activity was evident when increasing plant diversity using plant species commonly found as groundcovers in banana plantations. Increased activity of predatory and fungivorous nematodes in the vegetated groundcover treatments, and a significant increase the β-glucosidase activity were both associated with significant decrease in pseudostem discoloration caused by *Foc*. The relationship between β-glucosidase and pseudostem discolouration suggested that *Fusarium* wilt-susceptible bananas could still be part of a cropping system that supported an active and diverse soil biology, which occurs when vegetated groundcover is included into the banana production system.

A complementary study was carried out in Indonesia to understand the basis of soil suppressiveness on Foc Tropical Race 4. Soils from a commercial Cavendish banana plantation in Lampung with records of differential *Foc* disease incidence were characterized. Soil samples from Fusarium wilt ‘hot spots’ versus ‘healthy areas’ were collected and characterized. Comparisons of soil characteristics of these areas provided

insights into the nature of soil suppression, particularly with regard to enhanced biological activity. There is an indication that biological, chemical and physical properties of soils in the rhizosphere of infected plants differ compared to healthy plants, suggesting possible indicators and mechanisms of soil suppressiveness. Higher carbon and organic matter content, a better cation exchange capacity, and particular levels and composition of bacterial populations all seem to be associated with healthy soil. As in Australia, Foc soil-suppression was indicatively related to an increase in β-glucosidase and increase in actinomycete diversity and the presence of sufficient soil manganese and magnesium. This result suggested that there is potential for soil management practices that increase β-glucosidase activity and the number of actinomycetes as well as managanese/magnesium level could help with the suppression of Fusarium in the soil and possibly reduce the expression of Fusarium wilt symptoms. Modifying soil properties through organic amendments and planting intercrops like *Allium spp*. (e.g. Chinese Leek, *Allium tuberosum*) also suppressed *Foc* infection.

Results of these studies highlight the significant potential of pursuing further research towards developing the various elements of soil suppression in search for disease management tactics using biological agents and soil management as part of an integrated disease management approach in mitigating the damage of Foc. Using ground-cover coupled with other cultural practices such as soil microbe-amendments and even nutrition management should be validated in Foc TR4-infested Cavendish-monoculture systems. This may be also adapted for small Cavendish growers.

Host pathogen interaction as influenced by *Foc* strain virulence and cultivar resistance

In Indonesia, 12 strains or vegetative compatibility groups (VCGs) of *Foc* had been identified in the previous ACIAR-funded project. The most frequent isolate identified was VCG 1213/16, the strain commonly reported that is associated with the most virulent of *Foc,* known as Tropical Race (TR) 4. An important research component of the current project was to understand the nature of virulence of these strains with regard to important banana cultivars in Indonesia. This information would provide a basis for risk assessment as well as an opportunity to manage the disease through cultivar use. Screenhouse and field tests were done, providing the opportunity to validate the screening methodologies of screenhouse testings versus field evaluation. Screenhouse results were not always consistent with field evaluation results. Although VCG 1213/16 is generally considered the most virulent across the cultivars, VCG 0124/5 and VCG 0121 (considered Race 1 strains) were observed infecting Cavendish too in the screenhouse with disease incidence ranging from 85-100%. This finding is contrary to a long-accepted understanding that these strains cannot infect Cavendish and are only known as Race 1 Foc strains. This, however, proved that VCG 0124/5 can infect Cavendish and is consistent with data from the reported epidemic of *Foc* TR 4 caused by this strain on Cavendish in India. Mixed inoculation of various VCGs to a susceptible host showed that VCG0124/5 was more aggressive than VCG 01213/16 in certain varieties. One other interesting result of the screenhouse tests showed that Kilita (AAB French plantain) and Pisang Kepok (Saba, ABB) were susceptible to all strains tested under screenhouse conditions. However, field trials where varieties were challenged with VCG 01213/16 showed that Pisang Kepok was resistant while Kilita was susceptible, which was consistent with the screenhouse tests. Horn plantain (Klutoc, AAB) was highly resistant to VCG 01213/16 in the field trial. Few AA diploids showed resistance to most VCGs both under screenhouse and field tests. The results of these host-pathogen interaction studies on strains versus cultivars clearly demonstrate that screenhouse tests may not always be consistent with actual field experiment results. The interaction between cultivars and VCGs also clearly indicate that while TR4 strain VCG 01213/16 is generally the most virulent to most varieties, other VCGs previously designated as Race 1 strains can also infect Cavendish. Some Race 1 strains, like VCG0124/5, are more virulent to other varieties than VCG 01213/16. The

differential responses of the various cultivars to the various VCGs demonstrate the potential of using cultivar deployment in disease management. The work also indicates the limitations of current Foc classification based on the current Race classifications, and thus should be revisited.

Banana bunchy top disease (BBTD) was identified in the previous ACIAR-funded project as another major banana production constraint. A BBTD virus-indexing technique was validated and used to demonstrate that even asymptomatic infected plants can carry the virus and can be potential sources of infection and spread. Some alternate hosts of the virus were also identified. A survey of infection in some banana areas in Yogyakarta was carried out. Results showed that BBTB has increased incidence and distributions based on the survey and characterization carried out. It points to the increased importance of this disease as an emerging constraint to production on small scale and larger farms in Indonesia.

Bioversity recognizes the strategic importance of the Australian-Indonesian relationship, and that *increasing the productivity, profitability and competitiveness of Indonesian horticultural and other high-value plant products* is an articulated priority of the Indonesian Government. Recommended follow-up work should also be consistent with ACIAR's four priority research thematic areas - crops – and contribute both to increasing productivity, quality and market access for agriculture products, and to greater resilience and diversity of production systems.

The final section of the report offers ten recommendations (some of which are highlighted above) aiming to build on both the quality project outputs to date, and the strong relationships that have been developed throughout the project with Australian and Indonesian partners. These recommendations should also link to other related *Musa* regional research in the Philippines, China and beyond, as well as in Africa and Latin America and the Caribbean. Bioversity's regional networks, including both the Banana Asia-Pacific network (BAPNET) and the researcher network ProMusa, will play important roles in the scaling of this work, particularly in the global context of mitigating the threat of Foc TR4. BAPNET will discuss this important follow up work during its upcoming biennial meeting in November 2014.

4 Background

Fusarium wilt and banana blood disease (BBD) have caused major economic damage within the banana growing islands of Java, Sumatra, Sulawesi and Kalimantan. Stands of Kepok, a popular cooking banana cultivar, have been devastated by BBD epidemics. *Foc* TR4 has been attacking Cavendish-types as well as other local cultivars and this virulent strain is spreading pandemically. In their study, Hermanto et al (2008) showed that *Foc* and BBD were responsible for an average loss in banana production of 28%, equivalent to an estimated annual revenue loss of about 1.4 trillion Rupiah (AU$ 185 million).

This poses a serious threat especially as bananas are Indonesia's top horticultural crop and it is planned that they contribute to national development through the National Agriculture, Fisheries and Forestry Rehabilitation Programme. In fact, the Indonesian government targets boosting banana production from 5 million tonnes (2010 production) to over 11 million tonnes by 2025. This they intend to achieve by increasing the productivity of smallholder growers who produce for local markets and by enhancing the quality of export bananas (Hilman, 2008). But with the devastating impacts caused by these twin disease problems on the country's banana production, which trickles down to small-scale banana growers, this Indonesian productivity programme may be jeopardised.

In Australia, Foc Race 1 has been seriously affecting the production of the lady finger variety in the Atherton Tablelands, north Queensland. This disease threat requires effective disease management strategies to avoid major income losses. Disease management strategies are critical for preventing Race 1 from spreading, and they could also serve as pilot studies to help prepare for the possible arrival of *Foc* TR4 in Australian production areas. Effective disease management and biosecurity measures that would address transboundary pathogens (e.g. BBD and *Foc* TR4) in Indonesia would certainly be strategically relevant in reducing the risk of pathogen movement. Understanding the epidemiology and management of these diseases will help prevent disease spread and prepare for managing these pathogens should they reach Australia.

These were the underlying bases in the conceptualization of this follow-up project which builds on the results of two ACIAR-funded projects entitled "*Diagnosis and management of wilt diseases of banana in Indonesia*" (HORT/2004/034) and "*Mitigating the threat of banana Fusarium wilt: Understanding the agroecological distribution of pathogenic forms and developing disease management strategies*" (HORT/2005/136).

In HORT/2004/034, biological control organisms were identified and shown to be effective in suppressing *Foc* under screenhouse conditions but ineffective in field trials. While these antagonists were found to be ineffective at field level, it was considered that there may be soil physical, chemical, and biological factors that affect the biological effectiveness of these antagonists that require further s. In the same study, it was also observed that *Foc* symptoms varied despite a uniform TR4 distribution and the use of same variety, leading to the assumption that the observed difference was due to a suppressive relationship induced by the soil. Hence this initiative to conduct more in-depth studies investigating the relationship between soil health (in relation to biological, chemical and physical indicators) and *Foc* severity. This project tried to harmonise current understandings relating to mechanisms of *Foc* suppression under field situations, and carry out new research which also examines the effect of organic matter and other farm practices for improving soil health relative to *Foc* severity.

In the same project, the relationship between VCG and virulence to host cultivars had also been investigated where preliminary results indicated that VCG1213/16 (TR4) was the most virulent among the 7 VCGs identified in Indonesia. However, there were strong indications of variety by VCG interactions in terms of virulence/susceptibility, thus highlighting the strong potential for the development of a diagnostic tool in the form of host differentiation, and a great opportunity to use this as a basis of cultivar resistance

deployment. The new project brought further the validation of screenhouse results with field trials, with the ultimate goal of developing a useful tool for disease management of *Foc* through logical deployment of cultivar resistance

Under the same project, the socio-economic survey conducted with more than 500 farmer-participants in Indonesia revealed that *Foc* is the major cause of decline in productivity. HORT/2005/136 demonstrated that basic cultural practices such as cultivation, optimum planting densities, optimal fertilization, weeding, and water application as needed are essential to realize the benefits of using healthy plant materials regardless of the variety. Yet these farmers had not used proven agricultural practices known to improve yields, including disease management strategies, like the use of clean planting materials (e.g. tissue culture materials), which were not available. Suckers from other farmers and neighbouring fields were mostly used, which increases the chance of spreading soil- and corm-borne diseases. As such, the project introduced integrated disease management strategies and best-bet management cultural practices in order to improve productivity and income.

In the current project, the main objective was to improve the livelihoods of small-scale banana farmers in Indonesia and the income of banana producers in Australia by improving banana production practices including the effective management of banana wilts. Two main approaches were used to achieve these end: a) **Piloting** best-bet farm management practices and integrated pest management strategies (IPM) and b) **strategic research** that would clarify the roles of key factors that influence disease development and spread of banana wilt diseases, validate relationships occurring between *Foc* VCG/race relationships and confirm the extent of occurrence of an emerging banana disease, BBTD, through rapid and accurate molecular techniques.

5 Objectives

The overall goal of the project was to enhance the productivity and improve the livelihoods of small-scale banana farmers in Indonesia and Australia, and to effectively manage banana wilts through an integrated approach in banana crop production.

Specifically, the project focused on three main objectives:

1. Developing packages of Integrated Pest Management (IPM)/ Integrated Crop Management (ICM) guidelines for rehabilitating and improving the livelihoods of banana farmers.

2. Evaluating and adapting packages of IPM/ICM technologies to develop sustainable and profitable banana production systems; and

3. Undertaking research to refine management practices using IPM/ICM principles.

Objective 1 was carried out through a review-workshop for the (a) identification of relevant IPM/ICM technologies and practices based on previous ACIAR project outputs; (b) farmer-participatory appraisal processes and; (c) expert-review toward the identification of knowledge gaps, and formulation of strategic research areas that generated knowledge to improve the smallholders' ability to manage *Foc* and BBD.

Objective 2 was executed through the (a) implementation and evaluation of banana production best management practice guidelines and (b) analyses of the current constraints to banana supply chains within the two pilot communities.

Objective 3 was accomplished by conducting (a) research to identify and understand the critical control points in the dynamics of banana wilt management focusing on properties and mechanisms of soil suppression and soil health; (b) field validation of screenhouse tests of VCG/race pathogenicity relationships; and (c) validation and confirmation of risk and distribution of BBTD.

This report highlights the research results, impacts and progress that had been made. It also provides recommendations and justification to necessarily extend some activities to come up with a more holistic and comprehensive research analysis.

6 Methodology

6.1 Pilot Studies

Coordinated by Bioversity International's Commodity Systems and Genetic Resources Programme – Asia-Pacific Office, the project involved national partners, local authorities and research institutes in Indonesia and Australia namely, the Directorate General for Horticulture (DGH), with its extension arm, the Balai Pengkajian Teknologi Pertanian NAD (BPTP), Dinas, the Indonesian Centre for Horticultural Research and Development (ICHORD), Indonesian Tropical Fruit Research Institute (ITFRI), University Gadjah Mada (UGM), and the Queensland Department of Agriculture Forestry and Fisheries (DAFF), Australia and 35 farmer-cooperators.

The project started with an Inception Workshop held on August 18-22, 2009 in Bogor Indonesia where project partners from DAFF, ACIAR, ITFRI, GMU, ICHORD, DG Hort and Bioversity discussed the project methodologies, activities, responsibilities and timelines for project implementation. It was in this meeting where the partners agreed on the project pilot site selection criteria, specifically: a) the location's potential to become a major banana-producing centre; b) the site should be one of the buffer zones of Jakarta Special Capital in supporting fruits and foods; c) banana should be an important crop in the area; d) wilt diseases are a major production constraint for bananas in the area; and e) farmers and farmer groups in the area are willing co-operators. In view of these criteria, Serampad Village in Cianjur, West Java and Legundi Village in South Lampung, were chosen for the pilot studies. Also, these sites were chosen based on accessibility issues, in relation to access to extension agents and markets, and the locations' proximity to the site of the previous ACIAR project (Malang), which would provide the opportunity for information exchange between farmers.

In October and November 2009, participatory rapid appraisals (PRAs) were conducted among farmer groups Dinas, Balai Pengkajian Teknologi Pertanian NAD (BPTP) and ITFRI through the use of protocols and prepared questionnaires. The PRAs were conducted to determine local knowledge and existing farmer practices on banana production as well as current farmer needs. Production problems and opportunities were identified and discussed among the various stakeholders (Figure 1). Results of the PRAs were used to formulate plans for the pilot plots in each village among which include the following IPM/ICM options that were deemed relevant: (1) land preparation; (2) banana population management ; (3) crop/cultivar diversity; (4) use of healthy planting materials; (5) nutrient management; (6) soil-water management; (7) early disease monitoring and eradication; (8) plant protection (fruit bagging and deflowering); and (9) quarantine.

At the onset, the farmer-cooperators were presented with different POT options from which they chose the technologies that they agreed to implement on their farms (see annex tables 1&2). The farmers' choice of POTs was based on their capacities to implement such POTs. Some chose more intensive POTs than others. A total of 15 farmer-cooperators were identified from Cianjur and 20 from Lampung. A representative number of non-cooperating farmers from the pilot areas were also identified. On a monthly basis, the ITFRI and BPTP extension technicians visited, interviewed and monitored the farmer-cooperators and verified which management practices they continually apply to their respective farms, cost of inputs, severity of diseases, yield and income from harvests, including income from other crops along with their banana crops, and farm productivity.

In previous ACIAR projects, the farmer-cooperators attributed the relative poor productivity to inaccessibility of knowledge regarding appropriate management practices, alternative crop production practices and disease management tactics. Hence, capacity

building activities were embedded in project activities to build up the farmer-cooperators' knowledge specifically on the best-bet management practices and IPM strategies.

Figure 1. Intensive discussion with farmers, traders, society key person, priest, government officials, extension workers at Legundi

A major component of this pilot study was the provision and use of affordable and sustainable supply of disease-free tissue-cultured planting materials, grown with appropriate cultural practices. Thus, banana seedlings were provided to the farmer-cooperators, coupled with capacity-building activities on the production and care of banana seedlings, as well as cultural practices. Two seedling systems were 'adapted', namely seedlings from bits derived from corms and tissue culture seedlings. The farmers in South Lampung were taught the conventional propagation method of corm bits (Nkakwa and Yemin, 2003).

This activity was put into practice with the establishment of the banana bit nursery in Legundi in 2010. ITFRI, in collaboration with Dinas and the village farmer groups, facilitated a village-level training on corm-bits nursery establishment, from corm selection to seedbed preparation and nursery maintenance. Similarly, farmers were taught nursery management of seedlings derived from tissue culture. Tissue culture meriplants were sourced from ITFRI's tissue culture laboratory.

In Australia, a risk analysis tool was developed for the banana industry to determine the risk of developing Fusarium wilt on banana plantations in the continent. The tool comprised three sections which covered (a) checklist to assess the risk of Fusarium, (b) flow diagram indicating the risk of developing Fusarium wilt at different stages of the crop cycle, and (c) further information on how to manage the risk of *Foc*. The checklist was developed prior to project inception and had been validated in this study in managing *Foc* Race 1 in Australia.

6.2 Strategic Research

Research activities were carried out in Australia and in Indonesia focusing on elucidating the mechanism(s) that may lead to the suppression/promotion of *Foc.* These studies included understanding the role of soil environment—physical, biological and chemical properties of the soil—in disease development. Experiments were also carried out to determine the effects of some farm practices in relation to soil suppressiveness, thus, disease management and consequently, to improve the farmers' production. A study to understand the virulence of various *Foc* strains against various Indonesian banana cultivars was carried out to provide a rational basis for cultivar deployment.

6.2.1 Soil characterisation study to understand soil *Foc*-suppression to refine disease management approaches

A workshop was carried out in UGM on March 2010 with QDAFF and Bioversity researchers participating. A hands-on training on sampling and soil analyses to determine the physical, chemical, and biological properties in relation to soil suppression to *Foc* were conducted. Related protocols in conducting controlled experiments, as well as extent and type of field data to be collected from farmers' fields, were agreed upon.

In Indonesia, soil samples were collected from the small-scale banana farms in Lampung and Cianjur, and also from the commercial plantation of Nusantara Tropical Fruits (NTF) and Great Giant Pineapple Company (GGPC) in Central Lampung where disease severity in their infested fields is known. From 2010 to 2012, soil samples were collected from the rhizospheres of both healthy and *Foc*-infected banana plants.

GMU led the collection of soil samples from the (1) *Foc* infested soils of the private commercial banana plantation of NTF and GGPC, (2) pilot plots in Cianjur, West Java (3) small-scale co-operators and non-cooperators' banana fields in South Lampung and Cianjur and (4) from an institutional farm belonging to Balai Benih (Seed Institute) in Salaman, Central Java. The soil samples consisted of *Foc*-infested soil samples (based on recorded *Foc* incidence) and healthy soil samples (from where healthy plants thrive) taken from the banana plant rhizospheres. A total of 47 soil samples were collected and analysed based on physical, biological and chemical characteristics (pH, labile C, water holding capacity, N, P K, cation exchange capacity, fluorescin diacetate, carbon organic matter, electrical conductivity, β-glucosidase, bacterial density and total Fusarium).

Climate and weather data during the time of soil sampling from the pilot plots in Cianjur, South Lampung and from NTF in Lampung were likewise collected. Historical *Foc* incidence and farmers' practices were also gathered in the areas where the soil samples were collected.

Selection of soil indicators (work from Australia)

This section deals with the development of soil health indicators that were sensitive to farm management practices and development of disease suppression assays. The indicators were tested by conducting a farm survey where soils from five organic and five conventional banana farms were compared in terms of soil health characteristics and suppressiveness or conductivity of *Foc*.

Site selection

The area under survey covered Tropical North Queensland South of Cairns and North of Cardwell, and the Atherton tablelands. Five organic and five conventional banana farms were selected in paired sites. Selection of organic sites was based on willingness of the farmers to cooperate while the number of available organic farms was rather limited. The

conventional sites were selected based on proximity to the organic sites in order to eliminate pedoclimatic variation among sites.

Soil collection for laboratory analysis, tray assays and pot trials

Soil samples were collected using a shovel. Composite samples (*n=15*) were taken from the top 15 cm of soil within 30 cm from the banana plant, in front of the following sucker. Stones and large pieces of organic matter were avoided during sampling. Soil was placed in a bucket and thoroughly mixed with a clean trowel. At each farm, three of the older banana blocks were selected, in most cases plantings older than five years. Only in the case of one organic farm it was not possible to take samples from blocks older than five years because the farm used a rotation system of three years banana, and three years fallow of cattle pasture, cowpea (*Vigna unguiculata*) or lablab (*Lablab purpureus*) and sometimes sweet potato (*Ipomoea batatas*). At this site, samples were taken from a one-year stand, a recently slashed block that had been under bananas for three years, and a fallow block under pasture.

Soil analysis

Collected bulked soil samples were analysed for physical, chemical and biological soil health indicators and samples were sent to a commercial laboratory, Incitec Pivot Ltd. Weribbee, Victoria, Australia, for further chemical analysis. The following soil health indicators were measured at the Centre for Wet Tropical Agriculture (CWTA) laboratory: pH, electrical conductivity (EC), nitrate nitrogen (NO_3–N), labile C, fluorescin diacetate, β-glucosidase, bulk density, water stable aggregates (WSA), soil particle size and nematode community structure.

Bioassays for assessing soil suppressiveness to Fusarium *wilts*

Laboratory hyphal extension test

Hyphal extension was determined by growing *Foc* R1 on collected soil samples on laboratory plates in an incubator (Alabouvette *et al.* 2006). Petri dishes were filled with 30 g of 2 mm sieved air-dried soil which was spread evenly to obtain a smooth surface (approximate bulk density = 1.0 g cm^3). Sterile water (12 mL) was added to each Petri dish. Wetted soils were left to equilibrate for 7 days in the incubator. Using *Foc* R1 cultures on ¼ strength Potato Dextrose Agar (PDA), a 1 cm diameter, 4 mm thick plug from the growing margin of the fungal colony was inverted and placed centrally on top of the soil in the Petri dishes. After one week, the number of plugs that had hyphae growing on the soil surface was recorded. Each week the extension of the mycelium was determined using a binocular microscope and the area of hyphal extension was estimated for a 4-week period. The Petri dishes were maintained at 25ºC during this period. Table 1 shows the rating system that was used to give a single number score (1-5) for extent of hyphal growth.

Table 1. Rating system for hyphal growth of the hyphal extension test

Rating	Criterion
1	5 or less hyphae and hyphae ≤ 1cm
2	More than 5 hyphae and hyphae ≤1 cm
3	5 or less hyphae and hyphae > 1cm
4	More than 5 hyphae and hyphae > 1cm
5	Extensive hyphal growth

The test was replicated five times per soil sample. The area under the (growth) curve (AUC) for each plate was estimated in Genstat (Version 9, VSN International, Hemel Hempstead, UK). The AUC values were then statistically analysed using a one-way-ANOVA and tests of multiple comparison of means (Fisher's protected LSD test with *P*=0.05) to assess differences in *Foc* suppressiveness between collected samples.

Glasshouse bioassays with tomato

Composite samples from the upper 15 cm from selected organic and conventional field sites were used to test the inherent soil suppressiveness to Fusarium wilt using a standardized method as described by Alabouvette *et al.* (2006). According to Alabouvette (1990) and Alabouvette *et al.* (2006), soil suppressiveness to Fusarium wilt is general to *Fusarium oxysporum* and not specific to certain *formae speciales* of the fungus. For practical reasons tomato, susceptible cultivar Tiny Tim, with its associated Fusarium pathogen, *Fusarium oxysporum* f. sp. *lycopersici* R3 (*Fol* R3) was used for the assays which will be described in more detail below.

Polystyrene trays (72 x 20 x 7 cm) with 5 rows of 18 cells where used. Each hole was 4 cm^2 at the top and narrowing down to 5 mm^2 at the bottom allowing for good drainage but preventing soil from dropping out. Per cell 45 cm^3 of soil was added, equating to about 40 to 50 g of soil depending on soil moisture content.

Two methods of inoculation were used; inoculation with spore solution in liquid malt extract, and inoculation with colonised millet seed.

One litre of liquid malt extract was inoculated with one plug of agar from the edge of a *Fol* R3 colony and placed on a rotary shaker (150 rpm) for seven days. After four days the solution was placed under near UV light for five hours to stimulate sporulation and placed back on the shaker. The solution was sieved through a sterile funnel (40 µm) and diluted to$10^{3,}$ 10^4 and 10^5 concentrations. Soil samples of organic and conventional soils were infested with 4ml of malt-based inoculum suspension at concentrations of 1x10^3, 10^4, 10^5 propagules ml^{-1} soil. Controls were given 4 ml of tap water and each treatment was repeated five times. Non-inoculated controls were included for both organic and conventional systems.

Millet seed (500g) was placed in Erlenmeyer flasks, soaked in water for 24 hours and sterilised by autoclaving. The flasks were inoculated with agar plugs of *Fol* R3 from the edge of a colony. The flasks were incubated at room temperature for 14 days and were mixed daily by hand shaking. Colonised millet seed was added to 45 ml of soil at rates of 0.5, 1.0 and 1.5 grams of inoculum and thoroughly mixed with approximately 50 g of soil. Each treatment was replicated five times. Non-inoculated controls were included for both organic and conventional systems.

One-week-old susceptible tomato cultivar (Tiny Tim) transplants were planted into the tray cells. Plants were kept in a greenhouse under controlled conditions with air temperatures maintained at 27-32ºCelsius, 70-80% relative humidity, and 12h sunlight per day throughout the experiment. Plants were watered once a day.

Disease progress was recorded twice a week from day 21 to 58 after sowing. The following rating system was used:

1= healthy plant,
2= wilting of lower leaves,
3= whole plant affected and
4= plant dead/ terminal wilting

Since the tests were done with unsterilised soil from various farms, PDA-plate isolation was done from the roots of each dead plant to determine whether death was caused by *Fusarium* or by another pathogen. At the termination of the trials, in case there was no

plant death, diseased plants were used for isolation. From three paired sites, also healthy plants were used to make isolations in order to check whether *Fol* R3 was absent.

Dead, diseased and healthy plants were taken from the trays and the roots and lower stems were carefully washed. Roots and stem were dipped for 30 seconds in 1% bleach solution to remove fungi and bacteria growing on the surface of the plant. Then, bleach was washed off in sterilised water. The roots were then cut into small (< 0.5 cm) sections with a scalpel. A representative part of this root material was taken and divided up into 5 parts. Then, 5 small pieces were cut from the lower stem. The root and stem material was then placed on ¼ strength PDA plates with a division between roots and stem and placed in an incubator at 27ºC. When the colonies were approximately 1 cm in diameter the plates were taken from the incubator, taped with parafilm and placed under UV light at 23ºC. The plates remained in the incubator until the cultures showed sufficient morphological characteristics to be identified.

The AUC for each plant was estimated in Genstat. The AUC values were statistically analysed using a one-way-ANOVA and multiple means comparison (Fisher's protected LSD test with *P*=0.05). Disease, growth and soil health indicators were tested on two-sided correlations. Principle component analysis was carried out on the disease indicators.

Pot trials

A pot trial with bananas was carried out to determine whether the bioassays for assessing soil suppressiveness to *Fusarium oxysporum* (using *Fol* and a susceptible tomato host) indeed gave an indication of soil suppressiveness to *Foc* R1. For this trial, the paired sites with the most and the least disease suppressiveness to *Fol* at four weeks post-inoculation were selected.

As this trial was set up before the bioassays with tomatoes were finalised, selection was based on disease progress at 34 days post inoculation. For each tomato plant the AUC value was estimated in Genstat. The AUC values were then analysed using a one-way-ANOVA and multiple means comparison (Fisher's protected LSD test with *P*=0.05).

Soil samples collected from all three fields of the four selected farms was used, and each field had one inoculated treatment and a control. Each treatment had two replicates, making 48 pots in total. The pots were randomised in a split plot design.

Soils were inoculated with *Foc* R1 colonised millet seed. Millet seed (500g) was placed in Erlenmeyer flasks, soaked in water for 24 hours and sterilised by autoclaving. The flasks were inoculated with strips of *Foc* R1 infected banana (var. Ducasse) pseudostem. The flasks were left at room temperature to incubate for 14 days and were mixed daily by hand shaking.

Approximately 1.5 kg of soil was weighed and placed in plastic bags and 10 g inoculum was added to the soil in each bag. After thorough mixing by hand, the soil was placed in 2L pots. Control pots were filled with 1.5 kg uninoculated soil.

Tissue cultured Ducasse (*Musa* AAB) banana transplants were carefully taken out of their pots and the roots manually cleaned and washed from potting mix and placed in the pots. The pots were placed on trays, and soil moisture content was kept constant by filling the bottom of the trays with water. At five weeks after planting, the trays were left dry for 24 hours to create more favourable circumstances for symptom expression. Each pot received 5 ml of organic liquid blood and bone meal (dilution 1/100) once a week.

Disease progress was recorded once a week from day 21 to day 58 post-inoculation. Plant wilting was recorded following a rating system of 1-5 developed by INIBAP (Orjeda, 1998), where:

1= plant healthy
2= slight yellowing or wilting of lower leaves
3= extensive yellowing or wilting of lower leaves
4= yellowing or wilting of most or all of the leaves
5= plant dead

Leaf emergence and growth were measured once every two weeks. At the end of the trial, the leaf area of the last fully emerged leaf was estimated as described by Turner (1972), and the corm was dissected to assess vascular discolouration on a rating of 1-6 according to the INIBAP guidelines (Orjeda 1998), where:

1= rhizome completely clean
2= isolated points of discolouration
3= vascular discolouration of up to 1/3 of rhizome
4= discolouration affects between 1/3 and 2/3 of rhizome
5= greater than 2/3 of rhizome discoloured
6= total discolouration rhizome vascular tissue

At termination of the trial, the number of discoloured roots was counted from a random sample of five roots. Soil was washed off from roots and corm and roots, corm and leaves were separately dried in a drying oven for seven days at 75ºC.

The AUC for each plant was estimated in Genstat. The AUC values were statistically analysed using a one-way-ANOVA and multiple means comparison (Fisher's protected LSD test with P=0.05). Disease, growth and soil health indicators were tested on two-sided correlations. Principle component analysis was carried out on the disease indicators.

Paired Site- Australia

The banana fields were assessed in consultation with the growers and using the rating scale of 0-5 described in Table 2.

Table 2. Severity rating scale for banana fields assessed for symptoms of Fusarium wilt

Score	Description
0	No symptoms present. *Foc* absent (0) or no confirmation of the presence of the disease.
1	Individual plants show FW symptoms which do not persist throughout the season, suckers tend to grow out of the disease.
2	Individual plants with the symptoms persisting, the following sucker shows symptoms but the disease does not seem to spread.
3	Small clumps of plants showing symptoms, which persist throughout the year, but the disease seems slow to spread.
4	Small clumps of plants showing symptoms, which persist throughout the year and the disease appears to be progressing rapidly.
5	Large areas devastated by the disease which is rapidly progressing.

Soil sampling was conducted by collecting soil with a 50 d mm soil core to a depth of 100 mm at 20 locations within a banana field. Physical soil parameters, sand, silt and clay and bulk density were determined for the soils. Soil chemical measurements were conducted by a commercial laboratory (IncitecPivot) and included Organic C, pH, EC, NO_3-N, P, PBI,

K, Ca, Mg, Na, Cu, Fe, Mn, Zn and SO4. Biochemical analysis of soil samples were conducted at the DAFF, Centre for Wet Tropics Agriculture and Ecosciences Precinct and included soil enzymes, labile C and soil nematode community analysis (pH, EC, NO_3-N, FDA, β-glucosidase, Labile C, nematode trophic groups (parasites, fungivores, bacterivores, omnivores, predators), diversity, enrichment, structure and channel indices).

Samples and field assessments were conducted in October 2010 and again in October 2011. October was the period when Fusarium wilt symptoms were typically most severe. A VCG analysis of the isolates taken from each field sampled for confirmation of the disease revealed that one of the sites had a different VCG which was a group linked to race 4, where as the other VCGs were typical of race 1 of Fusarium wilt. Therefore, the site at Mol was disregarded from further analysis, leaving 7 sites all growing lady finger bananas (*Musa* AAB), with the VCG 0124.

Pathogen virulence and cultivar resistance studies (Indonesia)

Virulence studies of the various VCGs of *Foc* to different Indonesian cultivars were carried out at the screenhouse and on the naturally VCG 01213/16-infested field of ITFRI. Selected cultivars representing important genomic groups (see below) were inoculated with *Foc* VCGs that were collected and characterised in previous ACIAR projects. Eight VCG groups (0120/15, 0123, 0124/5, 0126, 01218, 0121, 01219, and 01213/16) were used to challenge 11 cultivars. Two-month old *in vitro* meriplants were carefully removed from the media to avoid root damage and washed with running tap water. Those with healthy white roots were selected for inoculation by dipping the roots of the meriplants in 10^6 conidia suspension for 5 minutes. Inoculated plantlets were planted in 220 ml plastic cups containing sterile sand and watered with liquid compound fertiliser (Hyponex). Each cup (with a plantlet) was then placed into another empty plastic cup (double cup method) to collect the excess water and thus prevent the spread of *Foc* infected soil from the inoculated.

Cultivars included were: (1) Berlin (AA); (2) Calcutta (AA); (3) Kilita (AAB); (4) Klutuk Awu (BB); (5) Barangan (AAA); (6) Ambon Hijau (AAA Cavendish type); (7) Ambon Kuning (AAA Gros Michel); (8) Ketan (AAB); (9) Perancis (ABB); (10) Kepok (ABB Saba); and (11) Tanduk (AAB Plantain). The virulence of the various VCGs vis-a-vis the resistance of the various cultivars was evaluated to identify potentially resistant cultivars to specific VCGs, which could then be important in cultivar deployment as a means of disease management, and also for the development of a tool using differential host cultivars for diagnostics of *Foc* Races.

Validation of this study was done in the field using the same set of cultivars, testing against TR4, the VCG 1213/16. Field evaluation was conducted in naturally *Foc* TR4 infested soil on the ITFRI Experimental Farm, Solok, West Sumatra. The experiment was set up following the randomised complete block design with 12 treatments (11 cultivars, plus uninoculated Pisang Hijau, as the control), with 3 replicates having 10 plants per replicate. To ensure *Foc* TR4 infection, hardened banana seedlings for field evaluation were inoculated with VCG 01213/16 before planting. Visible symptoms of *Foc* TR4 infection such as leaf yellowing, pseudostem splitting, petiole buckling and wilting were being observed among the test plants.

The following data were taken for both the screenhouse and field evaluation experiments:

(1) Incubation period: Observation was taken from the first external symptom, yellowing on the leaf margin. Incubation period is calculated from the time of inoculation to the first appearance of external symptom.

(2) Disease severity data was based on -- Disease severity on the leaf using the Leaf Symptom Index (LSI) and Corm Disease Severity Index (CDSI)

(3) Disease incidence (DI) was calculated as percentage of plants showing *Foc* symptoms against the total experimental plants in a treatment

7 Achievements against activities and outputs/milestones

Objective 1: To develop a package/(s) of sustainable and operational Integrated Pest Management (IPM)/Integrated Crop Management (ICM) guidelines for rehabilitating and improving the livelihoods of banana farmers

No.	Activity	Outputs/milestones	Completion date	Comments
1.1	Identification of relevant IPM/ICM technologies and practices based on previous ACIAR project outputs, other related research, and farmer participatory processes (Ind/Aus)	1.1.1: Hazard analysis tool and best management practice (BMP) guidelines from previous and current knowledge of banana wilt management	August 2009	A risk analysis tool was developed in Australia for the banana industry to determine the risk of developing *Foc* on banana plantations in Australia. The tool comprises three sections: (1) checklist to assess the risk of *Fusarium*; (2) flow diagram of the risk of developing *Fusarium* wilt at different stages of the crop production cycle; and (3) further information on how to deal with and manage the risk of *Foc*. The checklist was developed and reviewed by industry experts. The draft of the publication was presented in the Australian Banana Growers Congress and final publication.
1.2	Identification of knowledge gaps and formulation of strategic research areas that will generate knowledge to improve smallholders' ability to manage *Foc* and BBD (Ind/Aus)	1.2.1: Report detailing strategic research areas to be investigated by the project	August 2009	**Australia** A report detailing areas of research was incorporated in the risk analysis tool. In Australia, a plant protection plan was developed for the Australian banana industry through the Australian Banana Growers Council, incorporating areas of research. **Indonesia** The following important management practices were identified and applied for banana production in the two pilot sites in Indonesia. These 'Must Do' practices were: (1) land preparation; (2) banana population management; (3) crop diversity; (4) use of disease-free planting materials; (5) nutrient management; (6) soil-water management; (7) early disease monitoring and eradication; (8) plant protection; and (9) quarantine (prevention of spread). 'Should Do' practices identified were: (1) use of resistant variety; (2) site selection, (3) weed management; (4) application of biological control; (5) vector management; and (6) management of other insect pests.

PC = partner country, A = Australia

Objective 2: To evaluate and adapt packages of IPM/ICM technologies in order to develop sustainable and profitable banana production systems

No.	Activity	Outputs/ milestones	Completion date	Comments
2.1	To carry out research to identify and understand the critical control points in the dynamics of banana wilt management focusing on properties and mechanisms of soil suppression and soil health (Ind/Aus)	2.1.1: Protocols developed in conducting research on suppressive soils, soil health and use of biological control agents	December 2009	**Australia** A protocol manual was developed for testing soil for suppression to *Fusarium* wilt. The protocol manual details the tests used for soil characterisation including physical tests (texture and water aggregate stability; chemical test: pH, EC, nitrate nitrogen, and labile C), biological tests (nematode diversity, fluorescein diacetate hydrolysis, β-glucosidase activity); and *Fusarium* fungistasis and a small plant test for suppression of *Fusarium*. **Indonesia** Trainings were conducted among the research staff and students of UGM on the methodologies based on the protocol manual developed by the Australian partners.
		2.1.2: Understanding the guidelines of *Foc* management using risk analysis tool	March 2013	**Australia** A risk analysis tool was developed in Australia for the banana industry to determine the risk of developing *Foc* on banana plantations in Australia. The tool comprises three sections: (1) checklist to assess the risk of *Fusarium*; (2) flow diagram of the risk of developing *Fusarium* wilt at different stages of the crop production cycle; and (3) further information on how to deal with and manage the risk of *Foc*. The checklist was developed and reviewed by industry experts. The draft of the publication was presented in the Australian Banana Growers Congress and final publication.

No.	Activity	Outputs/ milestones	Completion date	Comments
		2.1.3: Use of identified biological control agents optimised	March 2013	**Australia** The increase in cellulolytic organisms consistently scored highly on key soil health indicators: pH, FDA, labile C, nematode diversity and proportions of fungal feeding and plant-parasitic nematodes. **Indonesia** From the survey conducted at NTF, Lampung, only one isolate was consistently suppressive to *Foc*. The antagonist isolate was identified based on the sequence of the 16S rDNA and by Blast analysis, which was highly similar at 98% homology with *Pseudomonas fluorescens*. An experiment on the different possible biocontrol agents against *Foc* was conducted in UGM using the isolated endophytic bacteria, chitinolytic Trichoderma and chitosan using the Barangan cultivar. New soil samples were collected from the pilot sites in Cianjur, and farmers in all sites were taught soil management and cropping practices. New soil samples were also collected from Lampung (NTF and GCPC). The following were analysed from these samples: soil pH, electrical conductivity (EC) and labile C. Other physical and biological characterisations (Fungistasis Assay, Fluorescein Diacetate, β-Glucosidase) of previous and new soil samples are still being conducted.

No.	Activity	Outputs/ milestones	Completion date	Comments
		2.1.4: Progress reports detailing some soil characterisations that may relate to soil suppression and soil health on wilt diseases	March 2013	**Australia** A field experiment was implemented in a highly infested field with *Foc* growing a susceptible banana cultivar. Four management systems were employed on the site, (A) "aspirational" practices, (B) "best practice" practices, (C) conventional practices and (D) "detrimental" practice (practices thought to encourage disease development). A and B used interplant vegetative plant covers, while C and D had bare soil. In an assessment conducted in May 2012, greater *Foc* incidence was observed in the farms with C and D practices relative to the A and B practices. There were significant changes in soil parameters, increased CEC, FDA, fungivores and β-glucosidase related to a decrease in disease severity and an increase in bunch weight. This suggested that general suppressiveness increased under the vegetative ground covers promoting *Foc* antagonists and possibly an increase in plant tolerance to *Foc*, reducing disease severity and allowing harvestable banana bunches. A survey of 52 banana farms was conducted, which included sites growing Cavendish, Ducasse and lady finger banana cultivars, using climatic and soil variables that could determine the optimal combination of these variables where banana cultivars were growing, selected from a stepwise discriminant analysis. The analysis demonstrated that lady finger bananas were more likely to be grown in zones with greater heat and drought stress and soils with greater clay, Al, Mg and Na content. However, the stepwise discriminant analysis could not accurately allocate lady finger sites based on the severity of *Foc* symptoms either absent, mild or severe. A site was identified as having Fusarium wilt suppressive soil and was confirmed in pot assays. Further analysis of the sites was carried out to determine the soil factors that are related to Fusarium wilt suppression.

No.	Activity	Outputs/ milestones	Completion date	Comments
		2.1.5: Progress reports detailing some soil characterisations that may relate to soil suppression and soil health on wilt diseases	March 2013	A glasshouse experiment was conducted to determine if infection by plant-parasitic nematodes could hasten the development of *Foc* wilt symptoms in susceptible plants or overcome resistance in non-susceptible cultivars. The experiment demonstrated that plant-parasitic nematodes did not increase the susceptibility of bananas to the development of *Foc*, as the resistant Cavendish cultivars were not infected with Fusarium Race 1 in the presence of high populations of plant-parasitic nematodes.
2.2	Field validation of screenhouse tests of VCG/race pathogenicity relationships (Ind)	2.2.1: Field resistance evaluation of selected Indonesian cultivars against VCG 1213/16 (TR4)	December 2009-March 2011	**Indonesia** Field evaluation was implemented in naturally *Foc* TR4 infested soil in Aripan Experimental Farm, Solok, West Sumatra. The experiment was set up following the randomised complete block design with 12 treatments (cultivars), with 3 replicates having 10 plants per replicate. To ensure *Foc* TR4 infection, hardened seedlings for field evaluation were inoculated with VCG 01213/16 before planting. The following were the cultivars used for the field evaluation: (1) Berlin (AA); (2) Calcuta (AA); (3) Kilita (AAB); (4) Klutuk Awu (BB); (5) Barangan (AAA); (6) Ambon Hijau (AAA Cavendish type); (7) Ambon Kuning (AAA Gros Michel); (8) Ketan (AAB); (9) Perancis (ABB); (10) Kepok (ABB Saba); (11) Tanduk (ABB Plantain); and (12) Ambon Hijau (control- no *Foc* TR4 inoculation). Visible symptoms of *Foc* TR4 infection such as leaf yellowing, pseudostem splitting, petiole buckling and wilting were assessed among the test plants. As of May 2012, all cultivars were already infected except Berlin (AA) and Klutuk Awu. The VCG that infected each variety was identified as 01213/16, except for Raja Kinalun which was infected by VCG 0124/5. Tanduk variety was also infected by Fusarium with 324 days incubation, however the VCG that affected the cultivar is still unknown. Highest disease incidence was observed on Ambon Hijau (60%), Kilita (46%), and Barangan (43%).

No.	Activity	Outputs/ milestones	Completion date	Comments
2.3	Validate and confirm mitigation strategies for the management of BBTD (Ind)	2.3.1: Laboratory experiments to conduct virus indexing and field trials to evaluate mitigation strategies for BBTD established; development of diagnostic protocol for BBTD	March 2010-May 2012	**Indonesia** BBTV samples were collected from the provinces of Yogyakarta, Central Java, West Java and Lampung. Five isolates were collected from Yogyakarta and three isolates from Central Java. DNAs of infected banana plants collected were extracted using two techniques: (1) DNA extraction kit and (2) CTAB manual technique. Both techniques resulted in good amplification of BBTV. Two primer pairs were used: S-CRF & S-CRR and C1-CRF & C1-CRR. The amplification using the primers of common region of BBTV or ABTV (S-CR primers) and the specific primers for DNA-R BBTV (C1-CR primers) suggested that all samples tested were infected by BBTV not ABTV. Based on the healthy banana leaf samples, it was found, using PCR, that plants which were seemingly healthy/ free from symptoms (symptomless bananas) of banana bunchy top disease may still be infected with BBTV. This demonstrated the importance of the use of tissue cultured banana planting materials to prevent further spread of the virus in the above named areas. Another finding was that, several plant species normally growing alongside banana plantations (i.e. Zingber officinale, Alpinia galanga, and Heliconia psittacorum) were host plants of *Pentalonia sp*.-- the vector of BBTV. It was also found that *Curcuma domestica*, *Strelitzia reginae*, and *Colocasia sp* are the host of *Pentalonia sp* for survival but not for multiplication of aphids.

Objective 3. To undertake research to refine management practices using IPM/ICM principles including the identification and understanding of critical control points for Foc, BDB and BBTD management and field validation of Foc VCG/Race relationship as diagnostic tool for disease management (Ind/Aus)

No.	Activity	Outputs/ milestones	Completion date	Comments
3.1	Implementation and evaluation of banana production best management practice guidelines (Ind/Aus)	3.1.1:Appropriate pilot sites and farmer- participants selected 3.1.2: Clarification of actual farmer practice and farmer needs	November 2009	**Australia** Surveys among banana growers in north Queensland and northern NSW cultivating banana cultivars susceptible to *Foc* were conducted. A demonstration site and field experiment was established on a highly infested field with Ducasse, a highly susceptible cultivar in north Queensland. Field day demonstrations were organized featuring management practices with high farmer participation and national media coverage. **Indonesia** Pilot sites were identified in Indonesia: Legundi Village, Lampung and Serampad Village, Cianjur. Fifteen (15) farmers were selected to participate in the pilot studies in Cianjur, and 20 in Lampung. The management options were the following: (1) land preparation; (2) banana population management; (3) crop diversity; (4) use of disease-free planting materials; (5) nutrient management; (6) soil-water management; (7) early disease monitoring and eradication; (8) quarantine (prevention of spread).

No.	Activity	Outputs/ milestones	Completion date	Comments
		3.1.3: Appropriate data collected, analysed, documented; and a model of sustainable crop management developed. 3.1.4: Report on the BMP implemented and adopted by farmers	May 2013	**Australia** A field experiment was implemented in a highly infested field with *Foc* growing a susceptible banana cultivar. Four management systems were employed on the site, (A) "aspirational" practices, (B) "best practice" practices, (C) conventional practices and (D) "detrimental" practice (practices thought to encourage disease development). A and B used interplant vegetative plant covers, while C and D had bare soil. In an assessment conducted in May 2012, greater *Foc* incidence was observed in the farms with C and D practices relative to the A and B practices. There were significant changes in soil parameters, increased CEC, FDA, fungivores and β-glucosidase related to a decrease in disease severity and an increase in bunch weight. This suggested that general suppressiveness increased under the vegetative ground covers promoting *Foc* antagonists and possibly an increase in plant tolerance to *Foc*, reducing disease severity and allowing harvestable banana bunches.
3.2	Identification of constraints to banana supply chains within the two pilot communities (Ind)	3.2.1: Participatory surveys and suppliers/trader/ consumer analyses undertaken to identify constraints occurring within the banana supply chains	May 2012	**Indonesia** Information gathered in relation to supply chain were fed back to the Indonesian Centre for Agriculture Socio-Economic and Policy Studies (ICASEP) for the development/ implementation of solutions to identified constraints. In collaboration with ICHORD, surveys were conducted in Cianjur and Lampung to interview farmers, local banana consolidators, traders and also banana processors to identify gaps in the banana supply chain. Interviews were also be conducted among the buyers of the banana fruits and banana chips in major cities such as Jakarta, Bogor, Lampung and Cianjur.

No.	Activity	Outputs/ milestones	Completion date	Comments
3.3	Establishment of two pilot sites that applied the BMP guidelines developed in Objective 1	3.3.1: Pilot sites developed; lead farmer-participants identified and, together with extension workers, trained; Identified lead farmer-participants that underwent 'skilling-up' to ensure transfer of management practices to other smallholders involved in banana production	June 2010-2013	**Indonesia** The pilot studies in two selected sites in Indonesia were established. The PRAs conducted in these villages increased awareness among farmers on the production concerns and corresponding management strategies that are included in the pilot studies. As a design of the pilot studies, farmer-participants implemented varied options. Some agreed to implement the complete range of options while others selected what were appropriate and feasible for them. These variations were recorded as a methodology of the experiment. In Cianjur, a total of 20 farmers participated in the study. Three farmers chose to apply the complete options. In Lampung, 20 farmers participated in the study. Other farmers who did not participate (non-cooperators) in the project were also interviewed and their farms were also monitored as a control. Some farmers integrated banana production with several management options in their banana farms mix-cropped with maize, pepper and other vegetables. The management options were the following: (1) land preparation; (2) banana population management; (3) crop diversity; (4) use of disease-free planting materials; (5) nutrient management; (6) soil-water management; (7) early disease monitoring and eradication; (8) plant protection (fruit bagging and deflowering); and (9) quarantine (prevention of spread). The major component of the intervention was the use of healthy banana seedlings grown in good agronomic practices such as appropriate land preparation, fertilisation and population management. Two seedling systems were introduced in both locations: TC and corm-bits seedling production. Farmers were taught on nursery management.

No.	Activity	Outputs/ milestones	Completion date	Comments
				Indonesia Capacity-building of farmer-participants was an integral element in the conduct of the pilot studies. The farmers were trained on the various technology interventions included in the study. For instance, they were taught how to take care of and grow TC seedlings, and the conventional method of banana propagation through bits from corms. ITFRI, in collaboration with Dinas and the village farmer groups, facilitated village level trainings on corm bits nursery establishment from corm selection to seed bed preparation and nursery maintenance.

No.	Activity	Outputs/ milestones	Completion date	Comments
3.3	Establishment of two pilot sites which will apply the BMP guidelines developed in Objective 1		June 2010-2013	**Indonesia** The banana bit nursery in Legundi was established in 2010. Farmers of Cianjur were also trained to handle tissue culture seedlings and nursery management. Collaborating farmers in both Lampung and Cianjur were trained by ITFRI staff - 'hands-on'- to hasten skills and technologies in banana production and disease management as the plants were growing in the farmers' fields. Four-block demo plots were also established in Cianjur to demonstrate to farmers the effect of the use of tissue culture seedlings versus suckers and to demonstrate other banana disease management techniques. Collaborating farmers in Cianjur visited the site every two weeks for training and discussions with the BPTP extension workers. The blocks are described below: *Block 1* was cleared of old banana plants and planted with tissue culture cultivars Tanjung (budless Kepok variety), Jawaka, Kinalun and suckers of Tanduk; The block was inter-cropped with maize. *Block 2* was cleared of old banana plants and planted with tissue culture Ambon Kuning and was inter-cropped with maize. *Block 3* was planted with suckers of Raja Bulu and the old banana plants were maintained. It was inter-cropped with maize. *Block 4* was planted with suckers of Raja Bulu and the old banana plants were maintained. It was intercropped with tea and coffee.

No.	Activity	Outputs/ milestones	Completion date	Comments
3.4	Follow-up and monitoring of pilot study sites	3.4.1: Appropriate data collected, analysed, documented and a model for sustainable crop management developed 3.4.2: Report on the BMP implemented and adopted by farmers	November 2009-May 2013	**Indonesia** The activities of collaborating farmers were regularly monitored and recorded by the ITFRI staff, Dinas and BPTP representatives. It was observed that not all management options were being implemented as agreed because of some limiting factors such as the lack of rainfall (as farmers of Lampung depend mostly on rain for crop irrigation) and farmers' preference to the use of suckers/ bits over tissue culture seedlings. However, some management practices taught to the farmers were mostly implemented. Actual management practices were recorded and set as bases of yield and income analyses. ITFRI staff regularly visited the pilot sites (every 2 months) to monitor the actual activities of farmers in their field. All cultural practices employed by farmers (land preparation, irrigation, fertilization, application of organic matter etc.) and all crops being planted by farmers alongside bananas (mixed cropping - corn, chilli, vegetables etc.) were regularly recorded.

8 Key results and discussion

8.1 Pilot Studies

The pilot studies were carried out in two locations, Cianjur in West Java, and Legundi in South Lampung, Sumatra (Annex Table 1 and 2). Data gathering was completed in the last quarter of 2013.

8.1.1 Farm and farmer profile of co-operators

Annex Tables 3 and 4 shows a summary of information among the cooperators and their respective farms in both pilot sites. On almost all counts, cooperators in Cianjur can be said to be more 'resource-rich' and advanced compared to those in Lampung.

In Cianjur, co-operators have an average land area ranging from 0.12 to 3 hectares per household. Each co-operator has an average of only 894 banana plants/ ha. Average land area per farmer is 8,800 m^2. Based on the survey, a farmer gets an average annual income of Rp 5.2 million of which 15% comes from banana farming. Other than banana production, most common village cropping system focuses on other high value crops such as vegetables and other fruits.

In Legundi, the average land area of co-operators ranges from 0.025 to 0.3 hectares per household. Co-operators planted in an average of 1,200 m^2 land area with very high density planting, such that 5000 plants may be found in a hectare of land. The average farmer income per year is Rp 2.8 million, of which farmers attribute 52% of this income to banana farming. The cropping system in the village is characterized as mainly banana but mixed cropped with maize, pepper and other vegetables.

Preliminary analyses showed that co-operators in Cianjur earn more income than the farmers in Lampung (Annex Table 3) which may be due to their having bigger land area, more fertile soil and use of crop diversity (e.g. high value crops such as vegetables). The farmers in Cianjur are more knowlegable of production technologies involving diversed cropping systems than their counterparts from Legundi,lampung. In Lampung, the share of banana production of the family income of co-operators was greater (52%) than those in Cianjur (15%).

8.1.2 Disease status in the pilot sites

Banana wilts (Fusarium wilt and blood disease) and BBTD were the most common banana diseases observed in the pilot sites. Compared to the total number of cultivated bananas in both areas, the observed disease problems were considerably low (Annex Table 4). Of the average number of cultivated banana plants per average land area in Cianjur, only 6% and 3% reportedly manifest wilt disease and BBTD, respectively. In Lampung, of the 643 banana plants per 1,200 m^2 average land area, reported disease incidences are very low at 2% and 1% to wilt disease and BBTD, respectively. Perhaps the farmers were already implementing eradication as a result of earlier informal meetings. Although disease incidence was relatively low in both sites, the risk of disease epidemic still exists because soil inocula are still present; thus disease management options still need to be considered in these pilot studies. Similarly, BBTD can potentially spread since the most common cultivar planted in the pilot sites is Pisang Berlin, a cultivar that is very susceptible to the banana bunchy top virus (BBTV). Also, most farmers in the

area use suckers and bits from possibly infected corms/ mother plants, which provides another avenue of disease spread.

8.1.3 Evaluation of farmer technology adoption

At the start of the project, the co-operators in the pilot sites selected a number of disease management options (Annex Tables 1 and 2) to employ in their farm, depending on their capacity and needs. Accordingly, the co-operators were trained on the various management options available to them.

Based on the PRA results, the Cianjur co-operators were more knowledgeable on banana farming than those in Lampung. Out of the 15 co-operators, three (20%) agreed to employ all the 15 farming practices. However, during project implementation, only 1-3 of the 15 management options were actually applied by the co-operators, indicative of low adoption.

In Lampung, 20 farmers initially agreed to participate in the pilot study and four (20%) committed to implement all of the 15 farming practices. During project implementation, of the 15 suggested management options, 40% of the options were actually implemented.

Annex Figure 3 further illustrate the Cianjur co-operators' technology-adoption behaviour. Figures show the similarity among the technologies applied by the farmer co-operators during the first months of project implementation. Only two major groups were identified and four outliers, these include two champion farmers, namely Deny and Harun. Figure 5a illustrates the group of farmer co-operators and non-cooperators. The following technologies were common to almost all farmers: utilization of sucker and bit, implementation of an annual cropping system and specific row arrangements, application of biofumigation, control of BBTD and other pests, and deflowering and bagging.

Annex Figure 3 and 4 show the natural adoption preferences of the farmers to the suggested technologies for banana production. Five groups were generated from the selected farmers' practices such as: integrated control, eradication, tool sterilization, ploughing, desuckering, row arrangement, control of BBTD, fruit bagging, weed management and the use of suckers. Figure 5b shows that both farmer co-operators and non-cooperators were implementing the suggested banana production technologies in Cianjur.

Annex Figure 5 illustrates the farmer-cooperators' and non-cooperators' adoption behaviour of suggested banana production technologies in South Lampung. Annex Figure 5 shows more dispersion in technological adoption which means that co-operators in Lampung applied differing farmer practices at the start of the project. Farmers in the village have very limited knowledge on banana farming technologies. These farmers initially implemented eight banana production techniques namely: site selection, utilisation of bit planting material, crop rotation, irrigation and drainage management, weed management, and wilt disease control. Complemented with capacity building activities by ICHORD and ITFRI staff, the figure shows more converged clustering on the co-operators' farming practices indicating that during project implementation, they tended to adopt similar practices. These farming practices that have become common to most co-operators were using suckers for planting, planting in rows and using a range of banana cultivars.

Annex Figure 6 shows the clustering of cooperating farmers and non-cooperating farmers at the beginning of the project implementation. Farmers 15 to 22 (non-cooperating) clustered with the rest of the cooperating farmers, showing similar practices among farmers at the beginning of the project. Figure 5b shows the evident grouping of the

cooperating and non-cooperating farmers. The gap indicates the farmer-cooperators' application of suggested technologies in contrast to the non-adopters of the technologies.

Annex Figure 7 shows the overall clustering of farmer-cooperators' and non-cooperators' banana production practices in Cianjur and Lampung over time. For Lampung, two separate clusters (A & B) were formed between the co-operators and non-cooperators indicating very different farm practices after the introduction of banana production technologies. With the introduction of technologies to the Lampung farmers, co-operators readily implemented the technologies, indicating positive technology adoption. On the other hand, for Cianjur, overlapping clusters (C & D) for co-operators and non-cooperators were observed, indicating that Cianjur farmers were already practicing the technologies that were taught to them.

These trends may be due to Cianjur co-operators and non-cooperators having bigger land areas which they utilise for other high value crops, thus, focus was more on these income sources, banana not being the priority cash crop. Thus, technology uptake on banana production held less significance. On the other hand, technology uptake was observed to be more favourable in Lampung maybe because 50% of the household income is derived from banana farming, hence, the greater the desire to improve production.

Field disease management options

The farm practices implemented by the farm collaborators have influenced disease severity in their areas. Figure 1 shows the clustering of farmers in Cianjur based on their different farm practices to manage disease. The first cluster of farmers had lower disease incidence, and this may be attributed to the following farm management options the farmers have:

Management option		zero tillage	corm-bits for planting	annual cropping	liming	deflowering	Biological control	Restricted inter-farm movement	Contour management	Crop rotation	Resistant TC variety	Irrigation and drainage management	Early removal of BBTV infected plants	Comments
Cluster	1													may have effectively controlled fusarium wilt, blood bacterial wilt and banana bunchy top disease
	2													

(1) Zero tillage, (2) used corm-bits for planting, (3) implemented an annual cropping system, (4) applied lime, (5) deflowered, (6) used biological control, and (7) avoided farm to farm plant material movement. The management activities applied by these farmers grouped in the first cluster have effectively controlled the three major disease problems, namely: Fusarium wilt, blood bacterial wilt and banana bunchy top disease as these practices all affect pathogen inoculums build up and spread. The second cluster is composed of farmer collaborators who managed their farms through the following practices: (1) contour management, (2) crop rotation, (3) use of resistant variety from tissue culture, (4) irrigation and drainage management, and (5) early eradication of BBTV infected plants. The use of wilt-resistant varieties was expected to solve the problem, but since there were three different diseases common to the area, the use of Fusarium wilt resistant varieties did not effectively address all disease problems. This happened since the Fusarium wilt resistant variety was not resistant to other banana diseases such as Pisang Kepok being resistant to Foc but susceptible to blood disease. In Cianjur, it was observed that most banana farmers implemented mixed cropping -- the use of several banana cultivars planted together with other

crops in the same land area. The farmers practice in using a range of banana varieties in the field is due to their adaptability to their climatic condition and consumer preference.

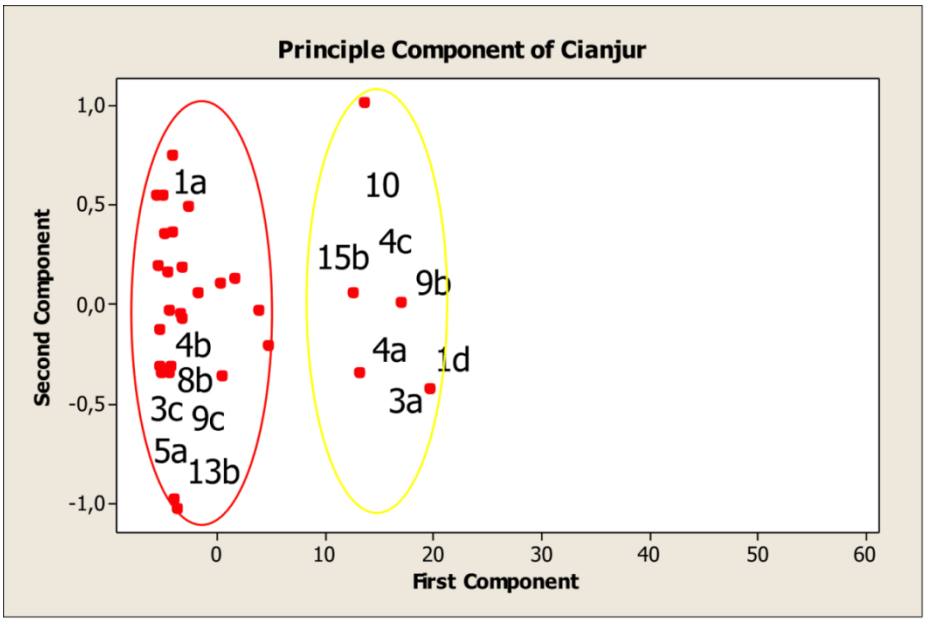

Figure 1. Clustering of farm collaborators from Sarampad Village – Cugenang County – Cianjur District. Numbers in the cluster indicate code of implemented management options (see list in annex pp77-78).

Farmers clustered together in the first group implemented (1) zero tillage, (2) row arrangement, (3) crop rotation, (4) annual cropping with multiple varieties, (5) using axilar shoot planting materials, (6) applied micro nutrient, (7) sterilized farm tools, and (8) early deflowering to control bacterial wilt. Among the approaches, the use of axilar shoot planting materials (clean planting materials) and multiple variety cropping systems were identified as the possible disease suppressing factors. In this case, farmers were trained to produce their planting materials through axillar shoot stimulation. As the production of planting materials was directly guided by the researchers, the materials produced for planting were better quality than existing planting materials usually used by farmers. Using a multiple variety cropping system including varieties with differential resistances avoids serious damage from all three diseases.

Farmers grouped in the second cluster implemented (1) minimum tillage, (2) managed planting space, (3) mixed cropping, (4) tissue culture and bit planting materials and (5) site selection. The performance of tissue culture materials in the field was not as expected because the site was naturally very dry and tissue materials need more intensive husbandry than is affordable for farmers in the site. Site selection was also implemented but it is not an appropriate option since most of the area in the village was endemically infested with Fusarium wilt pathogens. Based on the site experiments, the main disease suppressing management approach was mixed cropping with maize, chilli and estate crops.

The third cluster group of farmers implemented (1) desuckering, (2) applied farm manure, (3) fruit bagging, (4) use of Fusarium wilt resistant varieties, (5) biofumigation, and (6) insect vector management. While fruit bagging avoids the transmission of blood bacterial wilt, desuckering however facilitated transmission of both Panama and bacterial wilts. Based on the result of farmers' field practices, it was

seen that fruit bagging was not really needed in this area since most of the cultivated varieties were less affected by blood bacterial wilt. The main variety cultivated in Legundi was 'Muli' (Senorita – AA Sucrier) which is relatively resistant to panama wilt and is able escape insect transmission of blood disease, but is susceptible to banana buchy top virus.

In general, disease severity in both sites was relatively low (< 20%). The authors believed that the implementation of mixed- and multiple-variety cropping highly affected the disease incidence in both sites. Multiple banana variety cropping in the sites restricted the plant to plant transmission/distribution of the disease. Utilization of symptomless infected planting materials, such as BBTV infected asymptomatic suckers, on the other hand is the main contributing factor for disease spread in the area.

Management options for income generation

Analysis of Lampung farmers' income from banana resulted in four clusters of farmers (Figure 3). Farmers included in the first cluster, gained monthly banana income ranging from IDR 237,666 (~USD 20) to 314,000 (~USD 27). These farmers were those who (1) selected the planting site, (2) used an annual cropping system, (3) arranged plants in rows as part of plant density management, and (4) applied control practices of other pests. The second cluster had banana monthly income ranging from IDR 392,517 (~USD 34) to 583,750 (~USD 50), this cluster consists of farmer respondents who implemented (1) manuring (part of nutrient management), (2) tool sterilization (part of crop health), and (3) control of banana bunchy top disease. Farmers clustered in the third cluster used the following practices: (1) axilar shoot planting material, (2) minimum tillage for land preparation, and (3) deflowering for crop health management; and gained banana monthly income ranging from IDR 602.000 (~USD 52) to 851.642 (~USD 73). The farmers grouped in the fourth cluster gained IDR 979,315 (~USD 84) to 1,297,000 (~USD 111), these farmers implemented (1) appropriate planting space, (2) mixed cropping, (3) the use of biocontrol, and (4) insect vector management. The combination of management options implemented by farmers in the fourth cluster resulted in highest income from banana. Highest income observed in this cluster may be due to the synergistic combination of management practices. These field practices played an important role in controlling banana pests and diseases. The biological control application may have addressed the management of Panama disease Mixed cropping on the other hand controlled the spread of the disease in the area, where the mixed crop may have bordered an infected plant, preventing the spread of soil borne pathogen. The insect vector management controlled the transmission of banana blood disease bacteria and banana bunchy top viruses. The applied management options consequently influenced the increase of harvested banana due to increase in the number of uninfected banana plants.

Based on the income from banana, Cianjur farmer collaborators were grouped into four clusters as well. The first cluster per month only earned IDR 40,174 (~USD 3) to 77,026 (~USD 6) from banana production. These farmers implemented the use of (1) good planting space, (2) micronutrient application, (3) eradication of infected plants, and (4) biofumigation. Farmers from the second cluster per month earned IDR 82,083 (~USD 7) to 112,500 (~USD 9), these farmers (1) planted bits as planting materials, (2) implemented zero tillage for land preparation, and (3) controlled wilt diseases. Farmers in the third cluster per month earned an income of IDR 112,857 (~USD 9) to

226,500 (~USD 19), these farmers implemented the following, (1) annual cropping system, (2) irrigation, and (3) control of BBTD. Farmers in the fourth cluster per month earned an income of as much as IDR 274,750 (~USD 23) to 973,425 (~USD 83), these farmers implemented (1) contouring for land preparation, (2) planted axillar shoot planting materials, (3) bagged the fruit/bunch, and (4) applied suppressive soil approaches. Combination of management options implemented by farmers in the fourth cluster may have effectivelly reduced disease infected plants and increased number of harvested bunches per plant. However, unlike the farmers in Lampung where they generally cultivated the same cultivars, Cianjur farmers planted more banana cultivars including both low and high value bananas. Income of farmers may be influenced by amount of banana yield and price, but it was observed that income from banana of Cianjur farmers was not only influenced by implemented management options of the farmers that resulted in high yield, but was also influenced by cultivar market price. Lampung farmers income on bananas were higher than those from Cianjur because the former was mainly banana-based system while Cianjur was primarily a high-value cash crop cropping system. Cianjur farmers generally had higher household income derived from farming though.

There were other factors (e.g. banana farmers also have day jobs other than farming), that may have influenced or contributed to farmers' income in these sites but were not covered in this research. The following factors may have also influenced farmers' income: size of the business, variety (high / low value varieties), and competitiveness of banana with other fruit crops.

8.1.4 Profile of production and supply chain management for bananas in pilot sites

The collaborative study on banana supply chains between ICHORD, the Directorate General of Horticulture (DG Hort) and ITFRI, aimed at finding opportunities to improve price share for farmers and better fruit quality for consumers. From the PRAs conducted both in Cianjur and Lampung, the primary and secondary data were obtained to characterise the supply chain system in these provinces. Survey respondents included farmers, banana-processing cottage industry stakeholders, retailers, middlemen, and wholesalers.

The Banana Supply Chain and Market Status in Cianjur

The survey was conducted in Sarampad Village, Cugenang, District, Cianjur Regency, West Java Province on May 14-15, 2012. From the survey, the following data were derived:

- The respondents of the survey had ages ranging from 40-60 years old. Most of them have land areas of less than two (2) hectares per household on dryland areas and cultivated by mixed cropping systems.
- Banana farms are characterised by low yields due to pests and diseases, natural disaster (typhoon) and an undeveloped marketing system.
- Three women farmer groups run a home-based processing industry for banana chips. Despite a good market for banana chips, raw material supplies are limited especially regarding Nangka variety.
- Banana farmers have a relatively weak bargaining position compared to those of the traders. Even though the farmers tend to be the price takers normally, spot banana prices rose due to lack of supply caused by the typhoon in the last quarter of 2011.

- Most farmers sell their bananas to middlemen and wholesalers who in turn, sell these commodities to adjacent markets such as Cipanas and Cianjur, and to other markets in Cibedug, Ciawi, Bogor and to the central market in Kramat Jati, Jakarta. From these markets, the distributors sell bananas to retailers and final consumers (e.g. households, restaurants, hotels, and catering service companies). The total amount of bananas marketed by wholesalers to Cibedug and Kramat Jati markets by the wholesaler from Cugenang district is around 4,000 kg per week.

The Market Chain of Cianjur (Banana Processing Annex Figure 10) illustrates the prevailing market chain system. Most farmers sell their harvested bananas (e.g. *Ambon* and *Raja Bulu* cultivars etc.) to middlemen, wholesalers or retailers. The middlemen sell the bananas to wholesalers, who then sell the produce to retailers in town markets. In some cases, the middlemen themselves act as retailers and sell particular banana cultivars, such as Nangka, to banana chip processing plants at the village level. In other cases, some retailers purchase bananas directly from farmers and sell these to the town markets.

There are situations when the farmers sell bananas to the traders without fruit quality grading. Depending on the variety, bananas are priced at around Rp 2,000 per kg at farm level. Cash payment is usually carried out by the traders and the fruits are harvested from the farmers' fields by the traders themselves. Selling bananas to suppliers who then market these bananas to supermarkets is not common because these suppliers buy at a lower price than other wholesalers. This is due to the arrangement with supermarkets that their bananas are in supermarkets through a consignment agreement in which payments will be made two or three weeks after the commodity has been sold.

The Banana Supply Chain and Market Status in Lampung

In the supply chain of Lampung (Banana Processing Annex figure 11), village collectors harvest the bananas from the farmers' fields and sell them to bigger traders to nearby major cities such as Tangerang (Serpong Balaraja Banten Province), Jakarta, and Cirebon (West Java Province). The prices of bananas are decided based on the variety, fruit size and degree of maturity.

The most common cultivars in the market are Pisang Jantan and Muli. To maintain the connection with farmers, the village collector continues to buy bananas during the Eid Festival when the demand for banana is not very high in the cities of Jakarta, Tangerang and Cirebon.

Generally, village collectors pay the farmers in cash upon harvest of the bananas. However, some farmers request for advance payments from the village collectors even prior to harvest (called the 'ijon' system) to support their household needs. In such cases, farmers receive a lower price for their produce.

No locally sold bananas receive post-harvest treatments, as the traders do not require them, so post-harvest quality of banana produce is not much of an issue.

Depending on the volume, bananas are transported by an open-cup vehicle (e.g. L-300-capacity of 350 bunches at 6 kg) or truck (capacity of 900 bunches) for a distance of about 78 km. In normal conditions (light traffic), transporting the bananas takes about 4 hours from East Lampung Timur to Balaraja, 5.5 hours to Cibitung, and 12 hours to Cirebon. The transport cost is around Rp 400.000 (USD 40) per truck or Rp 210.000 (USD 21) per L-300. Poor roads constrain transport and lengthen travel times.

8.2 Strategic Research

8.2.1 Selection of soil indicators (Australia)

A range of soil indicators and their sensitivities were studied in comparing farms using different management practices, such as organic compared to conventional banana production as paired sites. Based on the analysis, physical soil properties in conventional and organic soils were very similar, especially within farm pairs. It appeared that water stable aggregates (WSA) and bulk density were mainly influenced by climate and soil type. It may also be that the soil management components including soil tillage that influence bulk density and WSA are analysed in a quite similar way in both management systems.

The chemical properties of organic and conventional soils were similar indicating a similar application of nutrients, though in different forms and in different ways. The higher sulphate levels in the conventional soils could be explained by application of sulphur-based pesticides and fertilisers (Balik *et al.*, 2009). The higher levels of labile C in the organic soils can be explained by application of organic soil amendments, especially thoroughly composted, soluble or liquid fertilisers. Observations during the survey suggested that organic banana farmers apply appreciable amounts of these types of fertiliser. Furthermore, the organic farms stimulated soil organic matter content by more cover cropping than conventional farms and retention of crop residues.

It was found that soil microbial activity indicators fluorescin diacetate (FDA) and β-glucosidase were both higher in organic soils than in conventional soils. The majority of nutrients in conventional production are applied in mineral form, which is readily available to the plant. Microorganisms involved in breakdown of organic materials thus lose a food source and their populations decline. Microorganisms engaged in the mineralization process often have symbiotic interactions with plants, exchanging soil minerals for carbon. However, as plants already receive readily available nutrients in mineral form they no longer benefit from investing carbon in sustaining symbiotic relationships with microorganisms, and these organisms thus may lose their functionality.

The soil nematode community is closely related to the soil microbial activity. This was evident from the relationship between the significant correlations between soil enzyme tests and nematode community indices. In this survey it was observed that the percentage of bacterial feeding, fungal feeding and predatory and omnivorous nematodes in the organic soils were often a factor two or more times greater than for conventional soils. Higher levels of bacterial and fungal feeding nematodes indicate greater levels of microbes, suggesting more intense cycling of nutrients. The higher level of predatory nematodes indicates a degree of self-regulation of the soil nematode community. This is also reflected in the diversity index that is significantly greater in the organic soils. Increasing both above-and below-ground diversity in an ecosystem increases dynamics, self-regulation and resilience of that system. In the conventional soil, the percentage of plant-parasitic nematodes was more than double that of the organic soils.

In conventional soils, there were less predatory nematodes to prey on the parasites, leaving the species that can cause economic damage to crops to proliferate. It is possible that pathogens can develop very rapidly in monocultures. Furthermore, pathogens and parasites are less reliant on decomposing organic substrates than other soil micro- and macro-organisms. Plant-parasitic nematodes were also positively correlated with *Foc* hyphal growth.

Bioassays were used to assess soil suppressiveness of soils to Fusarium wilt. Plate experiments were conducted to measure hyphal extension. Pot experiments were conducted to measure plant growth and disease progression. Based on the tests, it is questionable if the *Foc* hyphal extension test provides a useful insight in soil Fusarium suppression. Firstly, hyphal growth of Fusarium does not guarantee successful infection and subsequent disease expression of the host plant (Cook and Baker, 1983). Secondly, it was very hard to distinguish between different fungi on the plate, especially between *Foc* and other species of *Fusarium*. Especially in the second half of the test it was unclear if the *Foc* on the plug was colonizing the soil or that the fungi in the soil were colonizing the plug. The test did show that *Foc* hyphal growth was favoured by soil organic carbon, probably as well as any other fungal hyphae,.

From a management point of view, this means that to reduce *Foc* colonization, organic matter should only be applied if the material is well colonised by other saprophytic microorganisms or if there is sufficient microbial activity in the soil to colonise the material quickly and compete with the pathogen.

The presence of other fungi and possibly bacteria that are antagonistic to tomatoes makes it impossible to use the data for conclusions about soil Fusarium suppression. Tomato may not be the most suitable crop to test soil Fusarium suppressiveness because of its susceptibility to a wide range of plant pathogens. Also, plant health is an important factor in Fusarium infection. Growing tomatoes in a banana soil may therefore not give a clear picture of *Foc* suppressiveness of a soil. It is safer to use the specific host and pathogen one is looking at, or if impossible, to use a wider range of hosts and their associated (Fusarium) pathogens. Another issue was that halfway through the trial it was evident that the *Fol* R3 strain used had lost much of its pathogenicity. It would be advisable to do a pathogenicity test with the pathogen one wants to use before doing the actual bioassay. Furthermore, it is critically important to cancel out the possibility of cross-contamination.

The soil biological and biochemical measurements developed in this survey are sensitive to changes in management practices. The soil nematode community analysis and the biochemical tests, FDA, β-glucosidase and labile C were all greater on the organic sites relative to the conventional banana sites. The standard chemical soil properties, which are commonly measured, failed to detect differences between the two systems. However, it was not possible to determine if organic soils with greater microbial activity and diversity could suppress Fusarium wilt. The assays that were tested, hyphal extension and the tomato bioassay were not suitable for screening soils for their suppressive potential to Fusarium. In particular the hyphal extension was unsuitable as it was difficult to determine if hyphae were growing out of the agar block inoculated with Fusarium or into it. Further work on developing methods for measuring the suppressive potential of soil is needed.

Paired sites Indonesia

A preliminary survey of the soil factors associated with the suppression of Fusarium wilt of bananas caused by *Foc* was conducted in Indonesia by comparing paired sites. The sites were selected as either healthy with banana plants showing no symptoms of Fusarium or infected where the disease was present and causing wilt symptoms. Soil samples were collected from the top 10 cm from at least 10 sites in each area.

Soil samples were processed for physical, chemical and biological properties at the laboratory of the UGM, Yogyakarta. Physical properties assessed included water holding capacity of the 0.5 and 2.0 mm fraction of the soil. Chemical properties included pH, organic C, total N, total P, available K, cation exchange capacity (CEC) and electrical conductivity. Biochemical soil properties included labile C, fluorescein diacetate (FDA) and

β-glucosidase. Furthermore, biological properties were determined using selective media for bacteria, actinomycetes, microbial diversity and *Fusarium spp*.

Four locations were included in the survey, two locations in Central Java, one in West Java and one in Lampung, Sumatra. Due to differences in soil types in the four locations, the difference in soil properties were normalised by determining the difference between healthy and infected sites.

Based on the analysis conducted for the soil samples, it was found that in all locations the CEC, bacterial diversity and number of actinomycetes were greater in healthy soils than unhealthy soils. Conversely, the population of Fusarium species isolated from the soil was greater in infected sites compared to healthy sites. Since there was a significant difference in the number of Fusarium species between healthy and infected areas, the change in numbers was regarded as an indicator of disease potential and used in a regression analysis of soil properties.

The difference or change in the numbers of Fusarium species between healthy and unhealthy soil is not a conclusive method of determining disease suppression, but gives some indication of potential soil factors that may be involved. In this case, the decrease in with the suppression of Fusarium in the soil and possibly reduce the expression of Fusarium wilt symptoms. β-glucosidase levels indicate cellulolytic activity in the soil. It is the rate-limiting enzyme in the conversion of cellulose to glucose and therefore a good measure for the potential for the degradation of plant material. Greater β-glucosidase activity could mean that there are more organisms in the soil that can potentially degrade organic matter which may compete with the survival of Fusarium in the soil. The diversity of actinomycetes may similarly suppress Fusarium, and it is possible that actinomycetes produce β-glucosidase, which suggested that they were dominant in causing the difference in Fusarium numbers between health and infected areas.

The results from this limited survey suggested that analysing the differences between sites, rather than just the measurements, maybe a feasible method of investigating suppression. This is because there is high variability between locations due to soil types and climates and by investigating the relative difference between the sites at each location included in the survey some of the variability may be accounted for. This method appeared to be valid as there were greater amounts of Fusarium recovered from the soil in areas that were deemed to be infected relative to healthy areas. The factors associated with this change could then be determined, which turned out to be β-glucosidase activity and the diversity of actinomycetes.

The results are not conclusive but indicate that differences between healthy and infected sites may be due to a reduction in the amount of Fusarium in the soil, which is due to increase biological activity particularly cellulose degrading organisms and the different types of actinomycetes in the soil.

Paired sites Australia

A similar survey of paired sites was conducted in a sub-tropical banana production area in Australia. Sites were chosen at four locations where the disease was slow to progress and where the disease progressed rapidly. A follow up to the initial survey was conducted 12 months later and the fields reassessed for Fusarium and soil parameters.
The results from the initial survey show that Fusarium wilt was more severe at sites where the disease was considered to progress rapidly relative to slow progression of the disease.

For some of the parameters measured there was no change between the two sampling times. For other parameters there was an increase or a decrease from the initial sampling time. The Fusarium wilt ratings of symptoms increased in some fields where the disease was not initially present or was determined to be progressing slowly. The change in the severity of the disease was analysed with the initial soil properties to determine what soil properties could have led to the change in the disease between the two investigation periods.

It was possible to predict the change in Fusarium at the different sites with soil properties β-glucosidase, nematode community enrichment index, manganese and magnesium contents of the soil. This model suggested that an increase in the severity of Fusarium wilt in the field was associated with a decrease in the manganese and magnesium measurement and an increase in the enrichment index and β-glucosidase in the soil. The predicted change in Fusarium wilt symptoms using the model described in equation 2 was plotted against the actual change with a significant R^2 of 0.99.

One site included in the survey had a reduction in the Fusarium wilt symptoms between the two sampling periods, the Lar “slow” site. This suggested the site may be suppressive to the disease and therefore underwent further evaluation for levels of Fusarium and *Foc*. There were similar levels of both *Fusarium spp*. and *Foc* in the soil in both sites sampled, where the disease progressed rapidly and slowly. This suggested that differences in the appearance of the of wilt symptoms of banana plants was not due to differences in Fusarium inoculum levels. However, the assay using Fol demonstrated a significant suppression in the vascular discolouration on tomato in the soil where *Foc* was observed to progress slowly. When the soil was steam pasteurised the amount of discolouration increased in the slow soil but remained at a similar level in where the disease was observed to progress rapidly.

The change in symptoms within banana fields over time was a useful method of determining what soil properties may be associated with suppression of Fusarium wilt symptoms. Although banana growers considered different fields to have “rapid” or “slow” development of disease symptoms, they were not always consistent, as there seasonal differences between years. By matching the changes in disease symptoms with soil properties it was possible to develop a model of soil factors that were related to the enhancement or suppression of the disease. In this case the soil nutrients manganese and magnesium were related to changes in the severity of the disease. A decrease in the available elements led to an increase in the observed symptoms of Fusarium wilt (Annex page 127).

The change in Fusarium wilt symptoms was also associated with changes in biological soil properties, the soil nematode enrichment index and β-glucosidase. The nematode enrichment index is a measure of the resource available to the soil food web, which is considered to be a response of the nematode community to bacteria that can quickly multiply with the availability of nutrients. The enrichment index is typically greater in soils with a lot of available nitrogen. Therefore, it is not surprising that a change in the enrichment index is associated with a change in the Fusarium wilt. Fusarium wilt is considered to increase in situations with high nitrogen availability, which is the same situation where it would be expected to have a high enrichment index. However, the relationship between the β-glucosidase levels in the soil and the change in Fusarium wilt symptoms is opposite to what was expected. The previous findings in the Indonesia survey demonstrated that an increase β-glucosidase was associated decease in the amount of Fusarium. It would be expected that this relationship would follow in Australia, but the model developed suggested that an increase in β-glucosidase was associated with an increase in the severity of Fusarium wilt symptoms. Therefore the role that β-glucosidase has as an indicator for suppression of Fusarium wilt of bananas requires further investigation.

There was one field where the Fusarium wilt disease symptoms were observed to decrease, Lar “slow”. When this soil was compared to the paired site where there was a rapid progression of the disease there were similar levels of Fusarium and *Foc*. This suggested that the mechanism for suppression that led to a decrease in the disease symptoms was not affecting the survival of the pathogen in the soil. Therefore, it possible that a different suppressive mechanism of the disease may be occurring at this site relative to what was observed in Indonesia, where a decrease in the number of Fusarium in the soil was measured between healthy and unhealthy plants. The suppressive mechanism may have been having an indirect impact on the development of the disease symptoms, as the tomato bioassay demonstrated that the soil where Fusarium wilt of bananas declined could suppress the development of Fusarium wilt in tomatoes. Furthermore, this suppression was biological, because the steam treatment of the soil removed the suppression. It is noted that many different mechanisms may be involved in disease suppression. Direct antagonistic interaction with the pathogen is one mechanism, which may have been occurring in Indonesia, whilst indirect methods involving a plant may have been occurring in Australia. The indirect mechanism may involve competition on the plant roots, production of antifungal products by rhizosphere organisms or induced suppression in the plant caused by soil organisms.

8.2.2 Pathogenicity, virulence and cultivar resistance studies

Two sets of pathogenicity experiments were conducted in Indonesia, (1) screenhouse test and (2) field test of *Foc* VCGs found in Indonesia against different banana cultivars. Based on the screenhouse and field tests conducted, different banana cultivars responded differently to different *Foc* VCGs. Validation of Race concepts on *Foc* with pathogenicity tests of VCG group isolates against a range of *Musa* cultivars and cultivars was conducted in this study.

Screenhouse test

Based on the screenhouse tests, the variety Berlin (AA Sucrier) showed tolerance/ resistance to the VCGs 0123, 0124/5, 0126 and 01218 – all identified under *Foc* race 1. Leaf disease severity index (LDSI), corm disease severity index (CDSI) and percentage of disease incidence of this variety against VCGs 0123 and 0126 were very low. Response of this variety to VCGs 0121 and 01213/16 (*Foc* Tropical Race 4) varies from susceptible and highly susceptible against slightly resistant to VCGs 01219. Based on literatures, Sucrier types are only infected by Tropical Race 4. (Ploetz and Pegg, 2000).

Table 3. Response of banana cultivars toward various *Foc* VCGs under the screenhouse

Banana variety	VCG							
	Race 1				Race 1/4	Race 4		
	0123	0124/5	0126	01218	0120	0121	01213/16	01219
Rejang (AA)	HR	HS	HR	HR	MR		R	R
Berlin (AA)	R	MR	R	HR	R	S	S	R
Ketan 01 (AA)	R	S	R	R	R		S	MR
Ambon kuning (Gros Michel/ AAA)	HS	HS	HS	HS	HS		HS	HS
Ambon Hijau (Cavendish/ AAA)	MR	HS	MR	MR	MR	HS	HS	MR
Randah (Cavendish/ AAA)	MR	HS	S	MR	MR	HS	HS	MR
Ambon Putih Jambi (Gros Michel/ AAA)	MR	S			R	S	HS	R
Barangan (AAA)	MR	HS	S	R	MR		HS	MR
Roti (AAB)	HR	HS	HR	HR	R	HS	HS	R
Kilita (AAB)	HS	HS	HS	S	HS	HS	HS	HS
Kepok tanjung (ABB/BBB)	HS	HS	S	HS	HS		HS	HS
Raja kinalun (ABB)	MR	HS	HR	HR	HR		MR	HR

Simultaneous VCG inoculation

The virulence of the VCGs collected from all over Indonesia was tested using the simultaneous inoculation of different VCGs of Fusarium oxysporum f.sp. cubense on a banana variety (cv. Ambon Hijau). Seven inoculation treatments: were tested on 2 month old seedlings of Ambon Hijau: A=VCG 01213/16+0121, B=01213/16+0124/5, C=0121+ 0124/5, D=01213/16+0121+0124/5, E=01213/16, F=0121, G=0124/5)

Table 4 shows the percentage of wilted plants of Ambon Hijau observed when inoculated by the 7 treatments. After 60 days of observation, all test plants showed 100% wilting on all treatments. The shortest incubation period (6 days) was observed on Ambon Hijau plants inoculated with pure VCG 01213/16 and the longest incubation period (17 days) was observed on 0124/5. The rest of the treatments showed incubation periods ranging from 11-13 days. Ambon Hijau showed initial symptoms after 13 days incubation on VCG 0121 alone. This may mean that VCG 01213/16 is most virulent on Ambon Hijau, as the banana variety was immediately infected 6 days after inoculation. VCG 0124/5 based on this experiment also shows that it is the least virulent VCG on Ambon Hijau. The observation also shows that Ambon Hijau is susceptible to all tested VCGs and their combinations.

Table 5 shows the leaf and corm disease severity indices at 60 days after treatment of Ambon Hijau inoculated with pure VCG 0124/5 and 01213/16 and combinations of these VCGs with VCG 0121. No significant difference was observed between the indices derived from each of the VCGs and their combinations. The non significant differences of leaf and corm disease indices on the tested VCGs (01213/16 and 0124/5) shows that the damage done by these VCGs on the plant may not differ at 60 days after inoculation, but the variety Ambon Hijau succumbs earlier on VCG 01213/16 (Table 10).

Table 4. Percentage of wilted plants at 60 days after inoculation and incubation period of *Foc* VCG 01213/16, VCG 0121 and VCG 0124/5 in single and multiple inoculation on banana cv. Ambon Hijau

Treatment	Percentage of wilted plants (%)	Incubation period Day
0124/5	100	17
0121	100	13
01213/16 + 0121	100	12
01213/16 + 0124/5	100	12
01213/16 + 0121 + 0124/5	100	12
0121 + 0124/5	100	11
01213/16	100	6

CV of incubation period = 9%.

Table 5. Leaf and corm disease severity index of banana cv. Ambon Hijau infected by *Foc* VCG 01213/16, VCG 0121 and VCG 0124/5 in single and multiple inoculation, 60 days

Treatment	Leaf disease severity index (LDSI)	Corm disease severity index (CDSI)
01213/16 + 0121	5	6
01213/16 + 0124/5	5	6
0121 + 0124/5	5	6
01213/16 + 0121 + 0124/5	5	6
01213/16	5	6
0121	5	5
0124/5	4	5

CV LDSI= 3% and CDSI =4%

Table 6 shows the percentage of VCG detected on wilted Ambon Hijau plants as inoculated by a combination of VCGs 01213/16, 0121 and 0124/5. On the combination of VCG 0121 and VCG 0124/5, 100% of the detected pathogen was VCG 0124/5. For the combination of 01213/16 and 0124/5, only 8% of the detected Foc was VCG 01213/16 and 92% of VCG 0124/5 was detected. For the combination of 01213/16 and 0121, 86% of the Foc detected was VCG 01213/16. For the triple combination of VCGs, VCG 0121 was not detected at all, only 25% of VCG 01213/16 was detected and 75% was detected as VCG 0124/5.

Table 6. Percentage of VCG incidence on wilted plants inoculated by multiple VCG of *Foc*

Treatment	Percentage of VCGs detected* (%)		
	1213/16	0121	0124/5
A (01213/16 + 0121)	86	14	0
B (01213/16 + 0124/5)	8		92
C (0121 + 0124/5)		0	100
D (01213/16 + 0121 + 0124/5	25	0	75

*Note : * = VCGs detected tested from 3 leaves of plant wilted*
Wilted plant inoculated with mix VCG of Foc only detect one VCG.

From these screenhouse inoculated experiments, based on disease severity it was observed that VCG 01213/16 and VCG 0124/5 may cause the same degree of destruction on Ambon Hijau plants despite the fact that test plants may succumb earlier to VCG 01213/16. However when VCG 01213/16 and 0124/5 were combined & inoculated on one test plant, more 0124/5 was detected/recovered on the infected plant. This may mean that VCG 0124/5 is more competitive than 01213/16 when inoculated in combination.

Field test

Field test results were not always consistent with screenhouse results. Field test results for Pisang Berlin, Ketan and Kepok were not the same as the results in the screenhouse. These three cultivars seemed susceptible to VCG 01213/16 in the screenhouse experiment, but in field tests these cultivars showed good resistance.

Consistent with the screenhouse tests were the field test results with the following cultivars: Kilita, Ambon Hijau, and Ambon Putih Jambi.

Kilita, Ambon Kuning and Ambon Hijau were found susceptible to *Foc* VCG 01213/16 in this study. The result from screenhouse testing gave the same assessment of these cultivars against *Foc* VCG 01213/16. Ambon Hijau control treatments in the screenhouse were also infected by *Foc* VCG 01213/16 despite the fact that these plants were not artificially inoculated. The plants were likely infected by the indigenous *Foc* in the soil since the study site is Fusarium endemic area.

Barangan in the VCG 01213/16 infected field displayed resistance, where Fusarium wilt symptoms were observed at 4 months after planting on just one plant which then started to disperse gradually to other plants. Four cultivars (Berlin, Klutuk Awu, Raja Kinalun and Tanduk) were not infected by Fusarium wilt until the last observation at 19 months after planting. They were found to be highly resistant to Fusarium infection especially against *Foc* VCG 01213/16. Either inoculated or non inoculated Ambon Hijau showed the highest disease percentages compared to other cultivars, confirming that Ambon Hijau is a highly susceptible variety to *Foc* VCG 01213/16.

Table 7. Fusarium wilt performance on *Foc* VCG 01213/16 field test at Aripan Experimental Farm

No	Banana variety	Incubation period (days after planting)	Disease incidence (%)	Leaf disease severity index	VCG testing result
1.	Berlin (AA)	-	0.0	1.0	-
2.	Calcuta (AA)	200 – 326	14.8	1.29	01213/16
3.	Kilita (AAB)	192 – 435	38.5	2.15	01213/16
4.	Klutuk Awu (ABB)	-	0.0	1.0	-
5.	Barangan (AAA)	137 – 325	13.0	1.46	01213/16
6.	Ambon Hijau (AAA)	165 – 358	30.8	2.06	01213/16
7.	Ambon Putih Jambi (AAA)	259	3.6	1.07	01213/16
8.	Ketan (AA)	387	3.5	1.07	01213/16
9.	Raja Kinalun (ABB)	-	0.0	1.00	-
10.	Kepok (ABB/BBB)	193	3.3	1.10	01213/16
11.	Tanduk (BBB)	-	0.0	1.0	-
12.	Ambon Hijau Control (AAA)	165 – 268	48.2	2.39	01213/16

9 Impacts

The demonstrated increase in productivity and profitability of improved practices such as use of clean planting materials, fertilization, population management and adopting a mixed cropping system in reducing disease incidence and increase in yield may result in immediate impact to banana growers. This however should be accompanied by outscaling activities coupled with capacity building in technology transfer.

The use of identified crop management practices that increase soil-suppression can also be immediately validated or even outscaled to mitigate the destructive disease in areas where the disease is now causing damage The study was carried out in Australia on Foc Race 1 nd in Lady finger. An opportunity to validate and even outscale on Foc TR4 presents itself in Cavendsih monoculture systems, such as in the Philippines, and China.

9.1 Scientific impacts – now and in 5 years

As a result of the project activities in Australia, soil health and suppression of *Foc* and other soil-borne diseases were included in the Australian Banana Growers Council strategic plan for plant protection research. This created an impetus for further research on soil health and soil biological activity arising from the research developed within the project. The potential of different mechanisms as basis of soil suppression in managing Foc will encourage more research efforts in biological control, soil health management, plant nutrition and amendments towards sustainable management of Foc in different agroecosystem.

The important results of this study on host-pathogen interaction where different banana cultivars responded differentially to different Foc strains point to the importance of expanded cultivar/variety evaluation against different Foc strains in various agro-ecological situations. It justifies further studies to define the concept of Races which have been oversimplified through the years. It proved that VCG strains do not correspond to the Races that have been established earlier. An improved understanding on the concept of *Foc* races allows for the development of a range of diagnostic reference cultivars, which will be important in cultivar deployment and quarantine policy enforcement.

The importance of elucidating mechanisms of pathogen virulence and host resistance is highlighted by the differential variety-strain interactions.

The two pilot studies conducted within this project were designed to develop a model for sustainable community approaches to crop and disease management, with emphasis on managing BBD and *Foc*. The technical basis of the model will influence the rollout and approach of a regional adoption programme.

Research outputs of this project present fresh and critical knowledge in *Foc* and BBD etiology and epidemiology, as well as in the management of these internationally important diseases. Enhanced knowledge of plant and soil health management, and a better understanding of the disease-suppression mechanisms of suppressive soils, including the interactions of biological, physical and biochemical properties contribute to improved disease management strategies.

The project catalysed further the R&D on Fusarium wilt within the scientific community, and raised the interest of scientists on the extent of variation of races of *Foc*. Further research on *Foc* was initiated, such as the evaluation of East African highland bananas (EAHB) for resistance to *Foc* TR4 in China and the Philippines. A proposal to GIZ was prepared by Bioversity International to develop risk reducing strategies for Fusarium wilt through effective quarantine and integrated crop and disease management, based on

improved understanding of biological mechanisms, as well as policy research and the mobilization of stakeholders along the value chain

Furthermore, the links to the Banana Asia-Pacific Network (BAPNET) and other regional and research networks, including Pro*Musa*, facilitated the rapid transfer of knowledge and experiences to other producer countries, with the potential of expanding the impact relatively quickly.

The wealth of knowledge gained from all scientific and applied research will contribute greatly to understanding the mechanisms and management of Fusarium wilt in broader and holistic approach. This will be especially useful in manageing the risk of TR4 spreading to and within other global regions.

9.2 Capacity impacts – now and in 5 years

Capacity building was integral not just in the 'skilling-up' the farmer-cooperators involved in the project but also to ensure the transfer of management practices to other surrounding small-scale farmers. The extension workers and research scientists that were directly and indirectly involved in the project benefit from the capacity building activities (Table 13) as well.

Farmers in the pilot sites derived immediate capacity impacts through various trainings on field and disease management options (Appendix Table 5) which they have applied and can apply repeatedly as they see appropriate for varying farm conditions and situations. Additionally, crop production and IPM practices, technology-transfer activities, and development and application of research protocols were among the thematic areas covered in the training activities and these are invaluable inputs to enhancing farm productivity among the farmer-cooperators and effectiveness in extension workers and field scientists.

This project adopted participatory processes in its planning and implementation. The farmer-cooperators were the focus of attention from the beginning of the project and scaling-up their knowledge was vital. Hence, farmer education led to the identification of lead farmers (15 from Cianjur and 20 from Lampung) in the pilot sites who, hopefully, would facilitate the adoption of generated technologies by other farmer co-operators and even non-cooperators and feedback information to extension workers and researchers and technicians. This building up of the knowledge and scientific base of farmers effectively strengthens project ownership and sustainability.

The provision and use of affordable and sustainable supply of clean planting materials, grown under appropriate cultural practices was a major component of this project. Thus, capacity-building activities included trainings on the production and care of healthy banana seedlings and various cultural practices. In both pilot sites, the farmer-cooperators were taught the conventional propagation method of banana corm-bits, which included the actual protocol of bit production and the necessary nursery management. These capacity-building efforts steered and encouraged a farmer group in Legundi village to set-up their own village nursery which supplies the planting materials to local farmers. It also led to the creation of a livelihood opportunity for this farmer group, and hopefully, would expand to the entire village as well. The head of this farmer group is now being frequently tapped by Dinas as resource person for farmer trainings not only in the local village, but in nearby villages as well. Increased interest in soil health and suppression of *Foc* were generated as a result of the training conducted at the UGM in February 2010. Further trainings are envisaged for the future.

On the technical front, close cooperation between Indonesian, Australian and Bioversity International counterparts facilitated the development of new approaches to deploying biological control agents and suppressive soils for the control of *Foc*. These interactions have strengthened the capacity of all stakeholders involved in the project.

Table 14. Capacity building activities

DATE	TITLE	PLACE
25-27 February 2010	Training on Soil Health	University Gadjah Mada
October 2010	Farmers' Training on Corm Bit Nursery Establishment and Management	South Lampung
December 2010	Farmers' Workshop on Tissue Culture Seedlings Handling	Serampad Village, Cianjur
25 February 2010	Extension of banana wilt management on farm	Serampad Village, Cianjur
February 2010	Visit and sampling at Great Giant Pineapple Company (GGPC) and Nusantara Tropical Fruit (NTF)	Lampung
10-12 July 2010	Visit and presentation on banana wilt management	NTF Way Jepara East Lampung
15-16 July 2010	Workshop on Soil Microbial Activity I	UGM
17 July 2010	Extension on banana wilt management	Balai Benih Hortikultura Salaman Magelang Central Java
May 2011	Extension of banana wilt management on farm	South Lampung and Cianjur
30-31 May 2011	Workshop on Soil Microbial Activity II	UGM
14-18 November 2011	Mid-term Review and Planning Workshop; field visits at pilot sites in Cianjur and Yogyakarta	Sheraton Media Hotel and Towers, Jakarta, Indonesia

The capacity-building activities occurred at several levels across the project: researchers to extension officers, researchers-extension-farmers and farmer-to-farmer training. These learning systems operated as two- or three-way channels. The farms served as demonstration areas for 'agro-ecological learning', where cropping cycles provided learning opportunities for farmers, extension agents and the researchers. The pilot studies were the key focus of the capacity-building aspects of the project, where adoption of management practices occurred according to farmers' needs, and where champion growers were able to observe the full impact of the introduced cultural and disease management technologies. Partial analysis of the technology adoption behaviour points to the tendency of farmer-cooperators to adopt recommended farming practices and disease management tactics. In this sense, peer learning had proven once more to be an effective platform for farmers' education, thus, even the non-cooperators shifted to the farm practices and disease management tactics that were embraced by farmer-cooperators.

Capacity-building materials such as brochures and booklets have yet to be developed to report on the successful cultural and disease management practices for improved banana production. Participatory processes with champion farmers during the material design phase will ensure that materials meet the needs of farmers and will be successful in the up-scaling of results to additional banana growing communities.

9.3 Community impacts – now and in 5 years

The activities for the pilot studies in Cianjur, West Java and South Lampung, Sumatra increased the interest of village farmers on the application of more effective banana production technologies in their farms. The PRAs conducted in these villages increased awareness among farmers on the different banana cultivars, diseases and their management, and marketing.

As the pilot studies revolved around improving the banana production of farmers through a community-based approach, consequently, the livelihoods of these smallholder producers will be enhanced. An improved production system, brought about through more effective disease management would improve household food security and income. This approach involves stimulating the development of farmers' organizations, regular stakeholders meetings, interactions and capacity building. As said, the success of one is the success of all, as an effective integration of all these efforts would expectedly translate to positive outputs and propel the improvement of the village economy.

As an offshoot of the capacity building activities, it was mentioned that a farmer group in Legundi Village had established a village nursery that provides affordable and sustainable supply of clean planting materials among farmers in the local village and nearby villages. This created a livelihood opportunity to the farmer group. Eventually, the economic benefits derived from the village nursery spread and had been recognized by farmers and farmer groups in other communities. This stirred a considerable interest to set up the same kind of village nursery in other areas. With this mounting interest for this alternative livelihood opportunity, it may only be a matter of time when village communities can be sustainable base sources of affordable and accessible banana planting materials.

9.3.1 Economic impacts

Bananas are an important fruit crop in Indonesia and Australia, as demonstrated in Table 15, with a combined value of more than AU$1 billion. If the technologies are scaled out and adopted effectively, every extra percent of yield protection for the target countries is currently worth around Aus$12 million. In 5 years, if a modest 10% yield was protected by scaling out, this would be worth Aus$120 million

Table 15. Key figures relating to annual banana production in Indonesia and Australia

Country/variety		Production	Yield	Area	Value
		(t)	(t/ ha)	(ha)	(Aus $million)
Australia	Cavendish	264,772	24.84	10,659	310
	Lady finger	1,380	18	77	80
Indonesia		4,393,685	14.65	299,910	800
Total		**4,659,837**	**15**	**310,646**	**1,190**

Indonesia

Based on the survey conducted by Hermanto *et al* in 2008, a 28% average loss in banana production, due to *Fusarium* wilt and BBD, is equivalent to an estimated annual revenue loss of about 1.4 trillion Rupiah (AU$185 million). The majority of these losses come from smallholder farms where *Foc* and BBD epidemics are more prevalent, because farmers do not have the technical and economic capacity to manage these diseases. While commercial growers of Cavendish have also been severely affected in the past, commercial farms have recently recovered some productivity with the introduction of improved production practices and disease management strategies. Barangan and Kepok are two very popular local cultivars grown by smallholder farmers which are affected by

Foc and BBD, respectively. Improving banana productivity by managing the disease, coupled with appropriate production practices, will impact on the income, foods and livelihoods of small-scale growers, and contribute towards a significant reduction in the AU$185 million revenue losses currently occurring in Indonesia.

Australia

In Australia, Race 1 *Foc* has significantly restricted the expansion of lady finger banana in the sub-tropical production areas for many years, resulting in recent expansion in the region's tropical production around the tableland area near Mareeba, in Queensland. Similarly sub-tropical Race 4 has devastated the Cavendish production in the sub-tropics. Race 1 is considered to be endemic across most of the banana production regions of Australia; while sub-tropical Race 4 has been restricted to the sub-tropics due to very stringent quarantine practices.

TR 4 *Foc* and bacterial wilts (*Ralstonia* sp.) are of paramount quarantine importance to Australia and all other banana producing countries. Although TR4 has appeared in the Northern Territory of Australia, it has not spread to other banana growing states, as Australian scientists have focused on containing this disease through regulatory measures including quarantine, the use of disease-free planting materials and good farm practice.

The recent increased detection of Race 1 in new plantings of Lady Finger on the tableland is significantly diversifying the industry in terms of production regions and alternative cultivars. The outcomes of research findings from this project will expand the range of management opportunities for banana production in Australia.

9.3.2 Social impacts

As bananas form a staple food in Indonesia and a subsistence crop that supports a large number of smallholders, the impacts of the project are expected to touch on the rural poor. Improving the ability of smallholders to more effectively manage these severe disease risks will enhance the financial stability of smallholders. These stable and improved incomes will have knock-on benefits for smallholder families including improved nutrition and increased educational opportunities for the children of smallholder growers.

Bananas are considered a nutritious, cheap food which is readily available year-round for both rural and urban poor. Reduced fruit losses, through improved disease management, will improve the amount of fruit moving into the local trade sector, impacting not only on farmers but on all stakeholders involved in the value chain.

Three women farmer groups run a home-based processing industry for banana chips. Despite the good market for banana chips, raw material supplies are limited especially for the variety Nangka.

9.3.3 Environmental impacts

The project potentially encouraged the adoption of exotic disease-resistant cultivars and could consequently adversely affect the conservation of indigenous diversity. However, the disease equally presents the threat that growers will abandon unproductive traditional cultivars, so curbing its impact is expected to positively influence banana biodiversity. Bioversity's experience in in situ conservation-through-use of banana diversity in East Africa, suggests that strengthening local producer communities can both improve incomes and health, while improving the sustainable management of the natural resource base. In addition, Bioversity has a primary concern in the conservation of *Musa* diversity through both *in situ* and *ex situ* mechanisms, and a global conservation strategy has been developed for *Musa* that recognises the importance of indigenous diversity in both Indonesia and Australia. Furthermore the encouragement of sustainable integrated [pest management practices will reduce dependence on poorly used pesticides The particular integration of harnessing soil suppressiveness to help combat the disease would also

have positive environmental impacts in terms of healthier soils with more divers microbial communities.

9.4 Communication and dissemination activities

With pilot studies forming an integral part of the project, communication and dissemination activities occurred throughout the life of the project. As the target audience of this project, smallholders and researchers engaged in trainings with researchers from ITFRI, as well as local Dinas' extension agents and underwent trainings in appropriate best management practices. Training manuals and brochures that promote the success of management practices introduced in the two pilot communities have yet to be developed to encourage and aid in technology uptake in provinces outside Cianjur, West Java and Lampung.

Researchers from ITFRI, GMU and the DEEDI prepared and presented technical publications for scientific journals in relation to their work on biological control agents, suppressive soils and *Foc* /Race relationships for the benefit of the wider scientific community. Positive results occurring in relation to this work are being extended to pilot communities as the roll out of management strategies occur over the duration of the project.

A protocol manual was developed for testing soils for suppression to *Foc*. The protocol manual details tests used to characterise the soil. These include physical tests: texture and water aggregate stability; chemical tests: pH, EC, nitrate-nitrogen and labile C; biological tests: nematode diversity, fluorescein diacetate (FDA) hydrolysis, □-glucosidase activity; Fusarium fungistasis; and a small plant test for suppression of Fusarium. Some of the methods require modification from the original published methodology and revalidation.

A risk analysis tool (RAT) was also developed from this project.

As the coordinator of the project, Bioversity International ensured that the results of the project were disseminated at a regional level through the BAPNET newsletter, technical journals and involvement in relevant conferences. Moreover, for a broader global information dissemination pathway, project outputs were documented and integrated to the banana resource information centre, a web-based information resource platform developed and coordinated by Bioversity. Some of the project team were involved in the presentation of research results at relevant conferences within both Australia and Indonesia and elsewhere.

Scientific articles will be written and published in refereed journals such as *Australasian Plant Pathology*, and *Plant Disease*.

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10 Conclusions and recommendations

This project has yielded scientific evidence that soil-suppression can reduce the severity of Foc infection and epidemics. The nature of soil biological-physical properties affects soil-suppression. Production management practices such as ground-cover manipulation, soil amendments, and even intercropping can be implemented to enhance soil-suppression as a part of IPM in managing banana wilts. Below it is recommended to validate and outscale such concepts and results. Key production practices such as using clean planting materials, population management, mixed cropping to widen genetic diversity and reduce vulnerability should be validated in other areas where the disease is causing damage.

Several strains of Foc exist in Indonesia. The observed interaction between strains and banana cultivars provides new evidence that the host-pathogen interaction (as described by Race classification by earlier researchers) is not enough to effectively characterize the pathogen virulence and host resistance interaction. It also indicates that cultivar deployment can be used to help manage the disease and minimize risk of developing severe epidemics. The Foc strain by variety interaction highlights the importance of evaluating varieties against strains of Foc in different agro-ecological situations. Screenhouse results must always be validated with field results especially if practical recommendations are to be made out of these trials.

10.1 Conclusions:

The overall goal of the project was to *enhance the productivity and improve the livelihoods of small-scale banana farmers in Indonesia and Australia, and to effectively manage banana wilts through an integrated approach in banana crop production*. Cluster analysis shows that as a result of the project work, some targeted farmer-co-operators have been successful in reducing wilt levels, and boosting their productivity and income. This includes the production of a corm-bit nursery business and a cottage banana chip processing enterprise.

The project has largely fulfilled its three objectives focusing on developing, evaluating, and refining integrated crop and pest management practices to manage banana wilts and boost banana productivity. Project work has identified, evaluated and refined a range of 15 IPM/ICM options in two pilot sites in Indonesia, involving 35 farmer co-operators, and compared these practices with standard practices in the same areas. Up to eight of these management practices were adopted by the farmer co-operators, where using shoots for planting, planting in rows and using a range of banana cultivars were the most commonly adopted. Cluster analysis helped to understand which combinations of practices were the most successful in reducing disease levels, and boosting productivity and income. Results showed that farmers who use healthy planting materials produced as tissue culture or corm-bits, coupled with fertilization and population management, generated as much as a three-fold income increase over non-adopters. These practices were more adopted in Lampung than in Cianjur, where the former were more dependent on bananas compared with Cianjur where average landholdings are larger, and farmers were growing a wider range of cash crops together with bananas. Intercropping in bananas reduced disease levels and also earned higher income compared with bananas that weren't intercropped. This demonstrated the value of genetic and cropping diversity in disease management, and their contribution to a more profitable and sustainable production system

The proposed guidelines presenting the range of IPM/ICM options and how to combine them for best effect will soon be published. The work has also produced a risk analysis tool (RAT) to further support wilt management.

Research in both Indonesia and Australia has also greatly improved understanding on how certain combinations of physical, chemical and biological factors can significantly suppress Fusarium wilt. The work has shown that many different mechanisms may be involved in disease suppression. Direct antagonist interaction with the pathogen is one mechanism, which may have been occurring in Indonesia, whilst indirect methods involving a plant may have been occurring in Australia. The indirect mechanism may involve competition on the plant roots, production of antifungal products by rhizosphere organisms or induced suppression in the plant caused by soil organisms. The presence of magnesium and manganese may also play some role. Harnessing soil suppressiveness may offer real potential for contributing towards more effective management of banana Fusarium wilt in the future. A protocol manual has been developed for testing soils for suppression to Foc. The project has also built on our earlier work on characterising Foc virulence in terms of VCGs and their distribution, virulence, host-pathogen relations. Our new understanding will help better manage Foc within and beyond the target region.

The project has also collected some data on local Indonesian banana supply chains which will help inform any scaling out activities.

10.2 Recommendations

In offering these recommendations, Bioversity recognizes the strategic importance of the Australian-Indonesian relationship, and that *increasing the productivity, profitability and competitiveness of Indonesian horticultural and other high-value plant products* is an articulated priority of the Indonesian Government. The recommended follow-up work should also be consistent with ACIAR's four priority research thematic areas - crops – and contribute to both increasing productivity, quality and market access for agriculture products, and to greater resilience and diversity of production systems.

These recommendations aim to build on both the quality project outputs to date, and the strong relationships that have been developed throughout the project with our Australian and Indonesian partners. They must also link to other related *Musa* regional research in the Philippines, China and beyond and in Africa and Latin America and the Caribbean. Bioversity's regional networks, including BAPNET as well as the researcher network ProMusa will play important roles in the scaling of this work, particularly in the global context of mitigating the threat of Foc TR4. BAPNET will discuss this important follow up work during its upcoming biennial meeting in November 2014.

1. BAPNET discusses how best to follow-up on this important work.
2. Future work must also contribute to efforts to mitigate the global threat posed by Foc TR4 (and indeed any other virulent strains that may appear), by applying locally adapted ICM/IPM practices, incorporating the new knowledge on host-pathogen relations and soil suppressiveness, and linking also with the contingency and quarantine work implemented in both the Asia Pacific and Latin America and the Caribbean Pacific (e.g see TR4 contingency plan.) Such work may also be better informed by the experience of the Australians. Although TR4 has appeared in the Northern Territory of Australia, it has not spread to other banana growing states, as Australian scientists have focused on containing this disease through

regulatory measures including quarantine, the use of disease-free planting materials and good farm practice.

3. In order to fully achieve the overall goal of *enhancing the productivity and improving the livelihoods of small-scale banana farmers* (*in Indonesia and Australia*)*, and to effectively manage banana wilts through an integrated approach in banana crop production* at scale, the package of technologies developed in this project will need to be finalised and then transferred to and implemented within a wider national, regional and international context. This will need to be accompanied by developing the necessary capacities and other resources (as exemplified in our work in the Philippines). Indeed in the past we have considered a different possibility of testing this work further in Cambodia, where growth in horticultural farm-level productivity is also a priority articulated by Cambodian Government, Research Organizations and Civil Society, as is promoting diversification into non-rice crops
4. Capacity-building materials such as brochures and booklets must be developed as soon as possible, so as to be able to more effectively build-capacity for adopting successful cultural and disease management practices for improved banana production.
5. The project has evaluated the current level of ICM/IPM practice adoption. We recommend both an immediate assessment of how and why particular practices and combinations of practices have been adopted or have not been adopted, and an assessment of sustained adoption in (say) 3 years time. The immediate assessment should validate those practices and/or combinations of practices that prove most effective in managing banana wilts.
6. As part of the scaling out process, it is recommended that the lead farmers from the pilot sites be encouraged to help facilitate the adoption of generated technologies by other farmer co-operators and even non-cooperators and feedback information to extension workers and researchers and technicians. This will include participating in future trainings and other capacity-building events.
7. Similar VCG studies should be done in other countries to assess risk and impact of the disease and to develop a cultivar deployment strategy.
8. As a result of the VCG work, it is recommended that a range of diagnostic reference cultivars be developed, to better inform cultivar deployment and quarantine policy enforcement, as part of future measures to control incidence and spread of banana wilts.
9. The role that β-glucosidase plays in suppression of Fusarium wilt of bananas must be further investigated, in support of newly begun studies in the Philippines to validate learnings from Indonesia and Australia on suppressive soil.
10. Any future work should also consider market and gender dimensions. A more rigorous value chain analysis could build on the existing work some on supply chains

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12 Appendices

Annex Table 1. List of farmers and farming choices in Sarampad, Cianjur

No	Name	Name of farmer group	Type of intervention intended to be done by the farmer								
			1	2	3	4	5	6	7	8	9
1	Hidayat	Mekarsari				V					
2	Herman	Mekarsari	V				v				
3	Afud	Mekarsari							V		
4	M. Harun	Mekarsari	v	v	v	V	v	v	v	v	V
5	Asep	Mekarsari						v			V
6	Asep (3000m)	Mekarsari	v	v	v	V	v	v	v	v	V
7	Yus	Mekarsari					V				
8	Deni	Berkah		v		V					
9	Amin	Berkah		v		V					
10	Kakah	Bahtera	v	v	v	V	v	v	v	v	V
11	Muslih	Bahtera								V	
12	Oleh	Hegarmanah		v				V			
13	Kokoh	Hegarmanah		v				V			
14	Afud	Hegarmanah		v				V			
15	Sutowo	ILM			v	V					

Legend: 1 - land preparation; 2 - banana population management; 3 - crop diversity; 4 - use of disease-free; planting materials; 5 - nutrient management; 6 - soil-water management; 7 - early disease monitoring and eradication; 8 - plant protection; 9 – quarantine

Annex Table 2. Land ownership, crops, and type of intervention intended to be done by banana farmer in Legundi village – South Lampung

No	Name	Land area (ha)	Existing crop	Type of intervention intended to be done by the farmer								
				1	2	3	4	5	6	7	8	9
1	Wardiyanto	0.50	Pisang; umur bervariasi	v	v	v	v	v	v	v	v	v
2	Panji Sukisno	2.00	Pisang			v	v		v		v	
3	Jasmanto	0.50	Pisang; kelapa				v	v				
4	Kamiyo	0.50	Pisang; karet	v			v					
5	Poniman	0.50	Pisang; sengon				v				v	
6	Sumarno	2.00	Pisang; kakao, karet, sawit	v	v	v	v	v	v	v	v	v
7	Tono	0.50	Pisang; kelapa				v				v	
8	Mustofa	0.25	Pisang; coklat		v						v	
9	Sutari (Kampung Baru)	1.00	Pisang; kelapa di pinggir				v				v	
10	Nonok (Kampung Baru)	1.75	Pisang; kelapa pinggiran	v								
11	Suwito (Tanjung Jaya)	1.00	Pisang; kelapa pinggiran				v				v	
12	Jaliman (Kp. Baru)	1.00	Pisang; kayu tahunan				v	v	v	v	v	v
13	Parno (Tanjung Jaya)	0.75	Lahan kosong				v					
14	Suwanto (Tanjung Jaya)						v				v	
15	Sahri (Tanjung Jaya)	1.00	Pisang; jagung, kelapa				v				v	

No	Name	Land area (ha)	Existing crop	Type of intervention intended to be done by the farmer								
				1	2	3	4	5	6	7	8	9
			sawit									
16	Herianto (Kp. Baru)	0.25	Pisang			V				v		V
17	Parwono (Kp. Baru)	1.50	Pisang; jagung, kelapa sedikit		v		V				V	
18	Chamit (Kp. Baru)	0.75	Pisang; jagung, kelapa	v	v	v	v	v	v	v	V	v
19	Wasis (Kp. Baru)	0.50	Pisang; kelapa di pinggir	V			V				v	
20	Wayan Kumpul (Tri Darma Yoga)			v	v	v	V	v	v	v	v	V

Legend: 1 - land preparation; 2 - banana population management; 3 - crop diversity; 4 - use of disease-free; planting materials; 5 - nutrient management; 6 - soil-water management; 7 - early disease monitoring and eradication; 8 - plant protection; 9 - quarantine

Annex Table 3. Income situation of banana farmer cooperator at pilot site

Pilot site	Income situation			
	Average	Minimum	Maximum	Stdev
Sarampad – Cianjur[*)]				
Income (Rp)	5.289.175	379.500	9.000.000	2.202.384
Share of banana to income (%)	14,94	0,00	60,47	19,54
Expenses:				
Consumption (Rp)	973.684	300.000	2.000.000	457.763
Recreation (Rp)	262.500	150.000	400.000	110.868
Health (Rp)	519.444	25.000	2.150.000	771.745
Education (Rp)	265.000	100.000	1.000.000	263.576
Social (Rp)	195.833	25.000	500.000	235.805
Income - expenses (Rp)	3.301.763	(3.070.500)	22.100.000	5.914.736
Legundi - South Lampung				
Income (Rp)	2.756.632	44.000	12.875.000	3.473.228
Share of banana to income (%)	52,84	5,33	100,00	40,29
Expenses:				
Consumption (Rp)	506.111	200.000	800.000	234.700
Recreation (Rp)	111.111	-	1.500.000	366.042
Health (Rp)	24.167	-	150.000	39.267
Education (Rp)	319.444	-	2.150.000	532.513

Annex Table 4. Disease incidence in the banana farms of farmer-cooperators at pilot sites

Pilot site	Number of systemic-infected plant			
	Average	Stdev	Minimum	Maximum
Sarampad – Cianjur				
number of plant	786,74	790,21	100,00	3000,00
Wilt diseases	6,13	6,62	1,00	20,00
Bunchy top	2,67	2,08	1,00	5,00
Total of systemic-infected plant	2,85	5,97	0,00	25,00
Disease incident (%)	0,37	0,67	0,00	2,50
Legundi – South Lampung				
number of plant	642,75	595,11	30,00	2000,00
Wilt diseases	2,44	4,37	0,00	16,00
Bunchy top	1,20	2,68	0,00	10,00
Total of systemic-infected plant	2,85	5,04	0,00	16,00
Disease incident (%)	0,50	0,95	0,00	3,33

Annex Table 5. Nine compulsory management options and six more suggested management options implemented at Cianjur and Lampung

No	Nine management options	No	Six management options
1.	Land Preparation	1.	Utilization of resistant variety
2.	Plant density management	2.	Site selection
3.	Crop diversity	3.	Weed management
4.	Clean planting material	4.	Biological control
5.	Nutrient management	5.	Vector management
6.	Soil moisture management	6.	Management of other pests
7.	Early detection of pests		
8.	Healthy planting		
9.	Quarantine		

12.1.1 Summary of Indonesian Project Activities

Indonesian Tropical Fruit Research Institute and ICHORD

Abstract

Indonesia is one of the centres of origins and diversities of banana. An ACIAR-funded – Bioversity International project (HORT 2008/040) entitled "Integrated Crop Production of Banana in Indonesia and Australia" aimed to: 1) validate race concepts on *Fusarium oxysporum* f. sp. *Cubense (Foc)* with pathogenicity tests of VCG group isolates against a range of *Musa* cultivars and cultivars, and 2) Develop of integrated pest management (IPM)/integrated crop management (ICM) packages and evaluate and adapt best management practices in banana production. Two set of experiments were established, namely screenhouse and field test of pathogenicity of *Foc* VCG toward banana accessions, and validation of best-bet integrated crop production on pilot studies in Cianjur and Lampung. Banana cultivars responded differently to different *Foc* VCGs. Bananas grouped into the same subgroup such as Pisang Ambon Kuning and Ambon Putih (both are Gros Michel) performed differently on different VCGs. The results opened possibility to find resistant variety among the same banana subgroup. Further test of more VCGs against more cultivars would be valuable to completely develop new concept of differential races on banana. Higher disease incidence of wilt diseases happened in the banana fields of non-cooperating farmers.

Introduction

Indonesia is one of the centre of origins and diversities of banana in the world. Historical records and surveys showed that the existing commercial bananas were naturally composed in the country and the region. In the past, Indonesians grew banana for wrapper, medicine, and ethnical believes, besides source of food supply. The situation resulted in high variation of bananas grown. Being the biggest contributor to Indonesian fruit production, banana involves 21,482 million banana-growing households for home consumption and cash crop.

Banana is produced in three types of farming namely, monoculture by big plantations, mixed cultivars/crops that are mainly done by smallholder farmer, and backyard mixed crops as part of farmer's livelihood. The first system provides implementation of good management and good handling practices, which is basically profit-oriented. The system, however, holds significant risk to pests and diseases outbreak and impact to environment situation. Cote *et al.* (2009) stated that monoculture of banana can have a serious detrimental impact on the environment as pesticide treatments can lead to surface and ground water pollution. Upon evaluation and implementation of integrated approaches of pest management, the author further mentioned that new management system of integrated crop production have led to a 65% decrease of pesticide use over the last 10 years. The last two types of banana farming system were easily found in developing countries like Indonesia. Besides its weakness in terms of farm management, the mixed cropping system provide volatility in product utility, income security and plant protection from pests and diseases. The work aimed to study the effect of whole situation of banana cropping system to farmers' livelihoods.

Besides its significant contribution to world food agriculture, banana has been threatened by complex of pests and diseases (Jones, 2009). Discussions among banana scientists, mainly taxonomist and breeder, during the *Musa*Net meeting in Bogor – Indonesia in 2012 concluded that diseases remain to be the main problem on world banana production (adopted from Uma presentation in BAPNET meeting, 2012). The finding was supported in Hermanto *et al*. (2011) as it mentioned that pests and diseases were the main complain of banana farmers in Indonesia. For that reason, commercial banana plantations spend 40-50% of their investment to control diseases (mainly Sigatoka leaf spot) for their farm. Wardlaw (1972) listed no less than 350 microbes, insects, and abiotic factors, while

Stover (1987) elaborated no less that 46 pests, diseases, and disorders existing concomitantly with banana. In a survey conducted by Hermanto *et al*. (2011) in Indonesia, 25 pests, diseases, and disorder problems of banana production were found.

Methodology

Validation of Race concepts on Fusarium oxysporum f. sp. cubense with pathogenicity tests of VCG group isolates against a range of Musa cultivars and cultivars

Research was done at the laboratory and screenhouse of the Indonesian Tropical Fruit Research Institute (ITFRI) from July 2007 until March 2013.

Screenhouse test

The set up was arranged in randomized completely block design in factorial pattern, 3 replicates, and 10 plants as unit treatment. The first factor was 12 banana cultivars such as: Ketan 01 (AAB), Kepok tanjung (ABB), Raja kinalun, Ambon Hijau, Ambon kuning, Ambon kuning Jambi, Berlin, Kilita, Rejang, Barangan, Roti and Randah. Second factor was VCGs of *Foc*: VCG 0120/15, 0123, 0124/5, 0126, 0121, 01218, 01213/16, and 01219. Five minutes dipping was needed before the *vitro* plants were planted on 250 mL plastic cup arranged in double cup technique (Muhammed *et al.* 1999).The plants were cultivated for two months. Observation was focused on:

1). Incubation period: period from time of inoculation to the first appearance of symptom

2). Disease incidence was observed at the end of observation using this formula:

$$P = \left[\frac{T_1}{T_2}\right] \times 100\%$$

where, P=disease incidence, T1=number of infected plants, and T_2=number of observe plants

3). Leaf disease severity index (LDSI) was assessed using Mohamed *et al*. (1999) scoring system as follow: 1=No streaking or yellowing of leaves, plant appears healthy; 2=Slight streaking and/or yellowing of lower leaves; 3=Streaking and/or yellowing of most of the lower leaves; 4=Extensive streaking and/or yellowing on most or all of the leaves; 5=Dead plant.

4). Corm disease severity index (CDSI) was assessed through destructive sampling at the end of experiment (2 months after inoculation). Prior to the experiment, the corm was cleaned from roots and soil, and cross sectioned at the middle and basal end. Observation was done using Jones (1994) scoring system as follows: 1=Corm completely clean no vascular discolouration; 2=Initial points of discolouration in vascular tissue; 3=Discolouration of up to 1/3 of vascular tissue; 4=Discolouration between 1/3 and 2/3 of vascular tissue; 5=Discolouration greater than 2/3 of vascular tissue; and 6=Total discolouration of vascular tissue.

Index of disease severity on leaves and corm are calculated by the formula:

$$I = \Sigma \frac{\text{scale value x number of plants of each of the scales}}{\text{Number of plants}}$$

Virulence of isolates were categorised based on the percentage of wilted plants as well as disease severity index on leaves (LDSI) and corm (CDSI) as shown in Table 1.

Table 1. Category of resistance of banana cultivars against Vegetative Compatibility Groups *Foc* isolates

Percentage of wilted plant	Disease severity Index		Incubation Period	Category of resistance
	Leaf	Corm		
0	1	1		Highly resistant
≤20	<2	<2		Resistant
>20-50	≤2	≤2		Moderately resistant
>50-75	>2-4	>2-4		Susceptible
>75	>4	>4		Highly susceptible

Data were analysed by variance. If the results obtained are significantly different, it is further tested for the Least Small Differences (LSD) at 5% level.

Field test

The experiment was conducted at the Aripan experimental station and Plant Pests and Diseases Laboratory, ITFRI, Solok, from September 2010 to February 2012. A randomised block design was used with 12 treatments and 3 replications with 10 plants for each treatment unit. The treatments were 11 banana accessions which were artificially inoculated by isolates *Foc* VCG 01 213/16 plus 1 banana variety Ambon Hijau control (without inoculation by *Foc*). The accessions were: (A). Berlin, (B). Calcutta, (C). Kilita, (D). Klutuk Awu, (E). Barangan, (F). Ambon Hijau (G). Ambon Jambi, (H). Ketan, (I). Raja Kinalun, (J). Kepok, (K). Tanduk, and (L). Ambon Hijau (control).

Banana seedlings were artificially inoculated by *Foc* VCG 01213/16 16 at the density of 10^4 conidia/ml and control plants were grown in the field according to the research layout. Plant maintenance was performed based on recommendation, and weed control was done mechanically. Tissue samples were collected from infected plants to verify the infecting VCG. Observation was addressed to the following parameters:

1. Distribution of Fusarium inoculum in the soil was observed before treatment.
2. The incubation period, observed from one month after planting with monthly interval.
3. The percentage of infected plants, calculated based on monthly observation.
4. Index of disease severity on leaves or leaves disease severity index (LDSI), observed when visible symptoms of the disease appeared in the leaves. Level of damage was done by using modified Mohamed *et.al*. (1999) method was as follows:

   1 = no symptoms on leaves

   2 = leaf yellowing / symptomatic 1-10%

   3 = leaf yellowing / symptomatic 11-25%

4 = leaf yellowing / symptomatic 26-50%

5 = leaf yellowing / symptomatic > 50%

Development of Integrated Pest Management (IPM)/Integrated Crop Management (ICM) Package, and Evaluate and Adapt Best Management Practice in Banana Production

Pilot communities were established in West Java and South Lampung, two provinces where banana production provides an important source of income for smallholder growers, and where BBD and *Foc* constrain production. The pilot studies focused on a single community in a catchment area of each province. The single catchment area selection was based upon criteria such as geographical site location, grower attitude, local government boundaries, and ensuring that the selected community is representative of other major banana growing areas. Inception meeting involved banana scientists and policymakers identified 15 management options to be the interventions among farmer-cooperators and was evaluated for their implemention (Table 2). The options consisted of crop management options (i.e. land preparation, clean planting materials, plant density management, nutrient management, soil water moisture management, crop diversity (i.e. intercropping, crop rotation)), and disease management options (i.e. variety selections, use of clean planting materials, early detection and eradication, weed control, and vector management). Since each option consisted of more than one component, a total of 41 components were evaluated. To see the impact of the project, additional non-cooperator farmers were observed at the end of the research.

Baseline data from each farmer such as information on knowledge of disease symptoms and management, current farm cultural and disease management practices, pest and disease incidence, IPM options in place, and sources of information/technologies/funding and other parameters were collected. Accordingly, all interventions, practices, and subsequent data on productivity components (i.e. agronomic traits, yield, quality, and inputs) were also collected, analysed and documented.

Table 2. Management options and package of technologies (POTs) presented to farmer-cooperators as possible interventions for implementation at the pilot sites

Management options	Component of technology
1. Land preparation	a. Zero tillage
	b. Minimum tillage
	c. Ploughing
	d. Contour/farm architecture management
2. Plant density management	a. Planting space arrangement
	b. Row arrangement
	c. Desuckering
3. Crop diversity/farming system	a.Crop rotation
	b. Mixed cropping
	c. Annual cropping system
	d. Monoculture multicultivars

Management options	Component of technology
4.(Healthy) Planting material	a. Tissue culture
	b.Bit
	c.Axilar shoot stimulation
	d. Sucker
5. Nutrient management	a. Liming
	b. Manuring
	c. Un organic fertilisation
	d. Micro nutrient application
6. Water management	a. Irrigation
	b. Drainage
7. Pest early detection and eradication	a. Pest scouting/recognition
	b. Eradication
	c. Integrated control
8. Crop health	a. Fruit bagging
	b. Deflowering
	c. Tool sterilisation
9. Quarantine	a.No sucker movement
	b.Irigation and drainage management
	c.Isolation
	d.No plant material movement
10. Resistant variety	Resistant variety
11. Site selection	Site selection
12. Weed management	Weed management
13. Biological control	a. Suppressive soil
	b. Biofumigation
	c. Application of biocontrol
14. Vector management	Vector management
15. Management of other pests	a. control of wilt diseases
	b. control of banana bunchy top disease
	c. control of other pests

Results and Discussion

Validation of race concepts on Fusarium oxysporum f. sp. cubense with pathogenicity tests of VCG group isolates against a range of Musa cultivars and cultivars

Screenhouse test

Berlin (AA Sucrier) variety showed increased tolerance and resistance to *Foc* race 1 (VCGs 0123, 0124/5, 0126 and 01218). LDSI, CDSI and percentage of disease incidence of this variety against VCGs 0123 and 0126 for were very low. Response of this variety to *Foc* Race 4 varied from susceptible and highly susceptible against VCGs 0121 and 01213/16, and slight resistance to VCGs 01219. Ploetz and Pegg (2000) mentioned that the Sucrier is susceptible to TR4. Pisang Rejang (diploid *accuminata*), on the other hand, performed differently, from resistant to highly resistant. Its high susceptibility level toward VCG 0124/5 was followed by recovery phenomenon at later observation (Table 3).

Table 3. Incubation period (IP), Disease Incidence (DI), Leaf Disease Severity Index (LDSI) and Corm Disease Severity Index (CDSI) of Berlin (AA Sucrier), Rejang (AA cv Rose) and Ketan (AA) tested to 8 *Foc* VCGs, 3 months after treatment

Banana variety	VCG *Foc*	Incubation Period (day)**	Disease Incidence (%)	Disease severity index		Resistance category
				Leaf	Corm	
Berlin (AA)	0120/15	19.00	19.11	1.39	1.17	Resistant
	0123	54.33	16.67	1.133	1.1	Resistant
	0124/5	40.07	36.67	1.5	1.367	Moderately resistant
	0126	25.67	16.67	1.4	1.43	Resistant
	01218	≈	0	1	1	Highly resistant
	0121	21.81	96.67	2.77	2.73	Susceptible
	01213/16	45.29	93.33	3.07	3.63	Susceptible
	01219	61	10.00	1.07	1.0	Resistant
Rejang (AA)	0120/15	35.88	26.67	1.43	1.5	Moderately resistant
	0123	0	0	1	1	Highly resistant
	0124/5	17.03 (30 dai healthy sucker)	100 (MP) 43.33 (S)	3.83 (MP) 1.87 (S)	2.43	Highly Susceptible (recovery phenomenon)
	0126	0	0	1	1	Highly resistant
	01218	0	0	1	1	Highly resistant
	0121					
	01213/16	69.19	63.33	1.97	1.53	
	01219	54.2	16.667	1.27	1.067	Resistant
Ketan 01 (AA)	0120/15	20,56	20	1,19	1,16	Resistant
	0123	51,33	5,33	1,08	1	Resistant
	0124/5	13,01	96,67	2,25	3,33	Susceptible
	0126	40,83	15,83	1,24	1	Resistant
	0121					
	01218	35,67	15,88	1,11	1,06	Resistant
	01213/16	11,78	75,43	2,25	2,93	Susceptible
	01219	11,06	27,5	1,19	1,08	Moderate resistant

Note : MP = mother plant, S = Sucker

***** =** incubation period was not included in categorizing degree of resistance*

Screenhouse stage test result showed that the Cavendish cultivars (Randah, and Ambon Hijau) can be attacked by eight (8) known VCGs of *Foc,* while the resistance levels differed (Table 4).

Table 4. Incubation period (IP), Disease Incidence (DI), Leaf Disease Severity Index (LDSI) and Corm Disease Severity Index (CDSI) of pisang Randah (dwarf cavendish) and Ambon Hijau (Ripe green Cavendish banana) tested to 8 *Foc* VCGs, 3 months after treatment

Banana variety	*Foc* VCG	Incubation Period (day) **	Disease incidence (%)	disease severity index		Category of resistance
				Leaf	Corm	
Ambon Hijau (Cavendish/ AAA)	0120/15	14.80	38.33	1.47	1.57	Moderately resistant
	0123	34.42	40.27	1.60	1.29	Moderately resistant
	0124/5	13.37	96.67	2.92	4.09	Highly susceptible
	0126	22.32	47.5	1.51	1.27	Moderately resistant
	01218	29.07	33.33	1.33	1.11	Moderately resistant
	0121	12.37	85.00	3.73	5.38	Highly susceptible
	01213/16	12.57	95.83	4.40	5.90	Highly susceptible
	01219	16.48	34.73	1.39	1.48	Moderately resistant
Randah (Cavendish/ AAA)	0120/15	56.50	66.67	1.93	1.47	Moderately resistant
	0123	47.78	37.78	1.84	1.41	Moderately resistant
	0124/5	21.60	96.67	4.17	3.73	Highly susceptible
	0126	25.16	83.33	2.46	1.80	Susceptible
	01218	80.25	16.67	1.17	1.00	Moderately resistant
	0121	22.17	100.00	3.83	4.93	Highly susceptible
	01213/16	23.13	96.67	3.77	4.63	Highly susceptible
	01219	46.92	23.33	1.37	1.20	Moderately resistant

According to Stover (1962) and Ploetz (2006), Cavendish cultivars have been found to succumb only to *Foc* Race 4 (0121, 01213/16 and 01219). In this study, two cultivars (Randah and Ambon Hijau) were observed to be was highly susceptible to VCG 0124/5 (Race 1). Similarly, the Randah variety was susceptible to VCG 0126 and Ambon Hijau was also susceptible to VCG 0123 (both VCGs are Race 1). The response of these cultivars against *Foc* Race 4 (0121, 01213/16 and 01219) were susceptible and highly susceptible, except for Randah which was tolerant to VCG 01219. VCG 0120 is subtropical Race 4 which is said to be capable of infecting the Cavendish group in subtropical areas; whereas in tropical areas, these VCGs grouped into *Foc* Race 1. However, results showed that Ambon Hijau and Randah (Cavendish) were susceptible and tolerant to this VCG. This phenomenon was also found in the field, where Ambon Hijau was attacked by *Foc* VCGs 0120 in West Java.

Ambon kuning (AAA Gross Michel) was highly susceptible to the seven tested *Foc* VCGs as indicated by its short incubation period (14-36 days), high disease incidence (95-100%), and higher average of LDSI and CDSI. However, Ambon Putih Jambi performed differently with various level of susceptibility to different tested VCGs (Table 5).

Table 5. Incubation period (IP), Disease Incidence (DI), Leaf Disease Severity Index (LDSI) and Corm Disease Severity Index (CDSI) of Ambon Kuning and Ambon Putih Jambi (AAA Gros Michel) tested to 8 *Foc* VCGs, 3 months after treatment

Banana variety	VCG *Foc*	Incubation Period (day) **	Disease Incidence (%)	Disease severity index		Category of resistance
				Leaf	Corm	
Ambon kuning (Gros Michel/ AAA)	0120/15	18.56	95.23	4.67	5.07	Highly susceptible
	0123	14.49	100.00	4.11	4.68	Highly susceptible
	0124/5	36.46	100.00	3.51	3.84	Highly susceptible
	0126	21.17	100.00	5.07	5.87	Highly susceptible
	01218	28.35	95.56	3.39	3.28	Highly susceptible
	0121					
	01213/16	21.17	100.00	5.14	5.15	Highly susceptible
	01219	14.58	100.00	4.23	5.1	Highly susceptible
Ambon Putih Jambi (Gros Michel/ AAA)	0120/15	59.3	10	1.13	1.06	Resistant
	0123	47.6	40	1.4	1.4	Moderate Resitant
	0124/5	45.2	85	2.13	2.6	Susceptible
	0126					
	01218					
	0121	14.4	100	3.7	3.3	Susceptible
	01213/16	14	100	4.5	4.1	Highly Susceptible
	01219	58.6	10	1.13	1.13	Resistant

Susceptibility of Ambon Kuning to all the tested Race 1 and Race 4 VCGs was in line with the report of Ploetz and Pegg (2000). Different results of the same Gros Michel subgroup from Ambon Putih Jambi shows new opportunity in growing Gros Michel in Race 1 infected areas.

The best-tasting dessert banana Pisang Barangan (AAA) was one of the most susceptible to Fusarium wilt. However, the findings showed that although it was highly susceptible to TR4 *Foc* VCG 01213/16, Race 1 *Foc* VCG 0124/5 and susceptible to Race 1 *Foc* VCG 0126, the variety performed better on the other VCGs (Table 6).

Table 6. Incubation period (IP), Disease Incidence (DI), Leaf Disease Severity Index (LDSI) and Corm Disease Severity Index (CDSI) of Pisang Barangan (AAA) tested to 8 *Foc* VCGs, 3 months after treatment

Banana variety	*Foc* VCG	Incubation Period (day) **	Disease incidence (%)	disease severity index		Category of resistance
				Leaf	Corm	
Barangan (AAA)	0120/15	24.5	47.9	2.0	1.72	Moderately resistant
	0123	58	67.76	1.98	1.76	Moderately resistant
	0124/5	20.33	83.63	4.25	4.25	Highly Susceptible
	0126	10.9	54.4	3.32	2.1	Susceptible
	01218	33.1	15.5	1.51	1.34	Resistant
	0121					
	01213/16	14.52	96.6	4.6	5.62	Highly susceptible
	01219	16.66	33.33	2.11	1.93	Moderately resistant

The AAB-cooking Pisang Roti was observed to be resistant to all the tested VCGs except 0124/5 (Race 1), 0121 (Race 4), and 01213/16 (TR4). Pisang Roti is the best variety for banana chips. Different response to different *Foc* VCG gives opportunity to grow the variety. Other AAB-cooking, Pisang Kilita (AAB French plantain) was highly susceptible to all tested *Foc* VCGs except VCG 01218 (Race 1) which was susceptible (Table 7). This scenario should raise alarm in banana-growing areas in Papua eastward where this variety predominantly exists.

Table 7. Incubation period (IP), Disease Incidence (DI), Leaf Disease Severity Index (LDSI) and Corm Disease Severity Index (CDSI) of Pisang Roti tested to 8 *Foc* VCGs, 3 months after treatment

Banana variety	VCG *Foc*	Incubation Period (day) **	Disease insidence (%)	disease severity index		Category of resistance
				Leaf	Corm	
Roti (AAB)	0120/15	32.3	0	1.13	1.06	Resistant
	0123	≈	0	1	1	Highly resistant
	0124/5	15.5	100	5.67	4.6	Highly susceptible
	0126	≈	0	1	1	Highly resistant
	01218	≈	0	1	1	Highly resistant
	0121	11.9	100	5.73	4.13	Highly susceptible
	01213/16	12.3	100	5.93	4	Highly susceptible
	01219	44.11	18.67	1.24	1.2	Resistant
Kilita (AAB French Plantain)	0120/15	14.97	100	4.77	5.57	Highly susceptible
	0123	10.1	100	4.87	5.9	Highly susceptible
	0124/5	18.57	100	4.53	5.6	Highly susceptible
	0126	11.00	100	4.73	6	Highly susceptible
	01218	19.62	66.67	2.67	3.0	Susceptible
	0121	16.79	100	4.45	5.7	Highly susceptible
	01213/16	11.10	100	4.215	5.02	Highly susceptible
	01219	12.27	100	4.7	5.7	Highly susceptible

Contrary to previous studies, Pisang Kepok Tanjung was observed to be susceptible to highly susceptible to all the tested *Foc* VCGs. The results were different from situation from the field where the variety was rarely found infected. Other ABB cooking Raja Kinalun performed better to Fusarium wilt except to VCG 0124/5 (Table 8).

Table 8. Incubation period (IP), Disease Incidence (DI), Leaf Disease Severity Index (LDSI) and Corm Disease Severity Index (CDSI) of Kepok Tanjung (ABB) and Raja Kinalun (ABB) tested to 8 *Foc* VCGs, 3 months after treatment

Banana variety)	VCG *Foc*	Incubation Period (day)**	Disease incidence (%)	Disease severity index		Resistance category
				Leaf	Corm	
Kepok tanjung (ABB/ BBB)	0120/15	10,72	96,67	2,92	4,48	Highly Susceptible
	0123	10,7	100	3,95	5,41	Highly Susceptible
	0124/5	11,47	100	4,03	5,83	Highly Susceptible
	0126	13,79	100	2,93	3,97	Susceptible
	01218	13,93	95,83	2,62	4,21	Highly Susceptible
	0121					
	01213/16	9,89	100	2,58	4,09	Highly Susceptible
	01219	11,27	96,67	3,02	4,99	Highly Susceptible
Raja kinalun (ABB)	0120/15	≈	0	1	1,03	Highly resistant
	0123	76,33	36,67	1,4	1,17	Moderate resistant
	0124/5	14,18	96,3	4,44	5,37	Highly susceptible
	0126	≈	0	1	1,03	Highly resistant
	01218	≈	0	1	1	Highly resistant
	0121					
	01213/16	46,34	56,67	1,63	1,68	Moderate resistant
	01219	≈	0	1	1	Highly resistant

Ploetz and Pegg (2000) grouped the banana cultivars distinctly into races and based on their response to *Foc* VCGs. Table 9, however, shows different evidence of varietal response to different *Foc* VCGs. Pisang Randah and Ambon Hijau (AAA Cavendish subgroup) for example, responded differently on different VCGs of Race 1, as well as Race 4. Bananas grouped into the same subgroup such as Pisang Ambon Kuning and Ambon Putih (both are Gros Michel) performed differently on different VCGs. Results open the possibility of finding resistant variety among the same banana subgroup.

Table 9. Response of banana cultivars toward various *Foc* VCGs

Banana variety	VCG							
	Race 1				Race 1/4	Race 4		
	0123	0124/5	0126	01218	0120	0121	01213/16	01219
Rejang (AA)	HR	HS	HR	HR	MR		R?	R
Berlin (AA)	R	MR	R	HR	R	S	S	R
Ketan 01 (AA)	R	S	R	R	R		S	MR
Ambon kuning (Gros Michel/ AAA)	HS	HS	HS	HS	HS		HS	HS
Ambon Hijau (Cavendish/ AAA)	MR	HS	MR	MR	MR	HS	HS	MR
Randah (Cavendish/ AAA)	MR	HS	S	MR	MR	HS	HS	MR
Ambon Putih Jambi (Gros Michel/ AAA)	MR	S			R	S	HS	R
Barangan (AAA)	MR	HS	S	R	MR		HS	MR
Roti (AAB)	HR	HS	HR	HR	R	HS	HS	R
Kilita (AAB)	HS	HS	HS	S	HS	HS	HS	HS
Kepok tanjung (ABB/BBB)	HS	HS	S	HS	HS		HS	HS
Raja kinalun (ABB)	MR	HS	HR	HR	HR		MR	HR

Field test

Results of *Foc* TR4 VCG 01213/16 field test differ from the screenhouse test on Pisang Berlin, Ketan and Kepok. The three cultivars were susceptible to the VCG in the screenhouse but showed good resistance in the field test. The finding might be caused by physiological development of the cultivars that resulted in resistance. Consistency in results both in screenhouse and field test was observed in Kilita, Ambon Hijau, and Ambon Putih Jambi. Barangan on highly infected *Foc* VCG 01213/16 was resistant since the existing pathogen was other VCG. Good source of resistance of wilt banana was performed by Calcuta (AAw) and Klutuk Awu (BB) (Table 10).

Table 10. Fusarium wilt performance on *Foc* VCG 01213/16 field test at Aripan Experimental Farm

No	Banana variety	Incubation period (days after planting)	Disease incidence (%)	Leave disease severity index	Resistance category	VCG testing result
1.	Berlin	-	0.0	1.0	HR	-
2.	Calcuta	200 – 326	14.8	1.29	R	01213/16
3.	Kilita	192 – 435	38.5	2.15	S	01213/16
4.	Klutuk Awu	-	0.0	1.0	HR	-
5.	Barangan	137 – 325	13.0	1.46	R	01213/16
6.	Ambon Hijau	165 – 358	30.8	2.06	S	01213/16
7.	Ambon Putih Jambi	259	3.6	1.07	R	01213/16
8.	Ketan	387	3.5	1.07	R	01213/16
9.	Raja Kinalun	-	0.0	1.00	HR	-
10.	Kepok	193	3.3	1.10	R	01213/16
11.	Tanduk	-	0.0	1.0	HR	-
12.	Ambon Hijau Kontrol	165 – 268	48.2	2.39	S	01213/16

Kilita, Ambon Hijau and Ambon Hijau control were found susceptible to *Foc* VCG 01213/16 in this study. The result from screenhouse testing gave the same assessment of these cultivars against *Foc* VCG 01213/16 which in screenhouse testing these concluded as the highly susceptible cultivars. Ambon Hijau control was also affected by *Foc* VCG 01213/16 despite the plants were not artificially inoculated. The plants were most likely attacked by indigenous *Foc* in the soil since Fusarium is endemic in the study site.

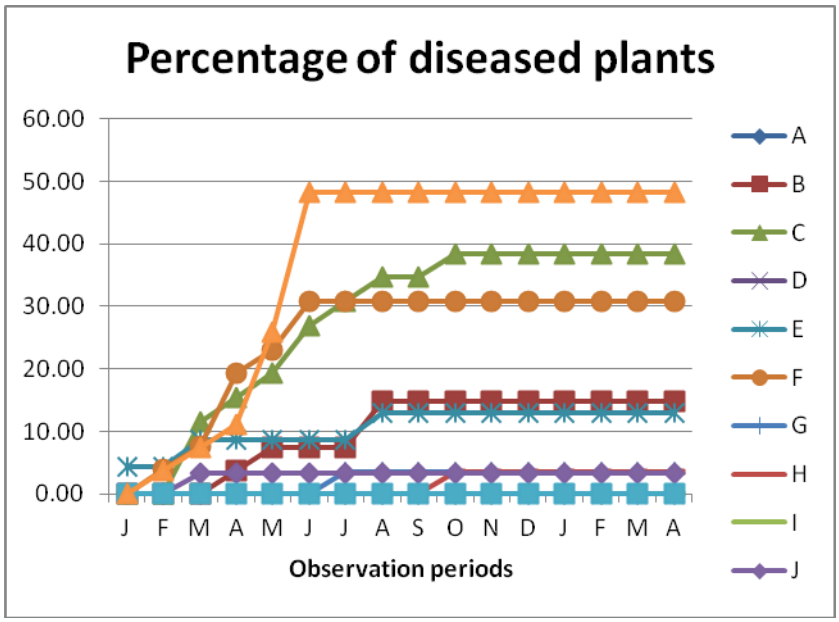

Figure 1. Development of fusarium wilt incidence in the field at 4-19 months after planting (MAP)

Fusarium wilt disease symptoms in the field were first observed four (4) months after planting on just one plant of Barangan accession, dispersing gradually to other plants. The diseased plants increased according to plant age. Four cultivars were not infected by Fusarium wilt until the last observation at 19 months after planting (i.e. Berlin, Klutuk Awu, Raja Kinalun and Tanduk). These cultivars were seemingly highly resistant accessions to Fusarium infection especially against *Foc* VCG 01213/16. Inoculated or non-inoculated, Ambon Hijau showed the highest diseased percentages compared to other cultivars, confirming that Ambon Hijau was the susceptible variety to *Foc* VCG 01213/16.

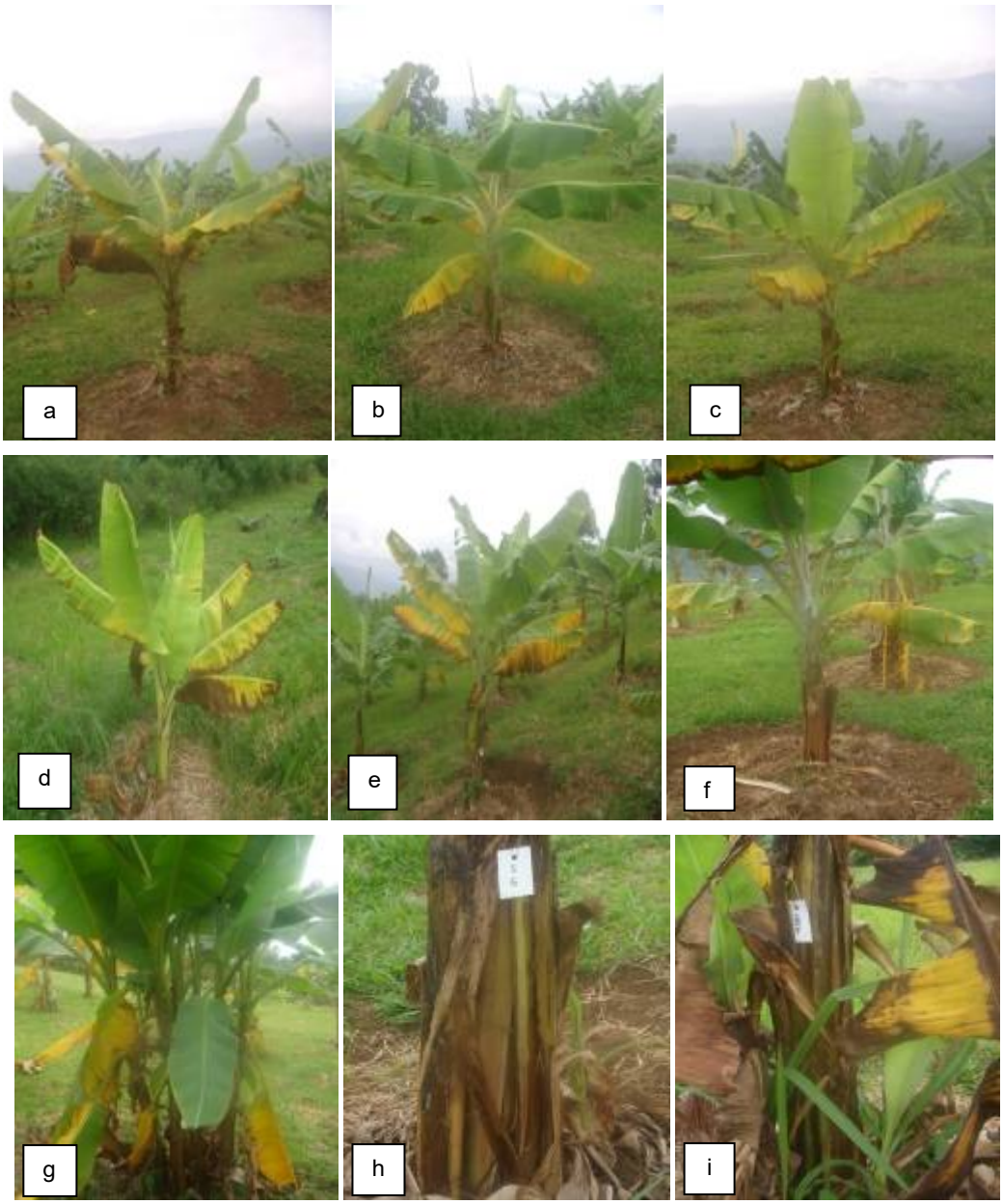

Figure 2. Fusarium wilt disease symptoms on banana accessions (a) Ambon Hijau, (b) Kilita, (c) Ambon Hijau Kontrol, (d) Barangan, (e) Ambon Jambi, (f) Ketan, (g) Calcuta, (h) pseudostem splitting on Ambon Jambi and (i) pseudostem splitting on Ambon Hijau.

Fusarium wilt disease symptoms clustered on some susceptible accessions while on the moderately resistant accessions, none or just a few of plants displayed the disease symptoms, although these accessions were located around the susceptible accessions. The disease symptoms spread quickly from one infected plant to another, on the susceptible accessions.

Development of Integrated Pest Management (IPM)/Integrated Crop Management (ICM) Package,and Evaluate and Adapt Best Management Practice in Banana Production

Implementation status of management option

Farmer-cooperators in Cianjur has wider land for banana cultivation than those in South Lampung, however, number of bananas they cultivated was almost the same (Table 11). The land ownership in Cianjur also varied, ranged from 0.12 – 3 hectares, while in South Lampung only ranged from 0.025 – 0.3 hectares. The situation consequently influences the share of banana toward family income.

Table 11. Number of banana plants and land width for banana cultivation

Pilot site	Number of banana plants and land width			
	Average	Minimum	Maximum	Stdev
Sarampad - Cianjur				
# of plant	786.74	100	3000	790.21
land width (m2)	8823.68	1200	30000	8177.99
Legundi - South Lampung				
Number of plants	633.75	150	2000	520.68
land width	1240	250	3000	771.64

Even though in the onset of the project (indicated by PRA result), the farmer-cooperators in Cianjur were known to have more advanced knowledge in banana cultivation in general, later on, they showed that they implemented the “must do” and “should do” management option less than those in South Lampung (Table 11). This situation might be due to the number of bananas they cultivate which was smaller in ratio vis-a-vis the land area (Table 11). While in South Lampung, the situation forces the farmer-cooperators to pay more attention to their banana farming.

Table 12. Number of management option that was implemented by the farmer-cooperators (based on data status on July 2011)

Pilot site		Number of management option that was implemented by farmer cooperators			
		Average	Deviasi	minimum	maximum
Sarampad - Cianjur					
9 +++ opsi management	Number	6.30	2.57	0	11
	Percentage	*20.32*	*8.31*	*0*	*35.48*
6 ++ opsi management	Number	1.25	1.37	0	4
	Percentage	*12.5*	*13.72*	*0*	*40*
Ketapang – South Lampung					
9 +++ opsi management	Number	15.75	5.51	9	28
	Percentage	*50.81*	*17.79*	*29.03*	*90.32*
6 ++ opsi management	Number	2.15	1.14	0	4
	Percentage	*35.83*	*18.95*	*0*	*66.67*

Status of pests problem

Compared to the total cultivated banana, disease problem in both pilot sites was very low (Table 12). However, the farmers should remain careful in managing their plants since banana wilt and bunchy top virus exist in the location. These diseases can be easily transmitted to existing healthy plants if not managed carefully. The existence of banana bunchy top in South Lampung can potentially spread since most of the farms are dominated by Pisang Berlin, a variety susceptible to BBTV.

Table 12. Disease incident of banana farm of farmer-cooperators at the pilot sites

Pilot site	Number of systemic-infected plant			
	Average	Stdev	Minimum	Maximum
Sarampad – Cianjur*)				
number of plant	786.74	790.21	100	3000
Wilt diseases	6.13	6.62	1	20
Bunchy top	2.67	2.08	1	5
Total of systemic-infected plant	2.85	5.97	0	25
Disease incident (%)	0.37	0,67	0	2.50
Legundi – South Lampung				
number of plant	642.75	595.11	30	2000
Wilt diseases	2.44	4.37	0	16
Bunchy top	1.20	2.68	0	10
Total of systemic-infected plant	2.85	5.04	0	16
Disease incident (%)	0.50	0.95	0	3.33

*) *There was one farm to be reported 100% devastated due to fusarium wilt that was excluded from calculation*

Income situation

The farmer-cooperators in Cianjur earned more income than those in South Lampung (Table 13) which may be a factor of the bigger land area that they manage. Aside from that, the land is more fertile and they grow other (high-value) crops such as vegetables. However, the share of banana to the family income of the farmer-cooperators in Lampung was higher than those in Cianjur. Farmers in South Lampung also spend more on education and social expenses, which might be influenced by culture of the society.

Table 13. Income situation of banana farmer-cooperators at the pilot sites

Pilot site	Income situation			
	Average	Minimum	Maximum	Stdev
Sarampad – Cianjur[*)]				
Income (Rp)	5,289.175	379,500	9,000,000	2,202,384
Share of banana to income (%)	14.94	0	60.47	19.54
Expenses:				
Consumption (Rp)	973,684	300,000	2,000,000	457,763
Recreation (Rp)	262,500	150,000	400,000	110,868
Health (Rp)	519,444	25,000	2,150,000	771,745
Education (Rp)	265,000	100,000	1,000,000	263,576
Social (Rp)	195,833	25,000	500,000	235,805
Income - expenses (Rp)	3,301,763	(3,070,500)	22,100,000	5,914,736
Legundi - South Lampung				
Income (Rp)	2,756,632	44,000	12,875,000	3,473,228
Share of banana to income (%)	5284	533	100	40.29
Expenses:				
Consumption (Rp)	506,111	200,000	800,000	234,700
Recreation (Rp)	111,111	-	1,500,000	366,042
Health (Rp)	24,167	-	150,000	39,267
Education (Rp)	319,444	-	2,150,000	532,513
Social (Rp)	530,556	-	3,000,000	822,861
Income - Expenses (Rp)	1,418,389	(935,000)	7,975,000	2,419,494

*) *There was one farmer that had income until Rp. 25.000.000,-, which was excluded from calculation*

Evaluation of farmer practices at Cianjur at the beginning of the project showed that most of the farmer-cooperators did not implement the recommended technologies, except for two, namely Deny and Harun. Both farmers were ‘champion farmers’ being innovative and curious to try out new technologies. On the average, at least 9 component of technologies were implemented from 41 components namely, utilisation of sucker and bit, implementation of annual cropping system and row arrangement, application of biofumigation, control of BBTD and other pests, and deflowering and bagging. After some time, the farmers’ attitudes were changing in response to the technology options. The farmers were grouped more diversely into five (Figures 3 and 4). The change also happened on what technology they implemented, namely integrated control, eradication, tool sterilisation, ploughing, desuckering, row arrangement, control of BBTD, fruit bagging, and weed management. Figure 4 also showed that several cooperating and non-cooperating farmers clustered into the same group of technological implementation. The evident proved that the technological interventions applied by the farmer-cooperators were likewise adopted by the non-cooperators.

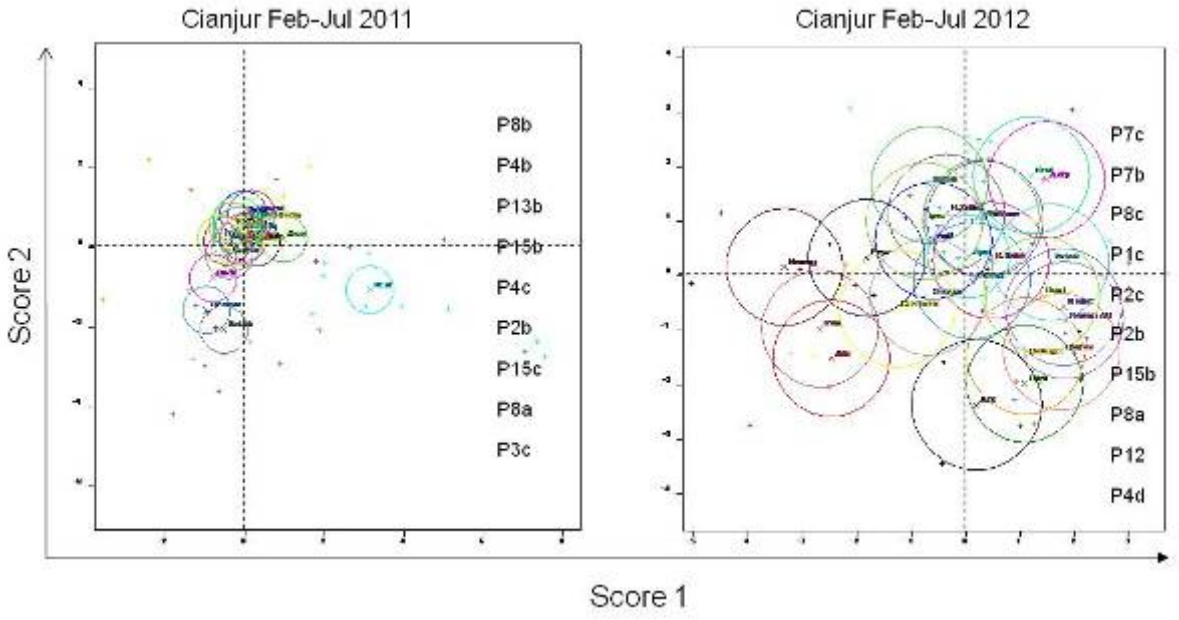

Figure 3. Overlap of banana management practices of co-operating farmers at Cianjur

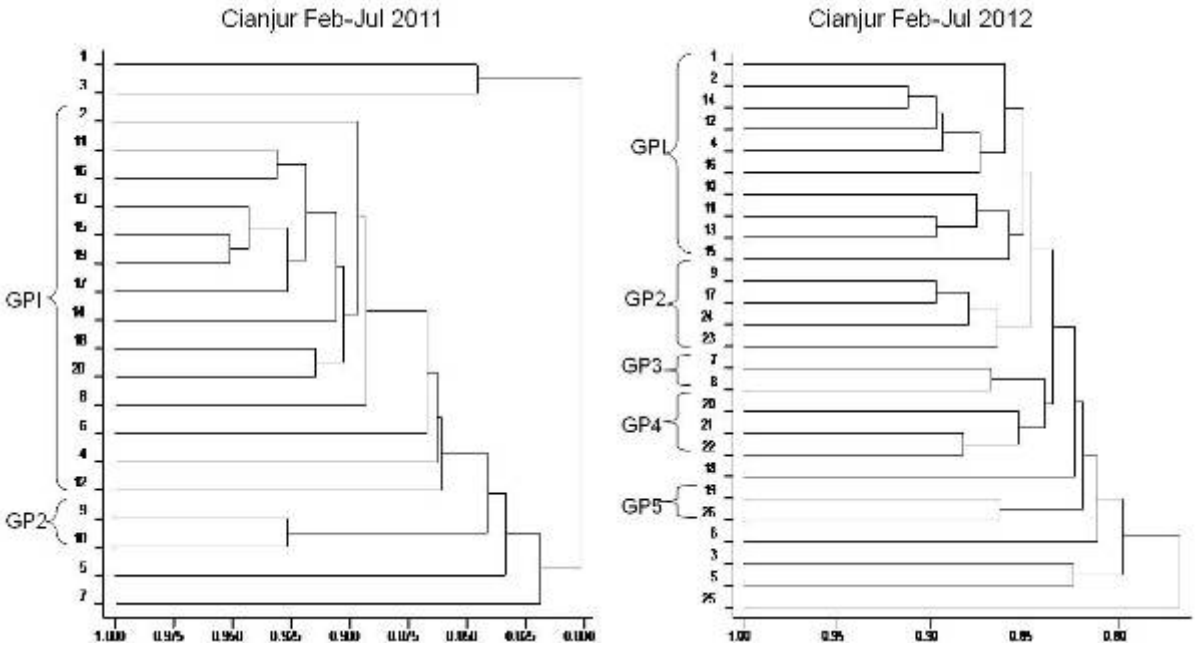

Figure 4. Similarity in management practices between cooperating and non-cooperating farmers at the demo plot in Cianjur

The practices of farmer-cooperators in Lampung at the beginning of the research were diverse (Figures 5 and 6), which may be due to less knowledge among Lampung farmers on banana cultivation and technology, and having more limited financial resources to implement the recommended technological options. The farmers implemented 8 out of 41 component of technologies, namely site selection, utilisation of bit planting material, crop rotation, irrigation and drainage management, weed management, and wilt disease control. As training and more frequent interaction occurred over time, the farmers shifted their farming practices from using bit initially, to axillar shoot; arranging planting density and rows, and implementing a multi-varietal, multi-species cropping system.

Analysis of end project data involved cooperating and non-cooperating farmers. Figure 6 shows that non-cooperating farmers (# 15-22) grouped separately from cooperating farmers. The gap indicates that the technologies known by cooperating farmer was not adopted yet by the non-cooperating ones.

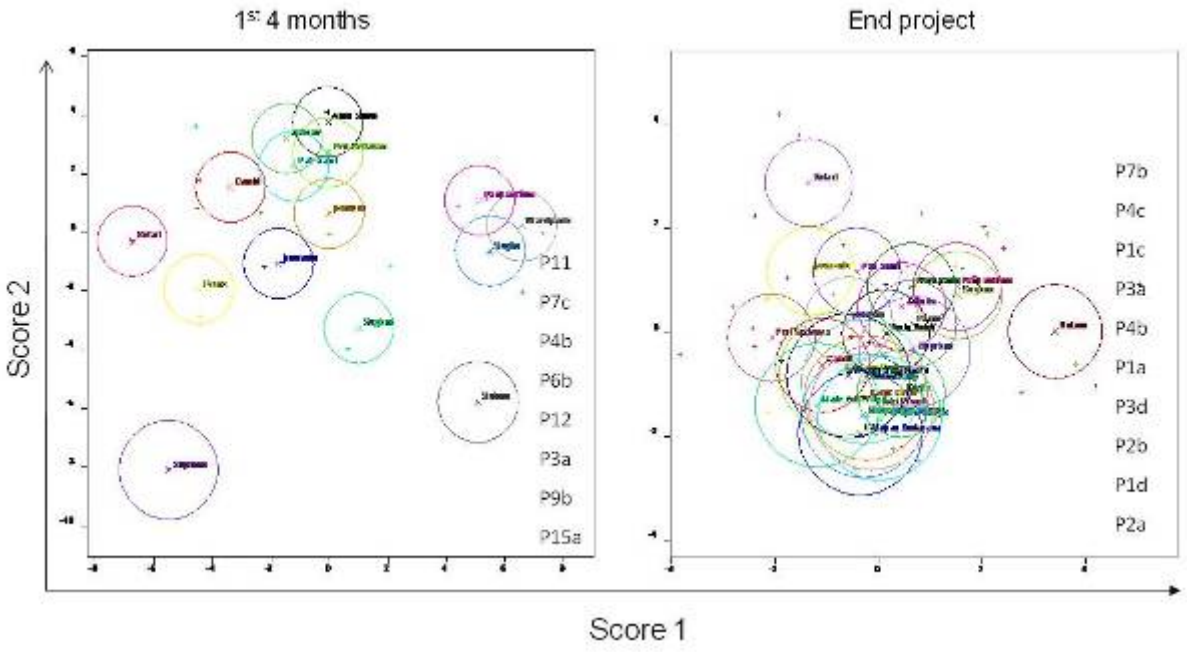

Figure 5. Cooperating and non-cooperating farmer practices of the pilot sites in Lampung

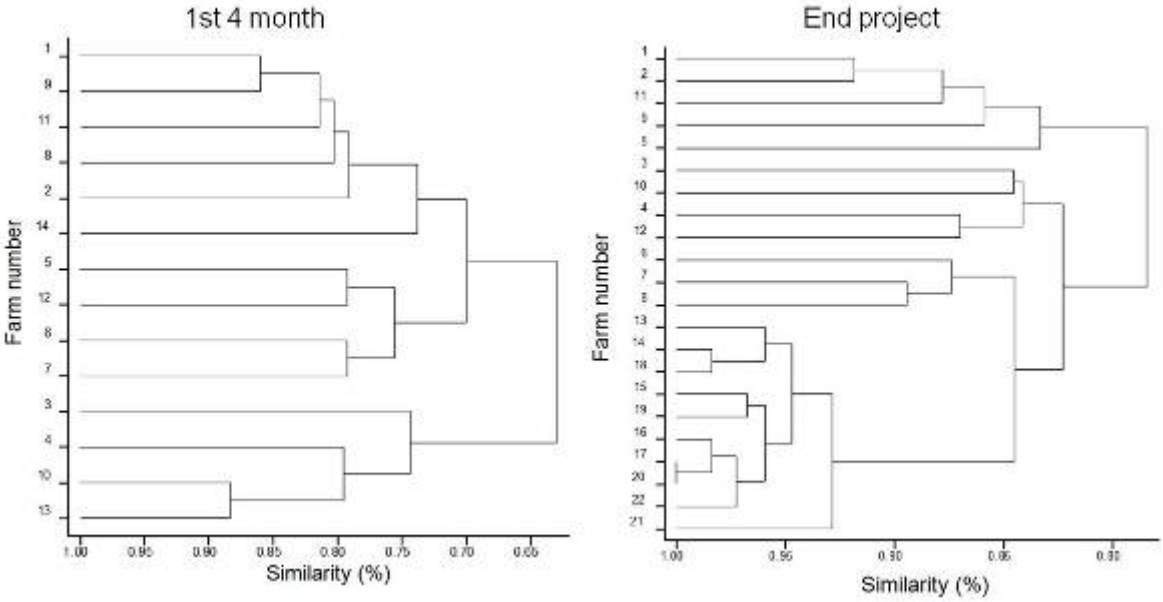

Figure 6. Similarity between cooperating and non-cooperating farmer practices at the first 4 months and end of the pilot sites in Lampung

Comprehensive analysis among farmers both from Cianjur and Lampung, and cooperating and non-cooperating farmers confirmed the previous finding. Overlap in technological implementation occurred among cooperating and non-cooperating farmers in Cianjur, while Lampung farmers did differently (Figure 7). The five technologies that farmers implemented most were tool sterilisation, planting space arrangement, site selection, organic fertilisation, and minimum tillage.

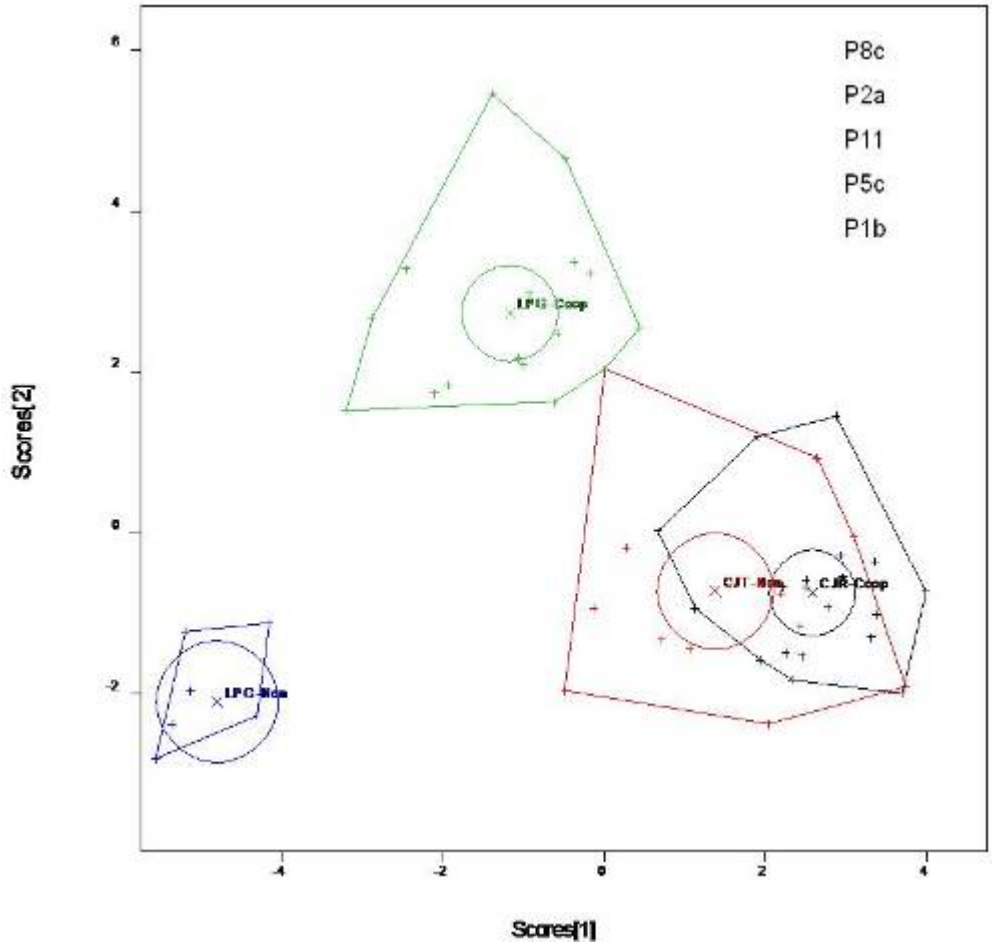

Figure 7. Difference in practice between cooperating and non-cooperating farmers in Lampung and Cianjur

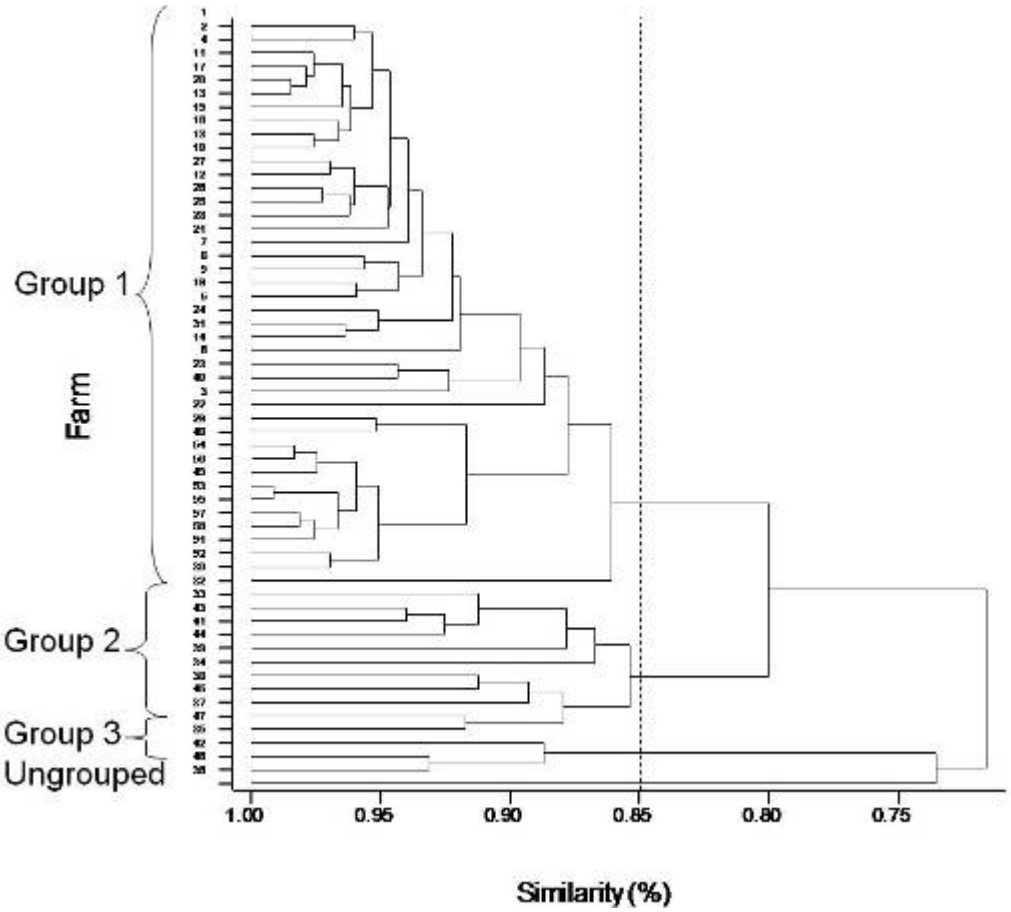

Figure 8. Similarity in implementing component of technologies among cooperating and non-cooperating farmers in Cianjur and Lampung

Boxplot analysis of the disease incidence among cooperating and non-cooperating farmers at Lampung and Cianjur shows that Cianjur farmers suffered more severely from Lampung (Figure 9). The analysis also shows that higher disease incidence of wilt diseases happened in the farms of non-cooperating farmers. The finding tends to prove the effectiveness of technological implementation of integrated crop production of banana.

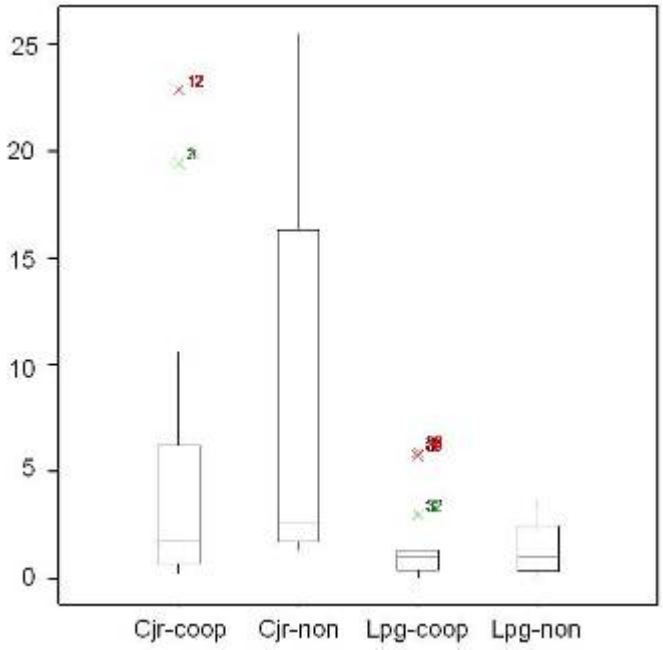

Figure 9. Boxplot for disease incidence of the cooperating and non-cooperating farmers in Lampung and Cianjur

Banana Processing

Cianjur District

In Sarampad, Cianjur there are five women's farmer groups (KWT) which is coordinated by Mrs. Lilis Sumarni . Each KWT produce banana chips that is marketed by themselves. KWT process banana chips 10 kg of bananas every day.

Cost of purchasing for 10 kg banana is Rp 18,000 or Rp 1.800/kg . Other costs for spices, cooking oil, gas, and labor are about Rp 27,000. From 10 kg of bananas can produce 2.5 kg banana chips which can be sold Rp 30.000/kg . (Table 4).

Table 14. Cost and revenue of banana chips production in Sarampad, Cianjur, 2013)

No.	Description	Volume	Unit	Price (Rp/unit)	Value (Rp)
A	**Cost**				
1	Banana var. Nangka	10	kg	1,800	18,000
2	Seasoning	1	unit	5,000	5,000
3	Cooking Oil	1	l	12,000	12,000
4	Gas	1	kg	5,000	5,000
5	Labor	1	person	5,000	5,000
6	Plastic for Packaging			None	-
	Total of cost				45,000
B	**Production**				
1	Banana chips	2.5	kg	30,000	75,000
	Revenue				30,000
	Per week				
	Production	12.5	kg		375,000
	cost				225,000
	Profit				150,000

Some household's make value added of banana to be banana chips. As mention in table 4 above, Mrs. Lilis can get additional income Rp. 150.000 per week. However, she get some difficulties to get raw material to proceed to be banana chips. It is because KWT use Var. nangka as raw material of Banana chips, while farmers rarely plan this banana, due to low price. Therefore KWT get raw material not only from Sarampad , but also from other villages .

Lampung District

There are two people for sample in Lampung that produce banana chips in household scale. Firstis Ibu Sunaryah which need one banana bunches per day as raw material with price is about Rp 15,000. Fresh banana chips are made without any seasoning. The additional cost is cooking oil , and plastic wrap. Labor is not paid because she work by herself assisted by her husband . The cost to make one bunch of bananas into banana chips is Rp 30,500. IbuSunaryah needs 6 banana bunches for a week to produce 18 kg banana chips, which can earn profit about Rp. 900,000 per week (Table5). She sells banana chips routinely to some schools near her home and her store.

Table 5. Cost and Revenue of Banana Chips Production in Lampung (Sunaryah), 2013

No.	Description	Volume	Unit	Price (Rp/unit)	Value (Rp)
A	**Cost**				
1	Pisang nangka	1	Bunch	15,000	15,000
2	Seasoning				
3	Cooking oil	1	Liter	10,000	10,000
4	Kayu bakar	1	Unit	5,000	5,000
5	Labor	1	Person		-
6	Plastic pack	5		100	500
	TOTAL Cost				30,500
B	**Production**				-
1	Banana chips	3.0	Kg	20,000	60,000
C	**Profit**				29,500
	PER week	6	Tandan		
	Production per week	9	Kg	18 kg	1,080,000
	Cost				183,000
	Profit				897,000

Another one is Ibu Murtini. She sells banana chips at her store. Every 10 bunches can produce 1,800 packs of banana chips which is sold Rp 400 per pack.. Cost to produce banana chips from 10 kg fresh bananas is about Rp. 280.000.

Table 6. Cost and Revenue of Banana Chips Production in Lampung (Murtini), 2013

No.	Description	Volume	Unit	Price (Rp/unit)	Value (Rp)
A.	**Cost**				
1	Pisang nangka	10	Kg	14,000	140,000
2	Seasoning				
3	Cooking oil				
4	kayu bakar	1	m3	15,000	15,000
5	Labor	1	orang		-
6	Plastic pack	1	kg	25,000	25,000
	Total Cost				**280,000**
B.	**Production**				-
	Banana chips	1,800	pack	400	720,000
C.	**Profit**				440,000
	PER WEEK	6 bunch			
	Production per week	4,320		400	1,728,000
	Cost				**456,000**
	Profit				**1,272,000**

Banana farming System in Cianjur and South Lampung are planted intercropped with other crops. However bananas farming can provide additional income for family farmers.

Processing banana chips are still in the household scale. The added value from banana can increase household incomes and provide employment, especially for women or a housewife.

Banana Distribution

Marketing Channels in Cianjur

Most farmers sell banana to the middle or big traders. Wholesalers sell bananas to the nearby markets in Cipanas and Cianjur, some of them had sold to Cibedug , Ciawi (Bogor) , and Pasar Induk Kramat JatiJakarta. From the markets, the banana will be distributed to the consumer, restaurants, hotels and catering enterprises. Every week the trader from Cugenang can distributed 2 tons of bananas to market Cibedug and Kramat Jati.

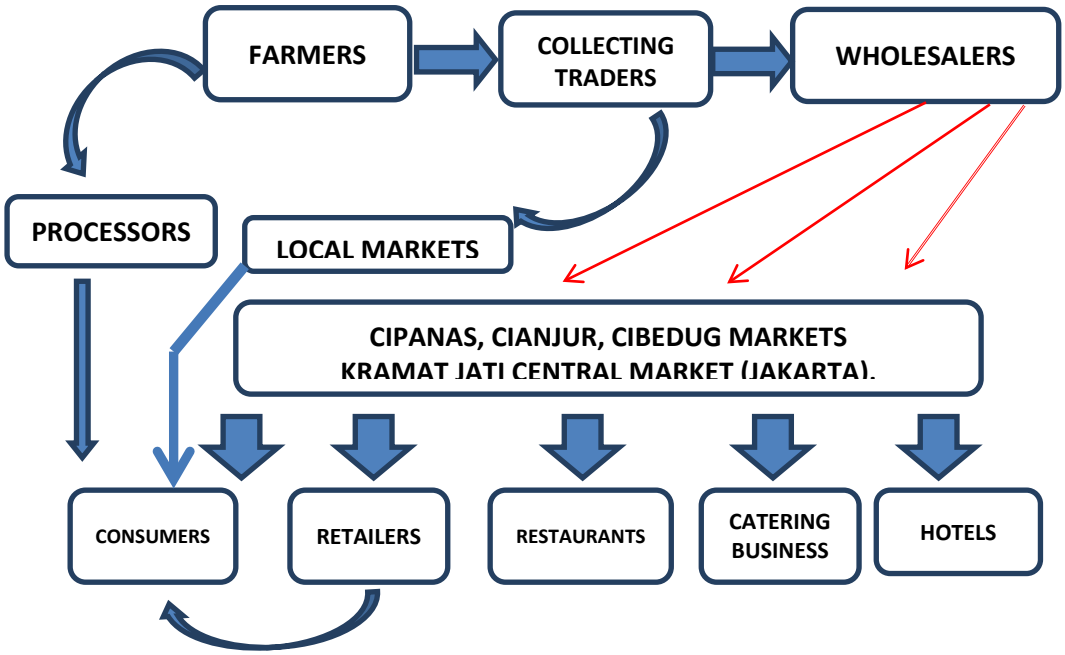

Figure 10 .Marketing Channels in Cianjur

Marketing Channels

1. Most farmers sell their harvested bananas, e.g. Ambon and Raja Bulu varieties, to collecting traders, wholesalers or processors. The collecting traders sell bananas to wholesalers. Bananas purchased by wholesalers are sold to retailers in some markets. Some collecting traders also act as retailers for their bananas not sold to wholesalers. A certain banana variety, such as Nangka, is sold by farmers to middlemen who sell it to banana-chips processing industry at village level. Some retailers purchase bananas directly from farmers and sell them to the markets.

2. The farmers sell bananas to the traders without fruits grading. Depending on the variety, bananas price is around Rp 2,000 per kg at farm level. Cash payment is usually carried out by the traders and the fruits are harvested by the traders themselves. Selling bananas to suppliers who sell this commodity to supermarkets is not common because the suppliers buy at lower price. It is possible because the suppliers do not cash payment from the supermarkets. They have to sell bananas in

supermarkets through a consignment agreement in which payments will be made two or three weeks later.

Marketing Channels in Lampung Selatan

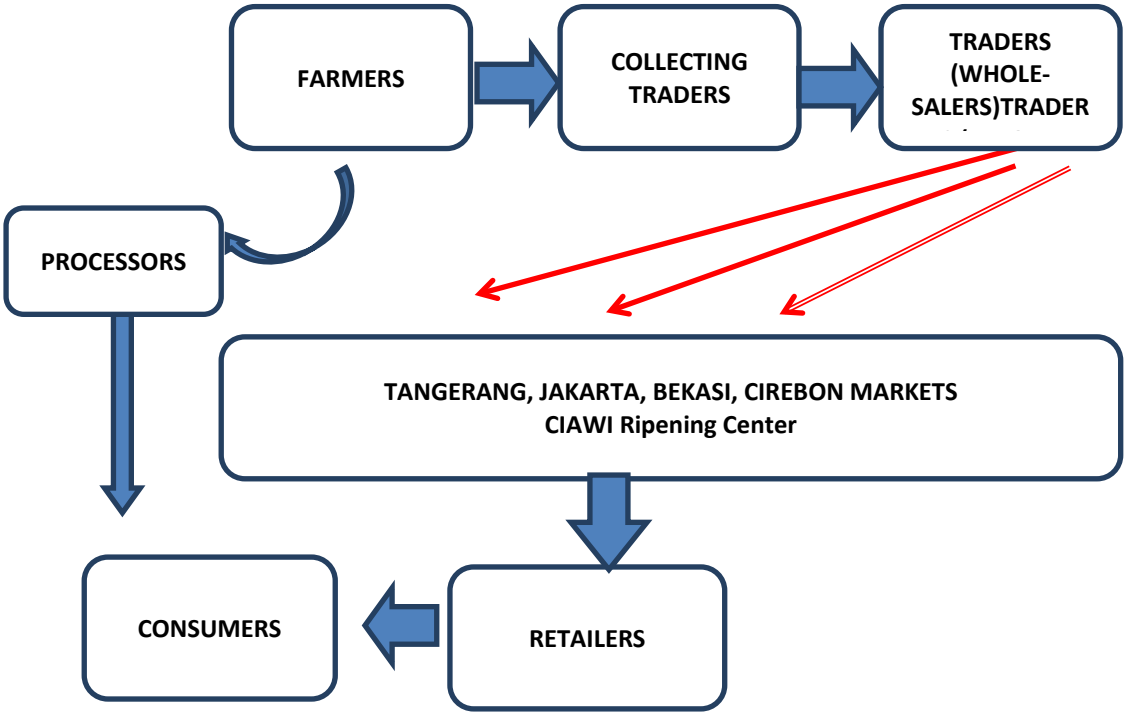

Figure 11 .Marketing Channels in Lampung Selantan

1. Collecting traders buy bananas (Janten, Pisang Ambon, Raja Sere, Mulivarieties) from farmers. The middlemen sell bananas to wholesalers. The traderssell to wholesalers. Furthermore wholesalers distribute bananas to Tangerang (Serpong , Balaraja) , Jakarta and Cirebon (Figure 2) which takes 4 – 4.5 hours to Tangerang from Lampung and 1 – 2 hours to Jakarta and Bekasi. Most bananas are sent to Ciawi (Bogor Regency) for ripening then marketed to Jakarta.

2. Payment from wholesalers to collecting traders is cash or credit (3-4 days). Payment from traders in Tangerang, Jakarta, and Bekasi to banana traders from South Lampung is cash or credit

3. The most widely planted varieties of banana farmers are Janten and Muli because they are resistant to pests and diseases and marketable.

CONCLUSIONS AND RECOMMENDATIONS

1. Banana cultivars responded differently to different *Foc* VCGs.
2. Bananas grouped into the same subgroup, such as Pisang Ambon Kuning and Ambon Putih (both are Gros Michel), performed differently on different VCGs. The result opens possibility to find resistant variety among the same banana subgroup.
3. Further tests of more VCGs against different cultivars would be valuable to completely develop new concept of differential races on banana.
4. Higher disease incidence of wilt diseases happened among the non- cooperating farmers.

ACKNOWLEDGEMENT

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University of Gadjah Mada

The following are the submitted abstracts of students in UGM, under the supervision of Dr. Siti Subandiyah and Dr. Arif Wibowo for the degrees of Bachelor of Science and Master of Science.

1. The Effect of Silica (Si) and Organic Material Addition into Suppressive and Conducive Soil on the Incidence of Fusarium Wilt Disease of Banana

Fusarium wilt caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*) is one of the most destructive diseases of banana. One potential method to manage fusarium wilt of banana is by manipulating nutrient status in the soil. This study was conducted to determine the quality of *Foc* suppressive and conducive soil, the influence of soil application of silica and manure on the incidence of fusarium wilt of banana. Surveys were conducted in 5 banana plantations in 3 provinces in Indonesia, i.e., Lampung-Sumatra, West Java and Central Java. From 5 locations, one location (Salaman-Central Java) was heavily infected by *Foc*, one location (NTF Lampung-Sumatera) was light infected by *Foc*, while 3 locations (Sarampad-West Java, Talaga-West Java and GGP Lampung-Sumatra) were healthy banana plantations without *Foc* infection. Labile carbon analysis showed that the *Foc* suppressive soil had greater labile carbon content than conducive soil. Also, the analysis of fluorescein diacetate hydrolysis (FDA) and β-glucosidase showed greater microbial activity in suppressive soil than the conducive soil. Observations of the incidence of necrotic rhizome of *Foc* susceptible Ambon Kuning (AAA) banana cultivar showed that in the suppressive soil taken from Sarampad-West Java, the application of silica and manure helped to suppress fusarium wilt disease development. In the conducive soil taken from Salaman-Central Java, silica and manure applications were not able to suppress the incidence of the disease. The result of this study indicated that in suppressive soil, the application of silica can increase plant resistance towards *Foc* infection, while manure application can increase soil microbial activity, and suppress *Foc* development.

Field Application of Bio*fertilizer*

Banana cultivar : Barangan, 3 Blocks with 5 plant replicates of each block
Fertilizer :

1. Organon commercial biofertiliser
2. Farmer formulation (*Foc*Off) from Bantul farmer group
3. Synthetic Fertiliser
4. Control

Field : UGM experimental field with *Foc* infected land
Observation : % *Foc* wilting development weekly and plant growth (number of leaves an plant height)
Results : up to the 24th week observation

Figure 12 .

2. Increased soil suppressiveness to banana fusarium wilt disease through banana intercropping with Allium spp

Fusarium wilt is one of the most destructive diseases of banana and has spread in many local plantations in Indonesia. Until now, the effective ways to control banana fusarium wilt disease have not been found yet. *Allium* sp. is one of many horticultural crops generally cultivated in Indonesia. This research was conducted to determine the effect of several species of *Allium* sp. intercropped with banana to improve soil suppressiveness to fusarium wilt disease of banana. The results showed that up to 12 months after planting, of 4 species of *Allium* spp. (*A. tuberosum/* chinese leek *, A. fistulosum*/ bunching onion*, A. cepa var. aggregatum*/ shallot and *A. wakegi*/ wakegi) intercropped with banana Ambon Kuning (AAA) cultivar, only chineese leek and shallot were able to suppress the incidence of fusarium wilt disease of banana by 46% and 33% respectively, when compared to the control treatment. This may be due to the different ability of bulb extract of *Allium* spp. to suppress the growth and spore germination of *Fusarium oxysporum* f.sp. *cubense*.

Conclusion: Allium tuberosum and A. cepa group aggregatum were growing well in the lowland banana experimental field and suppressing *Foc* well compared to A. fistulosum and A. wakegi which were upland allium with poorly growing in the lowland. However, the microbial activity by FDA assay suggested that the FDA value were less found on A. Tuberosum, A. Cepa group aggregatum, and A. wakegi compared to those of A. fistulosum and the control treatment, suggested that the FDA may resulted on total microbial activity including *Foc* population which was high in the unsuppressing soil. In the in vitro assay of *Foc* growth suppression by the allium extract suggested that A. Cepa group aggregatum, A. tuberosum, and A. fistulosum suppressed *Foc* significantly compared to A. Wakegi and the control treatment.

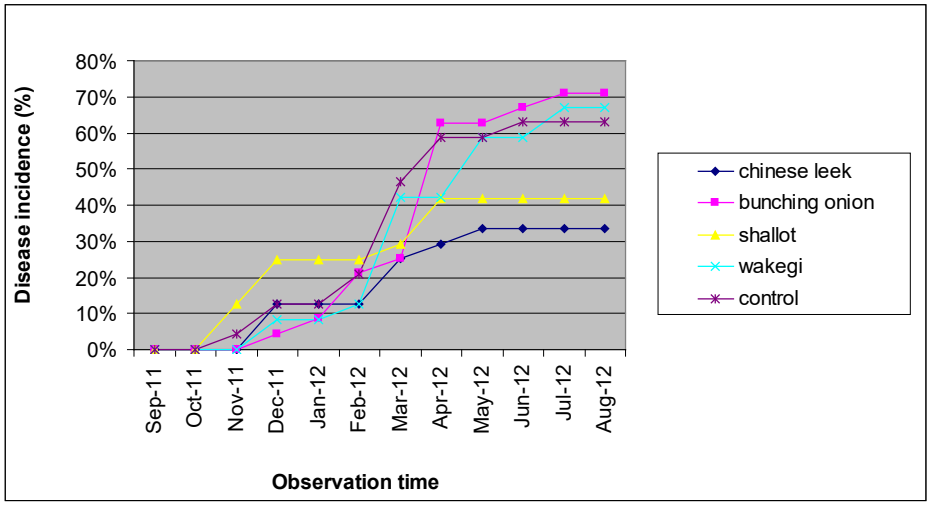


Figure 1. Development of fusarium wilt disease of Ambon Kuning (AAA) banana cultivar after intercropped with different *Allium* spp

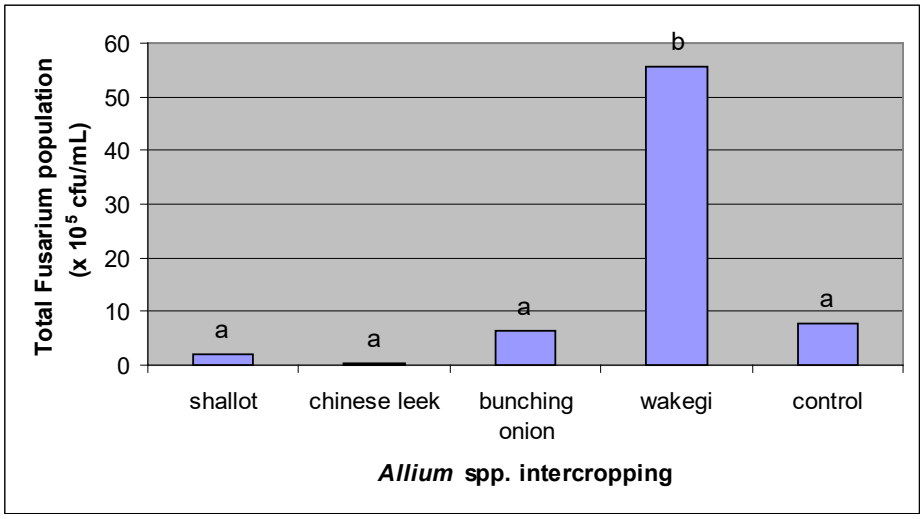


Figure 2. Total *Fusarium* spp. population in soil planted with banana intercropped with different *Allium* spp

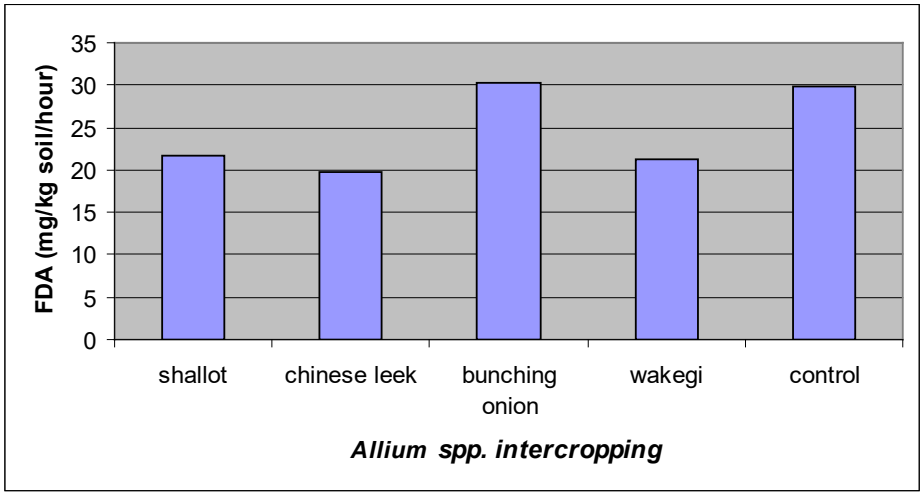


Figure 3. Total microbial activity in soil planted with banana intercropped with different *Allium* spp

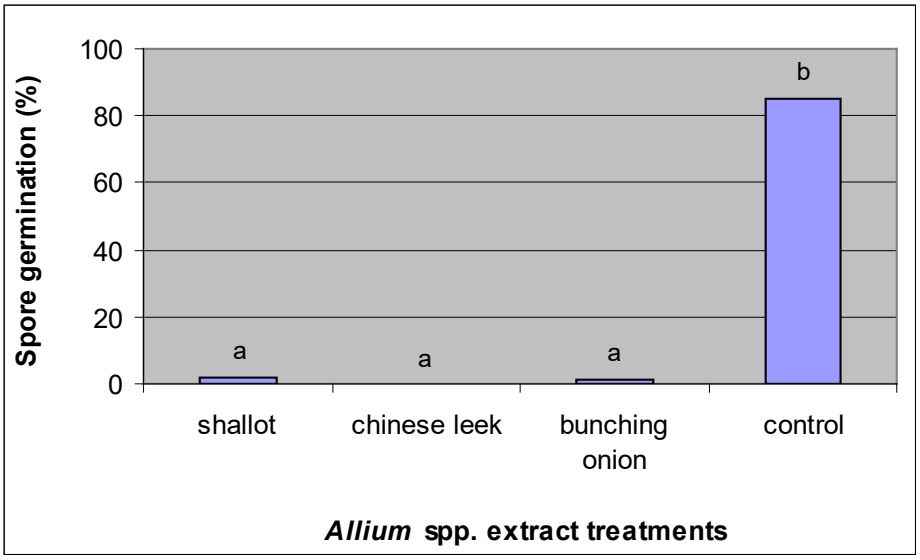


Figure 4. Percentage of spore germination of *Fusarium oxysporum* f.sp. *cubense* cultured on PDA amended with different *Allium* spp. Extract

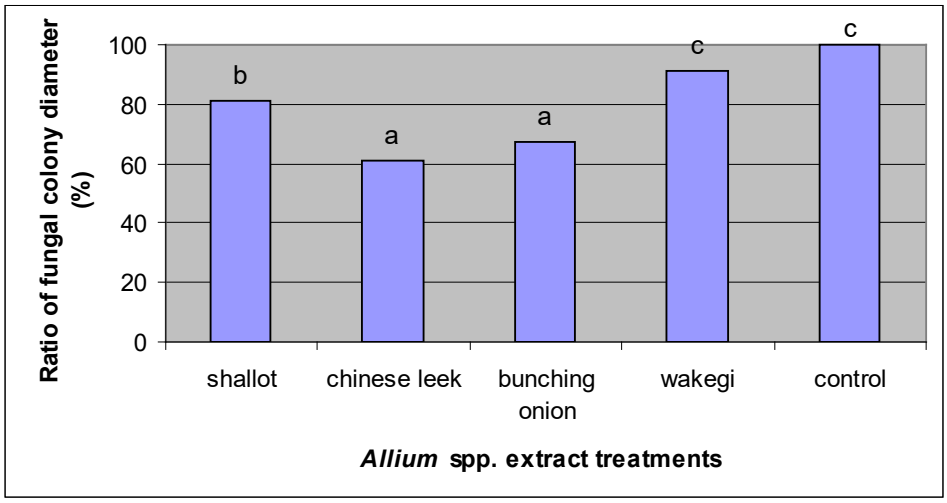


Figure 5. Ratio of *Fusarium oxysporum* f.sp. *cubense* colony diameter on PDA

3. Antagonistic mechanism of microbial community for wilt suppression

- *Foc* anatagonistic assay in vitro and collecting antagonist isolates of bacteria and actinomycetes
- Molecular identification of antagonistic agents by PCR and sequencing of ribosomal DNA
- Designing specific primer for detecting the antagonistic agents
- Bioassay of antagonistic agents against *Foc* in vivo using susceptible banana plantlets

Molecular identfication of the antagonistic bacteria and actinomycetes by sequencing of 16S-rDNA resulted on the identified antagonistic bacteria agiant *Foc*:

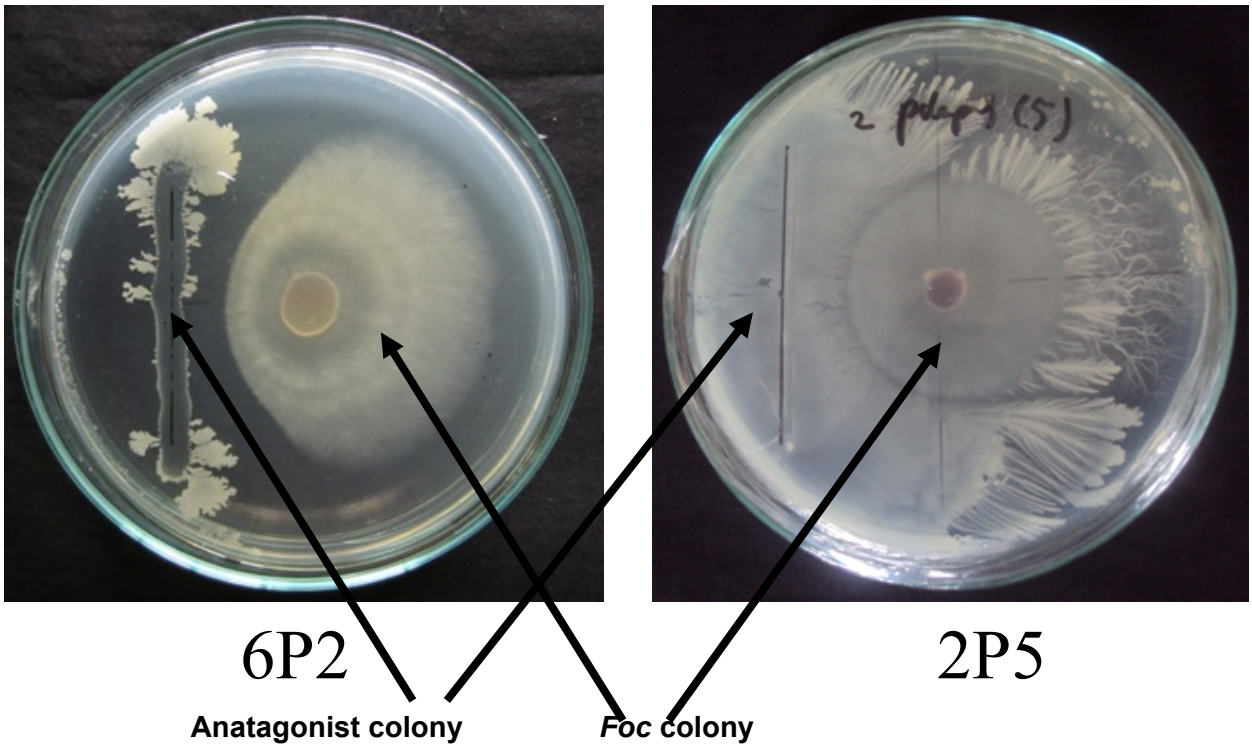
- *B. amylolyquefasciens* 5 strians of 1 strain Endophyte bacteria and 4 strains of rhizosphere bacteria
- *B. anthrachis* (Rhizosphere bacteria)
- *B. pumilus* (Rhizosphere bacteria)
- *B. thuringiensis* (Rhizosphere bacteria)
- *Lysibacillus fusiformis* (Rhizosphere bacteria)
- *Pseudomonas fluorescens* (Rhizosphere bacteria)
- Actinomycetes (Rhizosphere bacteria) in progress to be sequenced.
- The bacteria of *B. amyloliquuefasciens*, *B. pumilus*, and *Pseudomoanas fluorescens* strains were reported as commercial fungicides.

4. Antagonistic bacteria against Fusarium oxysporum f.sp. cubense were recovered from suppressive soil

Siti Subandiyah[1], Arif Wibowo[1], Tri Joko1, Rheni D. Puspitasari[1], Jacklyn Hendrita[1], Rahma Kusbari[1], Leane Forsyth[2], Antony Pattison[2], and Agustin Molina[3]

The previous research suggested that in *Foc* suppressive soil there was higher microbial activities compared to that in conducive soil. This research was conducted to analyse microbial community in the suppressive soil and to explore any antagonistic and endophytic bacteria against *Foc* in the rhizosphere and banana plant. Soil samples of suppressive without *Foc* infection and conducive soil with heavy *Foc* infection were obtained from East Lampung, Central Lampung, West Java and Central Java. The population density of bacteria in the suppressive soil were higher than those of the conducive soil samples. The diversity of the bacteria in the suppressive soil was higher with 4 groups based on morphological characteristics or 6 subgroups based on RISA (Ribosomal Intergenic Sequence Analysis) compared to those of conducive soil sample with only 2 groups based on morphological characteristics or 4 groups based on RISA. More than 100 bacterial isolates were collected from the soil samples and 19 isolates were isolated from banana plants. The bacterial isolates were tested to suppress *Foc* TR4 isolate Bnt2 *in vitro*. There were 10 bacterial isolates from the soil samples and 2 isolates from banana plants were antagonistic against *Foc* TR4 Bnt2 isolate. The antagonistic mechanisms were antibiosis and competitive against the pathogen. Molecular identification of the antagonistic bacterial isolates against *Foc* is in progress.

Conclusion: In traditional cultivated lands (Javanese soil samples) higher population of bacteria were found compared to those in intensive banana industry in Lampung, speculated due to more chemicals used in the industry reduce the bacterial population. In the healthy rhizosphere soils higher population and more diversity of bacteria were found than those in *Foc* infected rhizosphere soils. More *Foc* antagonistic bacteria were found in the healthy rhizosphere soils than those in the infected rhizosphere soils. *Foc* antagonistic endophytic bacteria were found in the healthy banana tissues. Population of antagonistic bacteria speculated to be responsible in conditioning of *Foc* suppressive soil *Bacillus amyloliquefaciens, B. pumilus*, and *Pseudomonas fluorescens* recovered in this research were reported before to be antagonistic against other fungal pathogens by other researchers and some of the bacterial strains have been commercialized as biofungicides or PGP (Plant Growth Promoting) Bacteria

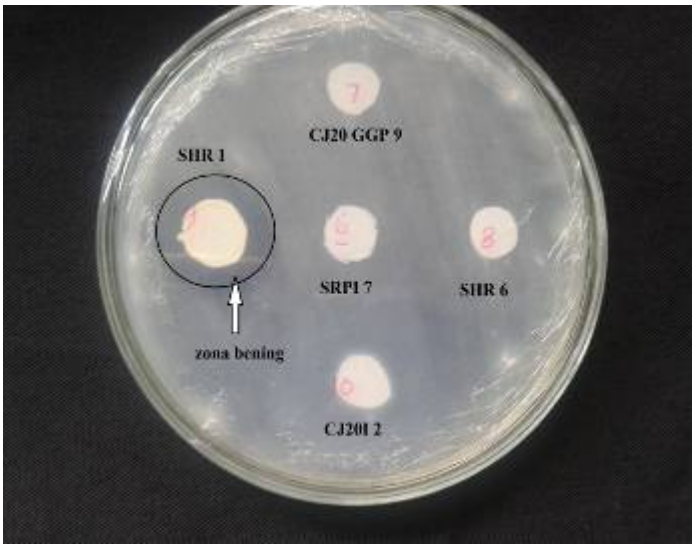


5. Abundance and antagonistic analysis of Actinomycetes related to banana fusaium wilt suppression

Siti Subandiyah, Sri Martina Wiraswati, Arif Wibowo, Tri Joko, Ngadiman, Tony Pattison, and Agustin Molina

Actinomycetes are the next in abundance after bacteria found in the soil. They grow slower than most bacteria and even compared to *Foc*, however among them may produce some antibiotic that suppress *Foc*. This research is conducted to analysed Actinomycetes community in the uninfected vs infected rhizosphere of banana planted in different cultural practices (location). Starch Nitrate Agar medium was used to recovered Actinomycetes from rhizosphere soil samples obtained from 3 different groups of banana cultivation, traditional farm in Central Java and Yogyakarta, banana industry in Lampung, Sumatera, and new setting of banana with different cultural practices in Cianjur West Java. *Foc* antagonistic assay was conducted by paper disc techniques of secondary metabolites of actinomycetes isolates. There is no strong relationship between *Foc* infection with actinomycetes abundance since in some actinomycetes populations were found both in *Foc* infected and healthy soil samples, however one isolate obtained from a healthy soil sample in Yogyakarta was found to produce secondary metabolite suppressing *Foc*. Further research is in progress for the possibility of using *Foc* antagonist actinomycetes for biological control of banana fusarium wilt.

Conclusion: Healthy banana rhizospheres have more abundance Actinomycetes. *Foc* antagonistic Actinomycetes are found both in the healthy and infected rhizosphere. Most Actinomycetes are slow growing with optimum growth at 6-7 days on nutrient medium with smaller colony than *Foc*, therefore they need to start growing earlier to suppress *Foc* development. Secoundary metabolite extracted from the antagonist actinomycetes were tested to suppress *Foc*, resulted that not all the antagonist isolates were recovered to produced secoundary metabolite suppressing *Foc* growth.



6. Diversity analysis of bacterial community in the rhizospere of infected and uninfected Fusarium wilt of banana

Rheni Dian Puspitasara

Banana (*Musa* sp) is an important tropical fruit commodity. The existence of pathogen, *Fusarium oxysporum* f.sp. *cubense* (*Foc*) causing Fusarium wilt has been devastating banana production. Some banana mats remain growing healthy on *Foc* infected lands, suggested that the soil is suppressive with microbial community suppressing the pathogen development. This study aims to find out the diversity of soil bacterial communities on the conducive soils and suppressive soils of *Foc*. The diversity of microbes in the suspected suppressive soil can be analysed for culturable and unculturable bacteria. The culturable bacteria were isolated on agar followed by morphological, physiological/biochemical and molecular characterization. The unculturable bacteria were directly analysed molecularly on the direct soil DNA extracts. Ribosomal Intergenic Spacer Analysis (RISA) was used for molecular analysis of soil bacterial community. Soil enzymatic analysis was conducted only through measuring the C labile concentration. The number of 15 composite soil samples were collected from the rhizospheres of *Foc* infected and healthy banana mats originated from East Lampung, Central Lampung, Cianjur, and Central Java provinces, consist of 9 samples obtained in rainy season and 6 samples obtained in dry season. Base on 35 morphological characteristics of bacterial colonies analysed by NTSYS version 2.1 (Numerical Taxonomy System, Applied Biostatistics, Inc., Setauket, New York), 4 groups of bacteria with similarity index of 71.11%, 74.28%, 65.71% and 65.07% were found in the healthy soil samples which was more diverse compared to those of *Foc* infected soil samples with only two groups of bacteria at the similarity index of 77.14% and 69.28% respectively. Furthermore, using the technique of RISA there were two groups with as many as six sub-groups at the similarity index of 78% on the healthy soil samples, whereas those on *Foc* infected soil samples there were 4 groups cleaved into 4 subgroups at the index similarity of 78%. The results suggested that there were more diverse microbial community found in healthy compared to *Foc* infected soil samples. The results on C labile measurement suggested that the concentration level of labile C was not consistently related to healthy or *Foc* infected soil samples.

7. Exploration and Identification of Antagonistic Rhizobacteria against *Fusarium oxysporum* f. sp*. cubense*, The Causal Agent of Fusarium Wilt from Suppressive and Conducive Soil

Raden roro jacklyn Hendrita

Fusarium wilt which also known as Panama disease, is the most important disease of banana caused by *Fusarium oxysporum* f.sp*. cubense* (*Foc*). The aim of this research was to explore and identify antagonistic bacteria against *Foc* from the banana rizosphere with or without *Foc* infection. The research methods were included the isolation of bacteria from soils, *in vitro* antagonism test, and amplification of 16S-rDNA using 16S-rDNA universal primers followed by sequencing of the amplified fragment DNA. DNA sequence analysis was conducted by BLASTN to find the homology sequencee of the antagonist isolates against *Foc* for bacterial identification related to the GenBank DNA sequence data. Soil samples were taken from the rhizosphere of banana originated from three different locations in Indonesia, included Central Lampung, East Lampung and Cianjur West Java. The number of 84 bacterial isolates from the soil samples were recovered, and 8 isolates of them them were antagonistic against *Foc* strain Bnt-2 by double layer analysis test. Further test was conducted to those 8 isolates using direct opposition method resulted on 1 isolate, Dry-2 from healthy banana rhizosphere in East Lampung remain antagonistic against *Foc*,. The result of DNA sequencing analysis showed that at the gene of 16SrDNA there were 1167 nucleotides of Dry-2 isolate which was homology to the nucleotide fragments of 100 bacterial strains of *Pseudomonas* from the GenBank. It showed that the bacterial isolate Dry-2 was closely related at 98% homology to 23 accession numbers of *Pseudomonas fluorescens* from 66 numbers of *Pseudomonas*.

8. Detection and differentiation of banana bunchy top virus in Java*

S. Somowiyarjo, .R.A. Priani and S. Hartono

In Indonesia, Banana Buchy Top Diseases (BBTD) was first recognized in 1980s in Bandung, West Java and as in other countries it has become the most important viral disease of banana in this country. Current research information provides a large amount of detail on the molecular characteristics of the virus, the mode of spread, and transmission of the virus on an international level, however limited information exists conforming the success of the diseases management in Indonesia. The research was conducted to employ the polymerase chain reaction-restriction fragment lengment length polymorphism (PCR-RLFP) to detect and differentiate the BBTV collected from Java (Yogyakarta, Central Java, and West Java). In the preliminary study, it was found that for extracting the total DNA from infected plants, CTAB method was better than Nucleon Phytopure DNA Extraction Kit. The best result of detection was obtained when the young infected leaf was used. Banana buchy top virus (BBTV) could be detected in all samples showing specific symptom of BBTD. However, no Abaca bunchy top virus (ABTV) could be detected from the same samples. The suitability of the technique for supporting to the pilot communities study on the integrated banana production which is now being conducted in West Java and South Lampung , is considered.

*Abstract for presentation in ACPP Darwin, April 2011

9. Molecular Detection of Banana Viruses in Indonesia

Sedyo Hartono, Rahma Ayu Priani, Siti Subandiyah, Agustin Molina, and Susamto Somowiyarjo

A survey of banana disease carried out in Java and Sumatra islands in 2011 found virus-like symptoms such as stunted growth plants, leaf streak and mosaic. To determine the presence of virus in plant tissues, PCR assay was performed on symptomatic plant samples collected from the fields and nurseries using primers for Banana bunchy top virus

(BBTV), Banana streak virus (BSV) and Cucumber mosaic virus (CMV). The results showed that BBTV and BSV were detected from farmer's fields and nurseries in Java and Sumatra. BSV are also detected in banana seedlings generated from tissue culture in nurseries of the banana company in Sumatra. The PCR amplified products of BBTV and BSV were then purified and sequenced. Sequence analysis showed the highest levels of sequence identity to BBTV Indonesian isolates (98%) and BSV Australian isolates (91%), respectively. To our knowledge this is the first report of BSV infecting banana in Indonesia.

MASTER'S THESIS ABSTRACT

10. Molecular characterization of complex viruses in banana

Rahma Ayu Priani

Banana (*Musa* spp.) is the most important fruit in almost every country, including Indonesia. Bananas can be infected by one or more viruses and the virus is able to infect multiple crops in a one family or other family. The research was conducted to detection and molecular characterization molecular of *Banana Bunchy top virus* (BBTV) and *Banana streak virus* (BSV), and determine host range of BBTV. This research can be used to monitoring of the epidemic and the evolution of virus is expected to become the basis for virus disease control strategy in bananas. This research can be used for monitoring the evolution of the epidemic and the virus that is expected to become the basis for virus disease control strategy in bananas. Infected plants were collected from Lampung, Cianjur and Yogyakarta. Host range plant of BBTV from Cannaceae, Zingiberaceae, Heliconiaceae, and Araceae family. DNA Extraction Kit Phytopure method were used to extract the total DNA. Two pairs primers of BBTV (BBTV-F and BBTV-R) and BSV (BSV5466 BSV6196-F and-R) were used for. BBTV and BSV PCR products were analysed by RFLP technique using the restriction enzyme *Dra* I and *Dde* I respectively. DNA amplified product of BBTV or BSV of approx 1100 bp and 750 bp respectively was successfully detected in plant samples from Lampung, Cianjur and Yogyakarta and one sample from Yogyakarta was double infected. *Zinggiber* sp. (*Zingiberaceae*) and *Heliconia* sp. (*Heliconiaceae*) are alternative host of BBTV. DNA sequences of BBTV samples of LMP-20 and CJR-28 were identified as replicase gene (DNA-1) BBTV is 1084 nt in length. The highest sequences homology samples of LMP-20 and CJR-28 were to Bali isolate with amino acid sequences identity of 99 % and 98,5 respectively and included in the Asian Group. DNA 1 BBTV sequence sample of YKT-A was identified as gene Reverse transcriptase (RT) and RNase H (RH) BSV is 709 nt in length. The highest sequences homology sample of YKT-A is to on China and another virus isolate with amino acid sequences identity of 94.9 % dan 86.4 % respectively. RFLP analysis showed the existence of genetic diversity in some isolates of BBTV DNA 1 and genetic uniformity in some isolates genes RT and RH BSV samples of Lampung, Cianjur, and Yogyakarta.

12.2 Summary of Australian Project Activities (as of May 2013)

Soil characterisation study to understand soil *Foc*-suppression to refine disease management approaches

Disease incidence for a given cultivar or variety is hypothesised to be the result of the inoculum levels of the disease causing pathogen and the external factors that make the plant conducive or suppressive to the disease (Equation 1). Disease suppressive and conducive factors are external factors that are associated with the environment in which the plant and the pathogen are present. They can include climatic factors, such as temperature and rainfall and soil factors of physical, chemical and biological origin.

Equation 1: $Disease\ incidence = fn\ [pathogen - suppressive\ factors_{tn} + conducive\ factors_{tm}]$

Soil biology, through organisms that are antagonistic to the pathogen is the most likely mechanism involved in suppression. However soil biology is constrained by soil chemical properties, which are further limited by the physical properties of the soil. Therefore, to determine the incidence of Fusarium wilt in bananas it is necessary to develop soil indictors which are soil properties that are related to changes in disease. The changes in Fusarium wilt can be determined by the incidence of disease, that is the proportion of plants showing symptoms or infected with the pathogen or the severity of the disease, that is how the plant reacts to the presence of the pathogen. Furthermore, it is possible to determine the potential for infection by determining the level of inoculum in the soil.

The expression of disease symptoms is a dynamic factor that changes with time in susceptible plants. Therefore it is necessary to understand external factors that may contribute to expression of the disease symptoms and the suppression or enhancement of the disease as a dynamic component over time. By determining the soil properties before measuring diseases symptoms, or measuring the symptoms of disease and relating back soil measured, has the potential to provide information on how external environmental influences impact on the suppression or enhancement of the disease.

Indicators are required that reflect the dynamic changes in the soil. While chemical properties may dictate the types of organisms in the soil it is necessary to validate biological measurements and changes that may be associated with changes in physical and chemical soil properties. Comparison of areas which are slow to show disease symptoms against those where the disease symptoms are expressed readily can also provide a static comparison. The static comparisons can be greatly enhanced if measurements can be conducted over time.

The work described below attempts to validate the hypothesis that incidence of fusarium wilt of bananas can be described as a function of inoculum levels, external suppressive and conducive effects which occur prior to the expression of the disease symptoms. To test the hypothesis firstly requires indicators that are sensitive to changes in management that can be related to disease expression, validation that external soil properties can be related to the incidence and expression of the disease and time factors which can demonstrate that changes in soil properties can increase or decrease disease expression.

Selection of soil indicators (work from Australia)

This section deals with the development of soil health indicators that were sensitive to farm management practices and development of disease suppression assays. The indicators were tested by conducting a farm survey where soils from organic and conventional banana farms comparing soil health characteristics and suppressiveness or conductivity of *Foc*.

Materials and Methods

Site selection

The area under survey covered Tropical North Queensland South of Cairns and North of Cardwell and the Atherton tablelands. Five organic and five conventional banana farms were selected in paired sites. Selection of organic sites was based on willingness of the farmers to cooperate while the number of available organic farms was rather limited. The conventional sites were selected based on proximity to the organic sites in order to eliminate pedoclimatic variation among sites

Soil collection for laboratory analysis, tray assays and pot trials

Soil samples were collected using a shovel. Composite samples (*n=15*) were taken from the top 15 cm of soil within 30cm from the banana plant, in front of the following sucker. Stones and large pieces of organic matter were avoided during sampling. Soil was placed in a bucket and thoroughly mixed with a trowel. At each farm three of the older banana blocks were selected, in most cases plantings older than five years. Only in the case of one organic farm it was not possible to take samples from blocks older than five years because the farm used a rotation system of three years banana, and three year fallow of cattle pasture, cowpea (*Vigna unguiculata*) or lablab (*Lablab purpureus*) and sometimes sweet potato (*Ipomoea batatas*). At this site, samples were taken from a one-year stand, a recently slashed block that had been under bananas for three years, and a fallow block under pasture.

Soil analysis

Collected bulked soil samples were analysed for physical, chemical and biological soil health indicators and samples were sent to a commercial laboratory, Incitec Pivot Ltd. Weribbee, Victoria, Australia, for further chemical analysis. The following soil health indicators were measured at the CWTA laboratory: pH, electrical conductivity (EC), nitrate nitrogen ($NO_{3-}N$), labile C, fluorescin diacetate, β-glucosidase, bulk density, water stable aggregates (WSA), soil particle size and nematode community structure.

Bioassays for assessing soil suppressiveness to Fusarium *wilts*

Laboratory hyphal extension test

Hyphal extension was determined by growing *Foc* R1 on collected soil samples on laboratory plates in an incubator (Alabouvette *et al.* 2006). Petri dishes were filled with 30 g of 2 mm sieved air dried soil which was spread evenly to obtain a smooth surface (approximate bulk density = 1.0 g cm^3). Sterile water (12 mL) was added to each Petri dish. Wetted soils were left to equilibrate for 7 days in the incubator. Using *Foc* R1 cultures on ¼ strength Potato Dextrose Agar (PDA) a 1 cm diameter, 4 mm thick plug from the growing margin of the fungal colony was inverted and placed centrally on top of the soil in the Petri dishes. After one week the number of plugs that had hyphae growing on the soil surface was recorded. Each week the extension of the mycelium was determined using a binocular microscope and the area of hyphal extension was estimated for a 4 week period. The Petri dishes were maintained at 25 C during this period. Table 1 shows the rating system that was used to give a one number score for extent of hyphal growth.

Table 2 Rating system for hyphal growth of the hyphal extension test

Rating	Criteria
1	Less that 5 hyphae and hyphae < 1cm
2	More that 5 hyphae and hyphae < 1 cm
3	Less than 5 hyphae and hyphae > 1cm
4	More than 5 hyphae and hyphae > 1cm
5	Extensive hyphal growth

The test was replicated 5 times per soil sample. The area under the (growth) curve (AUC) for each plate was estimated in Genstat (Version 9, VSN International, Hemel Hempstead, UK). The AUC values were then statistically analysed using a one-way-ANOVA and tests of multiple comparison of means (Fisher's protected LSD test with *P*=0.05) to assess differences in *Foc* suppressiveness between collected samples.

Glasshouse bioassays with tomato.

Composite samples from the upper 15 cm from selected organic and conventional field sites were used to test the inherent soil suppressiveness to Fusarium wilt using a standardized method as described by Alabouvette *et al.* (2006). According to Alabouvette (1990) and Alabouvette *et al.* (2006), soil suppressiveness to Fusarium wilt is general to *Fusarium oxysporum* and not specific to certain *formae speciales* of the fungus. For practical reasons tomato, susceptible cultivar Tiny Tim, with its associated Fusarium pathogen, *Fusarium oxysporum* f. sp. *lycopersici* R3 (*Fol* R3) was used for the assays which will be described in more detail below.

Polystyrene trays (72 x 20 x 7 cm) with 5 rows of 18 cells where used. Each hole was 4 cm^2 at the top and narrowing down to 5 mm^2 at the bottom allowing for good drainage but preventing soil from dropping out. Per cell 45 cm^3 of soil was added, equating to about 40 to 50 g of soil depending on soil moisture content.

Two methods of inoculation were used; inoculation with spore solution in liquid malt extract and inoculation with colonised milled seed.

One litre of liquid malt extract was inoculated with one plug of agar from the edge of a *Fol* R3 colony and placed on a rotary shaker (150 rpm) for seven days. After four days the solution was placed under near UV light for five hours to stimulate sporulation and placed back on the shaker. The solution was sieved through a sterile funnel (40 µm) and diluted to$10^{3,}$ 10^4 and 10^5 concentrations. Soil samples of organic and conventional soils were infested with 4ml of malt-based inoculum suspension at concentrations of 1x10^3, 10^4, 10^5 propagules ml^{-1} soil. Controls were given 4 ml of tap water and each treatment was repeated five times. Non-inoculated controls were included for both organic and conventional systems.

Millet seed (500g) was placed in Erlenmeyer flasks, soaked in water for 24 hours and sterilised by autoclaving. The flasks were inoculated with agar plugs of *Fol* R3 from the edge of a colony. The flasks were incubated at room temperature for 14 days and were mixed daily by hand shaking. Colonised millet seed was added to 45 ml of soil at rates of 0.5, 1.0 and 1.5 grams of inoculum and thoroughly mixed with approx. 50 g of soil. Each treatment was replicated five times. Non-inoculated controls were included for both organic and conventional systems.

One-week-old susceptible tomato cultivar (Tiny Tim) transplants were planted into the tray cells. Plants were kept in a greenhouse under controlled conditions with air temperatures maintained at 27-32 ºCelsius, 70-80% relative humidity, and 12h sunlight per day throughout the experiment. Plants were watered once a day.

Disease progress was recorded twice a week from day 21 to 58 after sowing. The following rating system was used:

1= healthy plant,

2= wilting of lower leaves,

3= whole plant affected and

4= plant dead/ terminal wilting.

Since the tests were done with unsterilised soil from various farms, PDA-plate isolation was done from the roots of each dead plant to determine wether death was caused by *Fusarium* or by another pathogen. At the termination of the trials, in case there was no plant death, diseased plants were used for isolation. From three paired sites, also healthy plants were used to make isolations in order to check wether *Fol* R3 was absent.

Dead, diseased and healthy plants were taken from the trays and the roots and lower stems were carefully washed. Roots and stem were dipped for 30 sec in 1% bleach solution to remove fungi and bacteria growing on the surface of the plant. Then, bleach was washed off in sterilised water. The roots were then cut into small (< 0.5 cm) sections with a scalpel. A representative part of this root material was taken and divided up into 5 parts. Then, 5 small pieces were cut from the lower stem. The root and stem material was then placed on ¼ strength PDA plates with a division between roots and stem and placed in an incubator at 27ºC. When the colonies were approx. 1cm in diameter the plates were taken from the incubator, taped with parafilm and placed under UV light at 23ºC. The plates remained in the incubator until the cultures showed sufficient morphological characteristics to be identified.

The AUC for each plant was estimated in Genstat. The AUC values were statistically analysed using a one-way-ANOVA and multiple means comparison (Fisher's

protected LSD test with P=0.05). Disease, growth and soil health indicators were tested on two-sided correlations. Principle component analysis was carried out on the disease indicators.

Pot trial

A pot trial with bananas was carried out to determine wether the bioassays for assessing soil suppressiveness to *Fusarium oxysporum* (using *Fol* and a susceptible tomato host) indeed gave an indication of soil suppressiveness to *Foc* R1. For this trial, the paired site with the most and the paired site with the least disease suppressiveness to *Fol* at four weeks post inoculation were selected.

As this trial was set up before the bioassays with tomatoes were finalised, selection was based on disease progress at 34 days post inoculation. For each tomato plant the area under the disease curve (AUC) was estimated in Genstat. The AUC values were then analysed using a one-way-ANOVA and multiple means comparison (Fisher's protected LSD test with P=0.05).

Soil samples collected from all three fields of the four selected farms was used, and each field had one inoculated treatment and a control. Each treatment had two replicates, making 48 pots in total. The pots were randomised in a split plot design.

Soils were inoculated with *Foc* R1 colonised millet seed. Millet seed (500g) was placed in Erlenmeyer flasks, soaked in water for 24 hours and sterilised by autoclaving. The flasks were inoculated with strips of *Foc* R1 infected banana (var. Ducasse) pseudo stem. The flasks were left at room temperature to incubate for 14 days and were mixed daily by hand shaking.

Approximately 1.5 Kg of soil was weighed and placed in plastic bags and 10g inoculum was added to the soil in each bag. After thorough mixing by hand, the soil was placed in 2L pots. Control pots were filled with 1.5 kg uninoculated soil.

Tissue cultured Ducasse (*Musa* AAB) banana transplants were carefully taken out of their pots and the roots were manually cleaned and washed from potting mix and placed in the pots. The pots were placed on trays, and soil moisture content was kept constant by filling the bottom of the trays with water. At five weeks post planting, the trays were left dry for 24 hours to create more favourable circumstances for symptom expression. Each pot received 5ml of organic liquid blood and bone meal (dilution 1/100) once a week.

Disease progress was recorded once a week from day 21 to day 58 post inoculation. Plant wilting was recorded following a rating system of 1-5 developed by INIBAP (Orjeda, 1998), where:

1= plant healthy

2= slight yellowing or wilting of lower leaves

3= extensive yellowing or wilting of lower leaves

4= yellowing or wilting of most or all of the leaves

5= plant dead

Leaf emergence and growth were measured once every two weeks. At the end of the trial the leaf area of the last fully emerged leaf was estimated as described by Turner (1972), and the corm was dissected to assess vascular discolouration on a rating of 1-6 according to the INIBAP guidelines (Orjeda 1998), where:

1= rhizome completely clean

2= isolated points of discolouration

3= vascular discolouration of up to 1/3 of rhizome

4= discolouration affects between 1/3 and 2/3 of rhizome

5= greater than 2/3 of rhizome discoloured

6= total discolouration rhizome vascular tissue

At termination of the trial, the number of discoloured roots was counted from a random sample of five roots. Soil was washed off from roots and corm and roots, corm and leaves were separately dried in a drying oven for seven days at 75ºC.

The AUC for each plant was estimated in Genstat. The AUC values were statistically analysed using a one-way-ANOVA and multiple means comparison (Fisher's protected LSD test with P=0.05). Disease, growth and soil health indicators were tested on two-sided correlations. Principle component analysis was carried out on the disease indicators.

Results

Description of the farms under study

Three of five pairs of farms were situated in the wet tropical coastal zone. One pair of sites was in the southern zone which received lower rainfall on an alluvial Dermosol (Table 2). The fifth pair was situated in the Atherton tablelands where temperatures are cooler and rainfall is considerably lower.

Table 3Location and soil description of surveyed farms in North Queensland, Australia (Simpson *et al.*, 2004, Isbell, 1996)

Farm	System[1]	Latitude	Longitude	Rainfall	Soil	Description	Drainage	Permeability	Erosion hazard
Ri1	O	17O 37' 33"	145O 56' 49"	3482	Ferrosoll	Friable clay	Poor[2]	High[3]	High[4]
Fr1	C	17O 37' 55"	145O 57' 02"	3482	Ferrosol	Friable clay	Poor[2]	High[3]	Moderate[5]
Fr2	O	17O 51' 29"	146O 05' 48"	3149	Ferrosol	Friable clay	Poor[2]	High[3]	High[4]
DoC	C	17O 51' 58"	146O 05' 57"	3149	Ferrosol	Friable clay	Poor[2]	High[3]	Moderate[5]
Wa1	O	18O 07' 17"	145O 48' 11"	2426	Dermosol	Friable clay	Imperfect[6]	Moderate[7]	Slight[8]
To1	C	18O 03' 50"	145O 49' 27"	2674	Dermosol	Friable clay	Well[9]	Moderate[7]	Slight[8]
Gr1	O	17O 34' 42"	146O 00' 03"	3390	Ferrosol	Friable clay	Poor[2]	High[3]	High[4]
Da1	C	17O 35' 11"	146O 00' 14"	3390	Ferrosol	Friable clay	Poor[2]	High[3]	Moderate[5]
DoO	O	17O 07' 39"	145O 22' 57"	967	Ferrosol	Friable clay	Imperfect[6]	Moderate[7]	Moderate[5]
Ho1	C	17O 06' 19"	145O 24' 47"	924	Ferrosol	Friable clay	Well[9]	High[3]	Slight[8]

[1] O = Organic, C = Conventional
[2] Water is removed very slowly in relation to supply
[3] Vertical transmission of water throught the soil profile would take 1-12 hours after thorough wetting to reach field capacity
[4] Significant erosion can occur
[5] Significant erosion can occur during development of a particular land use
[6] Water is removed only slowly in relation to supply
[7] Vertical transmission of water through the soil profile would take 1-5 days after thorough wetting to reach field capacity
[8] No appreciable erosion damage is likely to occur
[9] Water is removed from the soil readily but not rapidly

12.2.1 Comparison of soils under different systems

Physical indicators

There were no significant differences in physical soil health indicators between organic and conventional soils (Table 3). The similarity of the sand, silt and clay fractions between organic and conventional soils indicates that there was no significant difference in soil types between the two management systems. Bulk density and WSA did not show significant difference between systems either. This suggests that soil type and climate have more impact on bulk density and WSA than management system.

Table 4 Soil health properties for organic vs. conventional soils sampled at selected farms (n=5 for both farm type) in North Queensland. Mean values are followed by standard errors and were statistically compared using the Least Significant Different (LSD) test (P =0.05).

Group	Indicator	Unit	Organic ± (s.e.)			LSD	Conventional ± (s.e.)			LSD
Physical indicators	**Bulk density**	kg l^{-1}	1,14	±	0,017		1,10	±	0,014	
	Clay	%	18,5	±	1,00		24,0	±	2,10	
	Sand	%	50,0	±	1,30		43,4	±	1,70	
	Silt	%	31,5	±	0,900		32,6	±	0,800	
	WSA	%	90,1	±	0,700		85,2	±	0,900	
Chemical indicators	**Ammonium Nitrogen (KCl)**	mg kg^{-1}	2,64	±	0,300		2,08	±	0,100	
	Available potassium	mg kg^{-1}	167	±	25,3		431	±	132	
	Boron (Hot CaCl2)	mg kg^{-1}	1,30	±	0,100		1,70	±	0,300	
	Calcium (Amm-acet.)	meq $100g^{-1}$	9,10	±	0,700		10,1	±	0,800	
	Cation exchange capacity	meq $100g^{-1}$	12,2	±	1,20		13,6	±	1,00	
	Chloride	mg kg^{-1}	12,1	±	1,80		33,5	±	16,6	
	Copper (DTPA)	mg kg^{-1}	5,60	±	1,30		4,50	±	1,00	
	Electrical conductivity	µS cm^{-1}	51,0	±	2,60		106	±	6,80	
	Iron (DTPA)	mg kg^{-1}	51,0	±	10,1		24,8	±	5,30	
	Labile C	g kg^{-1}	0,622	±	0,0188	a	0,374	±	0,0201	b
	Magnesium (Amm-acet.)	meq $100g^{-1}$	2,30	±	0,200		2,40	±	0,200	
	Manganese (DTPA)	mg kg^{-1}	35,8	±	5,80		47,5	±	6,90	
	Nitrate	mg l^{-1}	7,40	±	0,700		11,6	±	1,20	
	Organic carbon	%	2,40	±	0,200		2,10	±	0,100	
	pH		6,65	±	0,100		6,75	±	0,100	
	Phosphorouos (Colwell)	mg kg^{-1}	129	±	24,5		241	±	42,3	
	Phosphorous buffer index		247	±	48,6		325	±	40,2	
	Potassium (Amm-acet)	meq $100g^{-1}$	0,420	±	0,100		1,25	±	0,300	
	Sodium (Amm-acet)	meq $100g^{-1}$	0,067	±	0,0084		0,047	±	0,0041	
	Sulphate sulfur	mg kg^{-1}	15,5	±	2,20	b	63,5	±	11,0	a
	Zinc (DTPA)	mg kg^{-1}	12,0	±	3,50		12,0	±	1,60	
Microbial indicators	**B-glucosidase**	µg pNP released g^{-1} soil h^{-1}	548	±	48,5	a	345	±	34,5	b
	Fluorescein diacetate	mg hydrolysed FDA kg^{-1} soil hr^{-1}	1,31	±	0,100	a	0,847	±	0,00	b
Nematode indicators	**Bacterial feeding**	%	32,0	±	1,60	a	17,0	±	1,30	b
	Bacterivore : Fungivore ratio		0,713	±	0,0211		0,826	±	0,0150	
	Channel index (CI)		24,1	±	1,50		18,8	±	1,10	
	Diversity index (H)		2,05	±	0,028	a	1,47	±	0,050	b
	Endophytes	%	27,0	±	3,80		28,0	±	4,30	
	Enrichment index (EI)		73,6	±	1,00		74,1	±	1,10	
	Fungal feeding	%	14,3	±	1,50	a	2,82	±	0,200	b
	Plant parasites	%	30,8	±	2,20	b	70,2	±	2,10	a
	Predatory and omnivorous	%	20,2	±	1,30	a	10,0	±	1,20	b
	Structure index (SI		67,8	±	1,80		69,1	±	1,60	
Disease indicators	**Fusarium plates**	AUC	8,20	±	0,400		11,5	±	0,300	
	Tomato trials	AUC	16,22	±	0,800	b	19,6	±	1,00	a

Chemical indicators

Most chemical soil health indicators did not show significant difference between management systems. Labile (active) C was significantly higher in organic soils and values were almost twice as high as for conventional soils. Sulfate levels were significantly higher in conventional soils at about four times the amount measured in organic soils.

Microbial indicators

There was a significant difference in microbial activity between management systems. Both FDA and β-glucosidase levels were higher in greater in soils. Organic soils appear to have greater microbial activity and higher activity of saprophytic microorganisms.

Nematode indicators

Nematode community structure was considerably different between organic and conventional soils. Percentage of bacterial feeding and fungal feeding nematodes were a factor two and five times higher in organic soils, respectively while the percentage of

predatory and omnivorous nematodes was also more than twice as high in organic soils compared to conventional soils. The percentage of plant parasitic nematodes, on the other hand, was only half of that for conventional soils. Lastly, the organic soils had a significantly higher diversity index, indicating that nematode community and likely the whole soil food web is more diverse and better balanced than in conventional soils.

12.2.2 Disease suppressiveness of the soils

Hyphal extension

The hyphal extension tests did not show a significant difference in *Foc* hyphal growth between organic and conventional soils (Table 3).

Incendence of Fol

Based on the results from the isolations of dead, diseased and healthy tomato plants it appears that *Fol* was not the only pathogen present in the soil that potentially affected tomato plant health. *Fol* was isolated from dead plants of all soils. Other plant antagonists isolated from dead or diseased plants included *Chalara* spp., *Curvularia lunata, Gliocladium* spp., *Phoma* spp., *Phomopsis* spp., *Pythium* spp., *Rhizoctonia* spp., *Sclerotinia* spp. *and Thielaviopsis* spp. The bacterial wilt *Ralstonia* was widespread and a serious limitation to tomato growing in the region.

Plant growth

The pot trial did not show any significant difference in growth or disease development between the management systems (Table 4). However, the averages for growth indicators were slightly higher and the averages for disease indicators were slightly lower in organic soils than in conventional soils.

Table 5 Averages of disease indicators of potted banana plants in a selection of soils used in the survey

Farm	System	Rhizome discoloration	Roots discoloration	Wilting	Growth cm wk^{-1}	Leaf emergence wk^{-1}	LA $cm2^{-1}$
Da1	C	5,00	3,40	6,67	2,63	2,06	56,70
DoO	O	6,00	3,20	6,50	1,79	2,00	50,25
Gr1	O	4,83	2,17	6,33	3,71	2,67	85,93
Ho1	C	5,33	3,67	8,00	1,88	2,29	56,56

Correlation between soil health indicators and plant health indicators

Area under disease curve (AUC) for tomatoes showed a negative correlation with nematode diversity, nematode community structure and also with soil magnesium. Disease in tomatoes was positively correlated with soil potassium and sulfate levels.

Hyphal growth of *Foc* on soil in petri-dishes showed a negative correlation with soil bulk density, nematode diversity, and fungal feeding nematodes. A positive correlation was found of *Foc* hyphal extension and Copper, EC, plant parasitic nematodes, organic carbon and sulfate sulphur (Table 5).

Table 6 Correlation of tomato disease progress and *Foc* hyphal extension to selected soil health indicators

Disease indicator x soil health indicator		P	r
Tomato AUC	Diversity H	0,0319	-0,4301
	Magnesium (meq 100 g^{-1})	0,0476	-0,4
	Potassium (meq 100 g^{-1})	0,0007	0,6323
	Structure index	0,0157	-0,4779
	Sulfate sulfur (MCP)	0,0168	0,4734
Fusarium plates	Bulk density	0,05	-0,3961
	Copper (DTPA)	0,0366	0,4201
	Diversity H	0,0464	-0,402
	Electrical conductivity	0,0096	0,5076
	Fungal feeding nematodes %	0,0297	-0,4352
	Plant parasitic nematodes %	0,0316	0,4308
	Organic carbon %	0,0363	0,4206
	Sulfate sulfur (MCP)	0,0424	0,4089

Growth of banana plants

Growth of potted banana plants was positively correlated with nematode diversity, organic carbon and phosphorus content of the soil while it was negatively correlated with soil sulfate content. The leaf area of banana pot plants was positively correlated with soil-P, and rhizome discolouration was negatively correlated with soil-P. Wilting of potted banana plants was negatively correlated with nematode diversity.

Table 7 Correlations between disease and soil indicators in banana pot plants

Disease indicator x Soil indicator		P	r
Growth	H	0,0323	0,62
	Organic Carbon (%)	0,042	0,59
	Phosphorous (Colwell) mg kg^{-1}	0,0173	0,67
	Sulfate Sulfur (MCP)	0,0343	-0,61
Leaf area cm^2	Phosphorous (Colwell) mg kg^{-1}	0,0093	0,71
Rhizome discoloration	Phosphorous (Colwell) mg kg^{-1}	0,0061	-0,74
Wilting	H	0,0181	-0,67

Correlation among soil health indicators

The β-glucosidase levels in the soil were positively correlated with nematode diversity and predatory nematodes and negatively correlated with plant parasitic nematodes (Table 2.9). Fluorescein diacetate (FDA) values were positively correlated with β-glucosidase levels (Table 7).

Table 8 Correlation between β-glucosidase and nematode indicators and FDA and β-glucosidase

Microbial indicator x nematode indicator		P	r
B-glucosidase	Diversity H	0,0375	0,4182
	Plant parasitic nematodes %	0,0301	-0,4342
	Predatory nematodes %	0,0256	0,4455

Microbial indicator x microbial indicator		P	r
FDA	B-glucosidase	0,0135	0,4872

The β-glucosidase values were negatively correlated with boron, phosphorus and P buffer index while they were positively correlated with soil iron, labile carbon C and sodium content. FDA was negatively correlated with available K, B, bulk density and clay and positively correlated with soil labile C, organic C, sand and sodium content (Table 2.11).

Table 9 Correlation between microbial indicators and chemical/ physical indicators

Microbial indicator x Chemical/ physical indicator		P	r
B-glucosidase	Boron (mg kg^{-1})	0,0038	-0,5576
	Iron (mg kg^{-1})	0,0031	0,568
	Labile Carbon (g kg^{-1})	0,0493	0,3971
	Phosphorous buffer index	0,0453	-0,4037
	Phosphorous Collwell (mg kg^{-1})	0,0304	-0,4334
	Sodium (meq 100 g^{-1})	0,0011	0,6137
FDA	Available potassium (mg kg^{-1})	0,0227	-0,4537
	Boron (mg kg^{-1})	0,0038	-0,5569
	Bulk density	0,0396	-0,4141
	Clay %	0,0104	-0,5026
	Labile Carbon (g kg^{-1})	0,003	0,569
	Organic carbon %	0,0337	0,4261
	Sand %	0,0432	0,4076
	Sodium (meq 100 g^{-1})	0,0024	0,5799

Discussion

Representative samples from both organic and conventional banana farms in the North Queensland banana production area were studied. Physical soil properties in conventional and organic soils were very similar, especially within farm pairs. It appeared that WSA and bulk density were mainly influenced by climate and soil type. It may also be that the soil management components including soil tillage that influence bulk density and WSA are done in a quite similar way in both management systems.

Chemical properties of organic and conventional soils were similar indicating similar application of nutrients, albeit in different forms and in a different way. The higher sulfate levels in the conventional soils could be explained by application of sulfur-based pesticides and fertilisers (Balik *et al.*, 2009). The higher levels of labile C in the organic soils can be explained by application of organic soil amendments, especially thoroughly composted, soluble or liquid fertilisers. Observations during the survey suggested that organic banana farmers apply appreciable amounts of these types of fertiliser. Furthermore, the organic farms stimulated soil organic matter content by more cover cropping than conventional farms and retention of crop residues.

Soil microbial activity indicators FDA and β-glucosidase were both higher in organic soils than in conventional soils. The majority of nutrients in conventional production are applied in mineral form, which tend to be readily available to the plant. Microorganisms involved in breakdown of organic materials thus lose a food source and their populations decline. Microorganisms engaged in the mineralization process often have symbiotic interactions with plants, exchanging soil minerals for carbon. However, as plants already receive readily available nutrients in mineral form they no longer benefit from investing carbon in sustaining symbiotic relationships with microorganisms, and these organisms thus may lose their functionality.

The soil nematode community is closely related to the soil microbial activity. This was evident from the relationship between the significant correlations between soil enzyme tests and nematode community indices. In this survey it was observed that the percentage of bacterial feeding, fungal feeding and predatory and omnivorous nematodes in the organic soils were often a factor two or more times greater than for conventional soils. Higher levels of bacterial and fungal feeding nematodes indicate greater levels of microbes, suggesting more intense cycling of nutrients. The higher level of predatory nematodes indicates a degree of self-regulation of the soil nematode community. This is also reflected in the diversity index that is significantly higher in the organic soils. Increasing diversity in an ecosystem, above-ground as well as below-ground increases dynamics, self-regulation and resilience of that system. In the conventional soil the percentage of plant-parasitic nematodes was more than double that of the organic soils.

In conventional soils there were less predatory nematodes to prey on the parasites, leaving the species that can cause economic damage to crops to proliferate. It is possible that pathogens can develop very rapidly in monocultures. Furthermore, pathogens and parasites are less reliant on decomposing organic substrates than other soil micro- and macro-organisms. Plant parasitic nematodes were also positively correlated with *Foc* hyphal growth.

It is questionable if the *Foc* hyphal extension test provides a useful insight in soil Fusarium suppression. Firstly, hyphal growth of Fusarium does not guarantee successful infection and subsequent disease expression of the host plant (Cook and Baker, 1983). Secondly, it was very hard to distinguish between different fungi on the plate, especially between *Foc* and other species of *Fusarium*. Especially in the second half of the test it was unclear if the *Foc* on the plug was colonizing the soil or that the fungi in the soil were colonizing the plug. The test did show that *Foc* hyphal growth, probably as well as any other fungi, was favoured by soil organic carbon.

From a management point of view this means that to reduce *Foc* colonization, organic matter should only be applied if the material is well colonised by other saprophytic microorganisms or if there is sufficient microbial activity in the soil to colonise the material quickly and compete with the pathogen.

The presence of other fungi and possibly bacteria that are antagonistic to tomatoes makes it impossible to use the data for conclusions about soil Fusarium suppression. Tomato may not be the most suitable crop to test soil Fusarium suppressiveness because of its susceptibility to a wide range of plant pathogens. Also, plant health is an important factor in Fusarium infection. Growing tomatoes in a banana soil may therefore not give a clear picture of *Foc* suppressiveness of a soil. It is safer to use the specific host and pathogen one is looking at, or if impossible, to use a wider range of hosts and their associated (Fusarium) pathogens. Another issue was that halfway through the trial it was evident that the *Fol* R3 strain used had lost much of it's pathogenicity. It would be advisable to do a pathogenicity test with the pathogen one wants to use before doing the actual bioassay. Furthermore, it is critically important to cancel out the possibility of cross-contamination.

Conclusion

The soil biological and biochemical measurements developed in this survey are sensitive to changes in management practices. The soil nematode community analysis and the biochemical tests, FDA, β-glucosidase and labile C were all greater on the organic sites relative to the conventional banana sites. The standard chemical soil properties, which are commonly measured failed to detect differences between the two systems. However, it was not possible to determine if organic soils with greater microbial activity and diversity could suppress Fusarium wilt. The assays that were tested, hyphal extension and the tomato bioassay were not suitable for screening soils for their suppressive potential to Fusarium. In particular the hyphal extension was unsuitable as it was difficult to determine if hyphae were growing out of the agar block inoculated with Fusarium or into it. Further work on developing methods for measuring the suppressive potential of soil is needed.

Soil characterization of infected and healthy plants

- characterization of soils (Bio, phy, chem. properties) with plants showing *Foc* symptoms

- characterization of soils with plants with out *Foc* symptoms

- Paired sites studies (Indonesia and Australia)

Paired sites Indonesia

A preliminary survey of the soil factors associated with the suppression of Fusarium wilt of bananas caused by Fusarium oxysporum f.sp. cubense was conducted in Indonesia by comparing paired sites. .The sites were selected as either healthy with banana plants showing no symptoms of Fusarium or infected where the disease was present and causing wilt symptoms. Soil samples were collected from the top 10 cm from at least 10 sites in each area.

Soil samples were processed for physical, chemical and biological properties at the laboratory of the University Gadjah Mada, Yogyakarta. Physical properties assessed included water holding capacity of the 0.5 and 2.0 mm fraction of the soil. Chemical properties included pH, organic C, total N, total P, available K, cation exchange capacity (CEC) and electrical cvonductivity. Biochemical soil properties included labile C, fluoresceine diacetate and β-glucosidase. Furthermore, biological properties were determined using selective media for bacteria, actinomycetes, microbial diversity and Fusarium sp.

Four locations were included in the survey, which included two locations in Central java, one in West Java and one in Lampung, Sumatra. Due to differences in soil properties between the location, the change in soil properties was normalised by determining the difference between healthy and infected sites.

The results of the soil properties from the paired sites at the four locations are given in Table 9. The changes in the soil properties, between healthy and infected plants at each location is given in Table 10. At all locations the CEC, bacterial diversity and number of actinomycetes was greater in healthy soils than unhealthy soils. Conversely the number of Fusarium isolated from the soil was greater in infected sites compared to healthy soils (Table 10). Since there was an increase in the number of Fusarium between healthy and infected areas, the change in numbers was regraded as an indicator of disease potential and used in a regression analysis of soil properties.

The change in Fusarium numbers at the four locations could be explained by:

$$\text{Fusarium (ln(cfu/g soil + 1)} = -3.612-(1.4698*\text{B-glucosidase}) - (1.983*\text{Actinomycete diversity})$$

The predicted change in the numbers of Fusarium, between the heathy and infected sites was significantly related to actual changes in numbers (Figure 1)

Table 10 Soil parameters measured at paired sites, that were infected and showing symptoms of Fusarium wilt and apparently healthy not showing any symptoms of Fusarium wilt in Indonesia

Parameter	KST		JPR		DLG		CJ20	
	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected
pH	5.45	5.93	5.87	6.15	6.90	6.71	6.13	7.28
C	1.55	1.82	2.96	2.98	2.03	1.32	2.22	2.23
N	163.1	195.3	259.4	177.3	632.8	96.2	265.1	626.2
P	28.9	7.1	25.0	32.3	40.4	139.2	62.1	57.9
K	1.69	2.60	1.33	2.62	2.29	1.53	0.77	2.47
CEC	28.9	26.1	21.2	20.8	33.1	26.3	8.6	7.7
WHC 0.5	10.50	16.42	5.52	5.96	8.34	5.71	1.86	2.22
WHC 2	11.17	16.63	5.75	6.23	9.02	6.01	2.36	1.50
EC	75	67	109	248	74	195	218	129
Lab C	0.19	0.20	0.83	0.84	0.19	0.14	0.09	0.77
FDA	33.0	5.5	13.5	16.3	6.6	8.7	12.7	29.1
B-glucosidase	12.8	16.7	6.6	6.6	91.1	88.8	138.0	132.6
Fusarium	3330	3330	1000	4000	0	7670	0	330
ln Fus	8.11	8.11	6.91	8.29	0.00	8.95	0.00	5.80
Bacteria	1.30^{10}	18.2^{10}	1.53^{10}	9.66^{10}	24.3^{10}	68.0^{10}	5.90^{10}	20.1^{10}
Ln(bacteria+1)	23.3	25.9	25.8	25.3	26.2	27.2	24.8	26.0
Bacteria diversity	3	1	1	1	2	1	1	1
No. antagonistic bacteria	2	0	0	0	0	0	0	0
Actinomycetes	7.67^{5}	2.00^{5}	0.10^{5}	0.20^{5}	0.10^{5}	0.10^{5}	0.00	3.00^{5}
Ln(Actin+1)	13.6	12.2	9.2	9.9	9.2	9.2	0.0	12.6
Actinomycete diversity	4	3	0	1	4	3	0	3
No. antagonistic acticonmycetes	1	0	0	1	0	1	0	1
Microbial density	14.0^{5}	9.50^{5}	3.00^{5}	3.85^{5}	2.00^{5}	1.60^{5}	1.36^{5}	3.00^{5}
Ln(mic+1)	14.2	13.8	12.6	12.9	12.2	12.0	11.8	17.2

Table 11: Changes in Soil parameters measured at paired sites, that were infected and showing symptoms of Fusarium wilt and apparently healthy not showing any symptoms of Fusarium wilt in Indonesia

Parameter	δ KST	δ JPR	δ DLG	δ CJ20
pH	-0.48	-0.28	0.19	-1.15
C	-0.27	-0.02	0.71	-0.01
N	-32.19	82.16	536.58	-361.04
P	21.76	-7.26	-98.78	4.22
K	-0.91	-1.29	0.76	-1.70
CEC	2.77	0.33	6.85	0.89
WHC 0.5	-5.92	-0.44	2.63	-0.36
WHC 2	-5.46	-0.48	3.01	0.86
EC	8.00	-139.00	-121.00	89.00
Lab C	-0.02	-0.01	0.05	-0.68
FDA	27.45	-2.87	-2.10	-16.48
B-glucosidase	-3.91	0.00	2.30	5.45
Fusarium	0.00	-3000	-7670	-330.00
ln Fus	0.00	-1.39	-8.95	-5.80
Bacteria	-16.9^{10}	5.67^{10}	-43.7^{10}	-14.2^{10}
Ln(bacteria+1)	-2.64	0.46	-1.03	-1.23
Bacteria diversity	2.00	0.00	1.00	0.00
No. antagonistic bacteria	2.00	0.00	0.00	0.00
Actinomycetes	5.67^{5}	-0.10^{5}	0.00	-3.00^{5}
Ln(Actin+1)	1.34	-0.69	0.00	-12.61
Actinomycete diversity	1.00	-1.00	1.00	-3.00
No. antagonistic acticonmycetes	1.00	-1.00	-1.00	-1.00
Microbial density	4.50^{5}	-0.85^{5}	0.40^{5}	-299.0^{5}
Ln(mic+1)	0.39	-0.25	0.22	-5.40

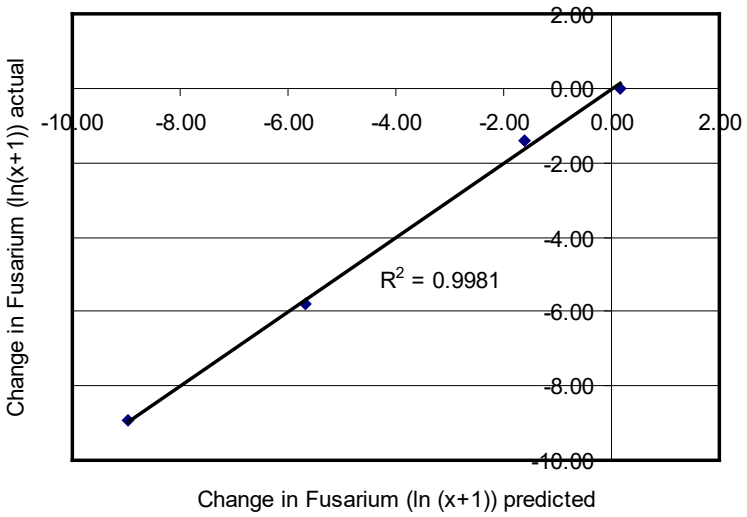


Figure 1: Linear regression model for the predicted change in the number of Fusarium compared to the actual change in Fusarium isolated from the soil of healthy bananas and bananas showing symptoms of Fusarium wilt.

The change in the numbers of Fusarium between healthy and unhealthy soil is not a conclusive method of determining disease suppression, but gives some indication of potential soil factors that may be involved. In this case the decrease in Fusarium was

related to an increase in β-glucosidase and increase in actinomycete diversity. This result suggested that there is potential for soil management practices that increase β-glucosidase activity and the number of actinomycetes in the soil could help with the suppression of Fusarium in the soil and possible the expression of Fusarium wilt symtoms. β-glucosidase measures cellulolytic activity in the soil and is the rate limiting enzyme in the conversion of cellulose to glucose and therefore a good measure for the potential for the degradation of plant material. Greater glucosidase activity could mean that there are more organisms in the soil that can potential degrade organic matter which may compete with the survival of Fusarium in the soil. The diversity of actinomycetes may similarly Fusarium, and it is possible that actinomycetes produce β-glucosidase, which suggest that they were dominant in causing the difference in Fusarium numbers between health and infected areas.

The results are not conclusive but indicate that differences between healthy and infected sites may be due to a reduction in the number of Fusarium in the soil, which is due to increase biological activity particularly cellulose degrading organisms ad the different types of actinomycetes in the soil.

The results from this limited survey suggested that analysing the differences between sites, rather than just the measurements, maybe a feasible method of investigating suppression. This is because there is high variability between locations due to soil types and climates and by investigating the relative difference between the sites ay each location included in the survey some of the variability may be accounted for. This method appeared to be valid as there was a greater number of Fusarium recovered from the soil in areas that were deemed to be infected relative to healthy areas. The factors associated with this change could then be determined, which turned out to be β-glucosidase activity and the diversity of actinomycetes.

Paired sites Australia

A similar survey of paired sites was conducted in a sub-tropical banana production area in Australia. Sites were chosen at four locations where the disease was slow to progress and where the disease progressed rapidly. A follow up to the initial survey was conducted 12 months later and the fields reassessed for Fusarium as well as soil parameters.

The banana fields were assessed in consultation with the growers and using the rating scale of 0-5 described in Table 11.

Table 12: Severity rating scale for banana fields assessed for symptoms of Fusarium wilt

Score	Description
0	No symptoms present. *Foc* present (0) or no confirmation of the presence of the disease.
1	Individual plants show FW symptoms which do not persist throughout the season, suckers tend to grow out of the disease.
2	Individual plants with the symptoms persisting, the following sucker shows symptoms but the disease does not seem to spread.
3	Small clumps of plants showing symptoms, which persist throughout the year, but the disease seems slow to spread.
4	Small clumps of plants showing symptoms, which persist throughout the year and the disease appears to be progressing rapidly.
5	Large areas devastated by the disease which is rapidly progressing.

Soil sampling was conducted by collecting soil with a 50 d mm soil core to a depth of 100 mm at 20 locations within a banana field. Sampling sites were selected by choosing plants at the same stage of development, such as bract fall, within the field. Physical soil parameters, sand, silt and clay and bulk density were determined for the soils. Soil chemical measurements were conducted by a commercial laboratory (IncitecPivot) and included Organic C, pH, EC, NO3, P, PBI, K, Ca, Mg, Na, Cu, Fe, Mn, Zn and SO4. Biochemical analysis of soil samples were conducted at the DAFF, Centre for Wet Tropics Agriculture and Ecosciences Precinct and included soil enzymes, labile C and soil nematode community analysis (pH, EC, NO3, FDA, β-glucosidase, Labile C, nematode trophic groups (parasites, fungivores, bacterivores, omnivores, predators), diversity, enrichment, structure and channel indices).

Samples and field assessments were conducted in October 2010 and again in 2011. October was the period when Fusarium wilt symptoms were typically most severe. A VCG analysis of the isolates taken from each field sampled for confirmation of the disease revealed that one of the sites had a different VCG which was a group as race 4, where as the other VCGs were typical of race 1 of Fusarium wilt. Therefore, the site at Mol was disregarded from further analysis, leaving 7 sites all growing lady finger bananas (*Musa* AAB), with the VCG 0124.

Sites that showed a reduction in the symptoms Fusarium wilt between the two sampling periods underwent further testing to determine the levels of Fusarium oxysporum f. sp. cubense in the soil and suppressiveness.

To determine the *Fusarium oxysporum* levels in the soil at each block a drop plate technique was used in combination with most probable number (MPN) tables. One gram sub-samples of soil were serially diluted in 9 mL aliquots of sterile distilled water and 5 drops of each dilution from 10^{-1} to 10^{-6} were plated onto Nash-Snyder selective media for *Fusarium* spp. Three replicates were plated for each dilution of 5 soil sub-samples, making a total of 15 dilution series for each soil. After 7 days incubation*, Fusarium oxysporum* colonies growing on the media were identified and counted (Figure 3). The mean MPN for each soil was used to calculate an approximate *Fusarium oxysporum* population (propagules) per gram for each site.

Fusarium oxysporum colonies were sub-cultured from the selective media onto potato dextrose agar before being single spored to generate monoconidial isolates. These pure cultures were then utilised for vegetative compatibility group (VCG) testing in order to determine the level of *Foc* within the *Fo* population at each site. VCG testing was carried out on at least 50 isolates from each site. The results from the biological characterisation is summarised in Figure 3.

Furthermore, to demonstrate the putative suppressiveness an experiment was set up comparing soil from the slow and rapid fields. The soil was placed into seedling trays with individual cells, 50mL of soil placed into each cell. The soil was inoculated with *F.o.* f.sp. *lycopersici* spore solution at 3 rates: 10^3, 10^4 & 10^5 spores/mL soil. The isolate used was BCPM 70 (race 3) which was grown on ¼ strength PDA for one week. Spores were harvested by irrigating the plates with sterile distilled water and scraping the colonies with a glass spreader. The spore solution was filtered through cotton wool and concentrations were determined using a haemocytometer. 4mL of inoculum suspension was added per seedling cell at the concentration required to reach those 3 rates, i.e. 1.25×10^6 (10^5 per mL soil), 1.25×10^5 (10^4 per mL soil) and 1.25×10^4 (10^3 per mL soil). 10 replicates (seedling cells) per treatment including untreated controls which received 4mL of sterile distilled water. A pasteurised treatment was included for both soils. Each cell of the pasteurised treatment was inoculated at the highest rate (10^5/mL soil). Tomato seedlings (cv. Tiny Tim) were transplanted into cells just prior to Fol inoculation.

Seedlings were examined for external symptoms and survival during the course of the experiment, but minimal external symptoms developed by the time the experiment was

terminated after 15 weeks. Plants were rated for vascular discolouration at the conclusion of the experiment according to the scale below;

0. No vascular discolouration
1. Discolouration restricted to below the cotyledons
2. Discolouration of stem above the cotyledons, but not to top of plant
3. Complete vascular discolouration of stem
4. Dead plant

Results

The results from the initial survey of the sites are presented in Table 12. Not surprisingly this suggested that Fusarium wilt was more severe at sites where the disease was considered to progress rapidly relative to slow progression of the disease. The results from the follow up survey conducted 12 moths later are presented in Table 13, with the change in soil parameters presented in Table 14.

For some of the parameters measured there was no change between the two sampling times. For other parameters there was an increase or a decrease from the initial sampling time, but for the Fusarium wilt rating however, there were changes with some fields where the disease was not initially present or was determined to be progressing slowly had an increasing in the symptoms of the disease. The change in the severity of the disease was analysed with the initial soil properties to determine what soil properties could have led to the change in the disease between the two investigation periods.

It was possible to predict the change in Fusarium at the different sites with soil properties β-glucosidase, nematode community enrichment index, manganese and magnesium contents of the soil as described in equation 2. This model suggested that an increase in the severity of Fusarium wilt in the field was associated with a decrease in the manganese and magnesium measurement and an increase in the enrichment index and β-glucosidase in the soil. The predicted change in Fusarium wilt symptoms using the model described in equation 2 was plotted against the actual change with a significant R^2 of 0.99 (Figure 2).

Table 13: Initial soil physical, chemical and biological soil properties at two field determined to have slow and rapid progress of Fusarium wilt at four locations in sub-tropical Australia and an assessment of the severity of Fusarium wilt in the field

Location	Hut		Lar		Mol	Pie	
Site disease progress	Rapid	Slow	Slow	Rapid	Slow	Slow	Rapid
Foc score (Table 11)	5	2	3	4	3	0	3
Silt	15	21	34	34	34	30	29
Clay	9	10	31	24	29	35	39
Sand	77	69	35	43	37	35	33
Bulk Density	1.28	0.93	1.03	1.00	1.11	0.81	0.89
Drainage	5	5	5	4	2	5	3
pH	5.4	5.7	5.7	5.1	6	5.2	4.8
EC	0.07	0.14	0.17	0.2	0.17	0.26	0.23
Cl	10	10	25	25	19	21	23
NO3	24	58	52	54	35	68	50
P	87	83	130	370	310	480	370
PBI	250	600	360	490	270	900	670
Ca	3.2	7	8.5	10	17	8.5	10
K	0.4	0.51	0.73	1.6	1.1	2.4	1.8
Mg	0.91	2.5	2.6	2.3	5.3	2.6	2.9
Na	0.04	0.09	0.18	0.13	0.1	0.09	0.1
CEC	5.36	10.3	12.1	14.5	23.6	14.4	16.5
Al	0.81	0.18	0.1	0.47	0.1	0.77	1.7
Cu	0.2	0.1	0.78	0.29	4	1.1	0.42
Fe	120	74	99	110	120	78	71
Mn	4.3	13	80	33	19	20	22
Zn	2.1	1.8	4.4	15	14	15	8.7
S	14	37	39	64	39	150	130
Organic C	2.9	5.9	3.4	4.6	2.6	5	4.1
Labile C	0.54	0.43	0.53	0.51	0.50	0.49	0.50
FDA	67.5	89.5	66	34.2	18.9	51	24.5
B-Glucosdiase	255.7	317.2	315.6	293.3	65	277.8	105.9
Plant-parasites/100g soil	289	191	319	462	331	329	541
Radopholus sp.	58	0	0	0	0	0	0
Pratylenchus sp.	0	0	260	256	127	123	236
H. dihystera	229	29	42	87	201	0	5
H. multicinctus	0	0	5	101	0	181	176
Meloidogyne	3	162	0	0	0	14	80
Criconematids	0	0	8	0	0	0	0
Fungivores/100g soil	58	131	79	34	7	22	44
Bacteriavores/100g soil	114	246	212	217	18	146	188
Predators & omnivores/100 g	73	41	60	102	9	61	97
Diversity H'	1.76	1.71	1.84	2.09	1.07	1.79	1.94
Enrichment index	75	83	81	82	89	87	84
Structure index	75	58	64	83	81	79	80
Channel index	19	16	14	7	12	5	10
B/(B+F) ratio	0.68	0.66	0.73	0.86	0.68	0.87	0.78
taxa	9	8	11	12	6	8	9
Detritus	37	72	46	33	13	33	29
Predation	27	12	14	20	5	17	19
Roots	36	15	41	47	82	50	52

Table 14 Repeated soil physical, chemical and biological soil properties at two field determined to have slow and rapid progress of Fusarium wilt at four locations in sub-tropical Australia and an assessment of the severity of Fusarium wilt in the field

Location	Hut		Lar		Mol	Pie	
Site disease progress	Rapid	Slow	Slow	Rapid	Slow	Slow	Rapid
Foc score (Table 11)	5	4	2	5	4	3	4
Silt	14	18	31	31	33	28	28
Clay	8	9	35	23	26	33	38
Sand	78	73	34	46	41	40	35
Bulk Density	1.28	0.93	1.03	1.00	1.11	0.81	0.89
Drainage	5	5	5	4	2	5	3
pH	5.6	5.9	5.6	5.6	6.9	5.4	4.9
EC	0.06	0.07	0.13	0.09	0.19	0.15	0.17
Cl	19	16	27	12	10	10	13
NO3	9.7	12	22	14	27	27	36
P	130	54	150	350	410	590	450
PBI	250	620	350	480	320	870	740
Ca	4	8	8.5	13	27	11	11
K	0.72	0.78	0.96	2.1	1.6	2.1	3.1
Mg	1.2	2.5	2.6	4	7.2	3	3
Na	0.04	0.1	0.2	0.17	0.16	0.13	0.12
CEC	6.49	11.5	12.4	19.4	36	16.7	19.4
Al	0.53	0.14	0.1	0.14		0.46	2.2
Cu	0.15	0.08	0.69	0.3	6.5	1.3	0.55
Fe	83	49	54	94	110	76	83
Mn	6.2	9.4	78	32	11	28	24
Zn	2	1.3	4.3	18	19	22	13
S	7.3	12	35	16	10	46	37
OC	3.1	5.2	3.4	4.9	4.1	4.8	4.5
Labile Carbon	0.36	0.29	0.37	0.32	0.31	0.30	0.32
FDA	65.3	78.3	64.8	35.8	20.2	50	22.3
B-Glucosdiase	117.3	17.3	149.1	135.1	59.2	269.1	161.9
Plant-parasites/100g soil	521	538	776	1443	145	393	1155
Pratylenchus	0	0	564	1031	41	206	818
H. dihystera	466	512	176	412	104	75	289
Meloidogyne	0	26	0	0	0	75	0
Fungivores/100g soil	192	128	212	550	104	150	241
Bacterivores/100g soil	329	358	529	825	414	356	1011
Predators & omnivores/100 g	548	486	564	1443	497	262	433
Diversity H'	2.02	2.05	2.16	2.23	2.17	2.26	2.08
Enrichment index	76	59	82	54	82	83	78
Structure index	90	88	91	87	94	84	77
Channel index	23	29	10	47	10	9	13
B/(B+F) ratio	0.63	0.74	0.71	0.6	0.8	0.7	0.81
taxa	10	11	12	11	11	12	11
Detritus	30	25	31	20	35	42	44
Predation	58	58	41	53	59	37	25
Roots	12	16	27	26	6	21	32

Table 15: Changes in soil physical, chemical and biological soil properties at two field determined to have slow and rapid progress of Fusarium wilt at four locations in sub-tropical Australia and an assessment of the severity of Fusarium wilt in the field

Location	Hut		Lar		Mol	Pie	
Site disease progress	Rapid	Slow	Slow	Rapid	Rapid	Slow	Rapid
δ *Foc* score	0	2	-1	1	1	3	1
δ Silt	-1	-3	-3	-3	-1	-2	-1
δ Clay	-1	-1	4	-1	-3	-2	-1
δ Sand	1	4	-1	3	4	5	2
δ BD	0	0	0	0	0	0	0
δ Drain	0	0	0	0	0	0	0
δ pH	0.2	0.2	-0.1	0.5	0.9	0.2	0.1
δ EC	-0.01	-0.07	-0.04	-0.11	0.02	-0.11	-0.06
δ Cl	9	6	2	-13	-9	-11	-10
δ NO3	-14.3	-46	-30	-40	-8	-41	-14
δ P	43	-29	20	-20	100	110	80
δ PBI	0	20	-10	-10	50	-30	70
δ Ca	0.8	1	0	3	10	2.5	1
δ K	0.32	0.27	0.23	0.5	0.5	-0.3	1.3
δ Mg	0.29	0	0	1.7	1.9	0.4	0.1
δ Na	0	0.01	0.02	0.04	0.06	0.04	0.02
δ CEC	1.13	1.2	0.3	4.9	12.4	2.3	2.9
δ Al	-0.28	-0.04	0	-0.33	-0.1	-0.31	0.5
δ Cu	-0.05	-0.02	-0.09	0.01	2.5	0.2	0.13
δ Fe	-37	-25	-45	-16	-10	-2	12
δ Mn	1.9	-3.6	-2	-1	-8	8	2
δ Zn	-0.1	-0.5	-0.1	3	5	7	4.3
δ S	-6.7	-25	-4	-48	-29	-104	-93
δ Organic C	0.2	-0.7	0	0.3	1.5	-0.2	0.4
δ Labile C	-0.18	-0.13	-0.16	-0.18	-0.12	-0.19	-0.18
δ FDA	-2.2	-11.2	-1.2	1.6	1.3	-1	-2.2
δ B-Gluc	-138	-300	-166	-158	-6	-9	56
δ PP 100	232	347	457	981	-186	64	614
δ *Radopholus sp.*	-58	0	0	0	0	0	0
δ *Pratylenchus sp.*	0	0	304	775	-86	83	582
δ *H. dihystera*	237	483	134	325	-97	75	284
δ *H. multicinctus*	0	0	-5	-101	0	-181	-176
δ *Meloidogyne*	-3	-136	0	0	0	61	-80
δ *Criconematids*	0	0	-8	0	0	0	0
δ Fungivores/100g soil	134	-3	133	516	97	128	197
δ Bacterivores/100g soil	215	112	317	608	396	210	823
δ Predators & omnivores/100 g	475	445	504	1341	488	201	336
δ Diversity H'	0.26	0.34	0.32	0.14	1.1	0.47	0.14
δ Enrichment index	1	-24	1	-28	-7	-4	-6
δ Structure index	15	30	27	4	13	5	-3
δ Channel index	4	13	-4	40	-2	4	3
δ B/(B+F) ratio	-0.05	0.08	-0.02	-0.26	0.12	-0.17	0.03
δ taxa	1	3	1	-1	5	4	2
δ Detritus	-7	-47	-15	-13	22	9	15
δ Predation	31	46	27	33	54	20	6
δ Roots	-24	1	-14	-21	-76	-29	-20

Equation 2 Prediction of changes in Fusarium wilt symptoms using a multiple linear regression model from initial measurement of soil properties

FW predict =-29.4-(Mn 10*0.031)-(Mg 10*0.753)+(El 10*0.390)+(B-gluc*0.00379)

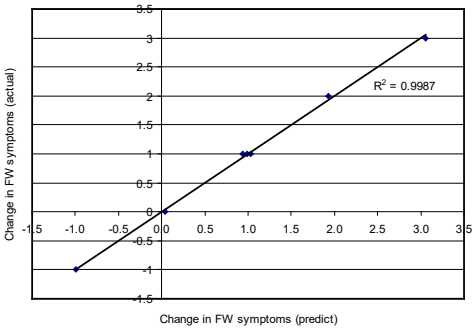


Figure 2: : Linear regression model for the predicted change in the severity of Fusarium using equation 2, compared to the actual change in Fusarium wilt severity at 7 sites in sub-tropical Australia.

One site had a reduction in the symptoms Fusarium wilt between the two sampling periods, the Lar “slow” site (Table 14). This suggested the site may be suppressive to the disease and therefore underwent further evaluation for levels of Fusarium and *Foc* (Figure 3). There were similar levels of both Fusarium spp. and *Foc* in the soil where the disease progressed rapidly and slowly (Figure 3). This suggested that differences in the appearance of the of wilt symptoms in the disease was not due to differences in inoculum of Fusarium. However, the assay using Fol (Figure 4) demonstrated a significant suppression in the vascular discolouration on tomato in the soil where *Foc* was observed to progress slowly. When the soil was steam pasteurised the amount of discolouration was observed to increase in the slow soil but remained at a similar level in the soil from the field where Fusarium wilt was observed to progress rapidly (Figure 4).

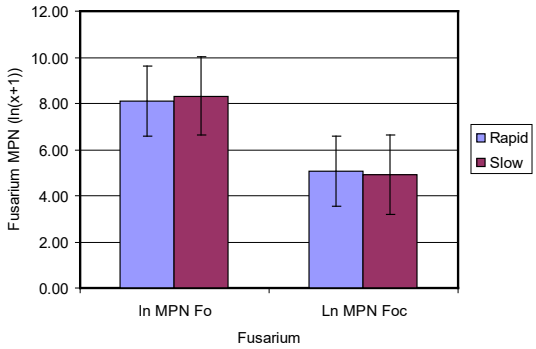


Figure 3 Most probable number of Fusarium oxysporum and calculated Fusarium oxysporum f. sp. cubense from VCG testing of 50 randomly selected isolates

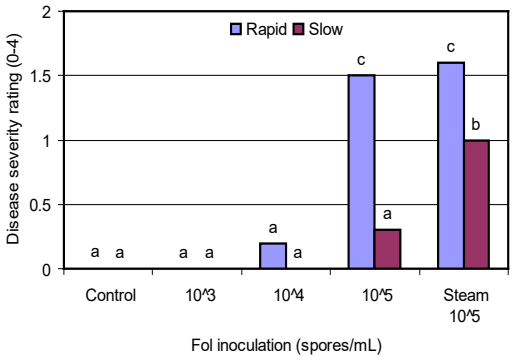


Figure 4 Severity of vascular discolouration of tomatoes inoculated with different concentrations of F.o. f.sp. lycopersici in soil where symptoms of Fusarium wilt of bananas were slow or rapid to develop

Discussion

The change in Fusarium wilt symptoms in banana fields over time was a useful method of determining what soil properties may be assocaiated with the development or suppression of Fusarium wilt symptoms. Although banana growers considered different fields to have “rapid” or “slow” development of disease symptoms they were not always consistent as seasonal changes occurred between years. By matching the changes in disease symtoms with soil properties before changes occurred it was possible to develop a model for soil factors that were related to the enhancement or suppression of the disease. In this case the soil nutrients manganese and magnesium were related to changes in the severity of the disease. A decrease in the elements led to an increase in the observed symptoms of Fusarium wilt. This seems counter to what would be expected as manganese is often associated with waterlogged soils and anaerobic conditions, which are considered to promote Fusarium wilt symtoms.

The change in Fusarium wilt symptoms were also associated with changes in biological soil properties, the soil nematode enrichment index and β-glucosidase. The nematode enrichment index is a measure of the resource available to the soil food web, which is generally considered to be a response of the nematode community to bacteria that can quickly multiply with the availability of nutrients. The enrichment index is typically greater in soils with a lot of available nitrogen. Therefore, it is not surprising that a change in the enrichment index is associated with a change in the Fusarium wilt. Fusarium wilt is considered to increase in situations with high nitrogen availability, which is the same situation where it would be expected to have a high enrichment index. However, relationship beween the β-glucosidase levels in the soil and the change in Fusarium wilt symtoms is opposite to what would be expected. The previous finding in the Indonesia survey demonstrated that an increase β-glucosidase was associated a decease in the number of Fusarium. It would be expected that this relationship would follow in Australia, but the model developed would suggest that an increase in β-glucosidase was associated with an increase in the severity of Fusarium wilt symptoms. Therefore the role that β-glucosidase has as an indicator for suppression of Fusarium wilt of bananas requires further investigation.

There was one field where the Fusarium wilt disease symptoms were observed to decrease, Lar “slow”. When this soil was compared to the paired site where there was a rapid progression of the disease there were similar numbers of Fusarium and *Foc*. This suggested that the mechanism for suppression that led to a decrease in the disease symptoms was not affecting the survival of the pathogen in the soil. Therefore, it possible that a different suppressive mechanism of the disease may be occurring at this site relative to what was observed in Indonesia, where a decrease in the number of Fusarium in the soil was measured between healthy and unhealthy plants. The suppressive mechanism may have been having an indirect impact on the development of the disease symptoms, as the tomato bioassay demonstrated that the soil where Fusarium wilt of bananas declined could suppress the development of Fusarium wilt in tomatoes. Furthermore this suppression was biological, because the steam treatment of the soil removed the suppression. It is noted that many different mechanism may be involved in disease suppression. Direct antagonistic interaction with the pathogen is one mechanism, which may have been occurring in Indonesia, where indirect methods involving a plant may have been occurring in Australia. The indirect mechanism may involve competition on the plant roots, production of antifungal products by rhizoshere organisms or induced suppression in the plant caused by soil organisms.

12.2.3 Understanding soil suppression against *Foc*

Introduction

Fusarium wilt of bananas is caused by the pathogen *Fusarium oxysporum* f.sp. *cubense*, which has had a conspicuous impact on banana production worldwide, altering the global banana trade and cultivar deployment (Pegg et al., 1996; Ploetz, 1994; Stover, 1962). The fungus colonises the xylem tissue of susceptible banana cultivars causing the plant to wilt and die (Ploetz, 1994). Since the first description of the disease in 1876 and its formal recognition in 1910 (Bancroft, 1877; Smith, 1910), there have been various methods attempting to control and manage the disease (Pegg et al., 1996; Ploetz, 1994; Stover, 1962). Investigation into the suppression of Fusarium wilt of bananas have been taking place for almost 100 years with the first reports of studies taking place in 1922 (Bruehl, 1975). There have been many suggestions on the soil conditions that make banana plants more receptive or suppressive to the pathogen (Bruehl, 1975; Dominguez et al., 2001; Peng et al., 1999; Pittaway et al., 1999; Stover, 1962). More recently, studies have focused on developing microorganisms that suppress or compete with the fungus to prevent disease development (Cao et al., 2005; Forsyth et al., 2006; Getha et al., 2005; Jie et al., 2009; Sun et al., 2008; Zhang et al., 2011). The management of Fusarium wilt in commercial banana production has relied on the development of resistant or tolerant cultivars (Ploetz, 1994). However, banana cultivars that are susceptible to Fusarium wilt retain important cultural value in many countries, allowing the development of important niche markets with the fruit retaining high value (Sudarama and Suprapta, 2011). As a consequence, there is increasing interest in the development of integrated disease management systems that enable production of susceptible banana cultivars in the presence of the pathogen (Saravanan et al., 2003).

New agricultural production systems, incorporating multiple practice changes and requiring a paradigm shift from conventional production methods are contentious, but have also proven to be successful in crops such as rice (Turmel et al., 2011; Uphoff, 2003). The development of an integrated management system for suppression of Fusarium wilt of bananas would similarly require changes in current banana production systems, incorporating multiple changes based on knowledge gained from previous research. The suppression of Fusarium wilt in susceptible banana cultivars would be reliant on a three basic factors; a reduction in carry over inoculum, increased plant tolerance and suppression of the pathogen.

A reduction in Fusarium inoculum in infected banana fields can be achieved by removing inoculum, such as infected plant material and decreasing survival of the fungus within plant material. Various methods of removing plant material have been tried such as burning or physical relocation of the plant material. Similarly, chemicals such as carbendazim have been used to reduce the amount of infection, by reducing the survival of the fungus in the soil (Saravanan et al., 2003). Other methods of reducing inoculum carry over include crop rotation, flooding and bare fallows (Stover, 1962).

Increasing plant resistance and reducing plant stress relies on crop management and environmental factors. Environmental factors that induce plant stress such as temperature extremes, water logging and drought have been found to enhance the disease (Aguilar, 1998; Pegg et al., 1996). Therefore, soil water management becomes a critical factor in management of the disease. Furthermore, increased nitrogen applications, particularly in the form of chicken manure, have been found to enhance disease symptoms (Nasir et al., 2003; Pittaway et al., 1999). Different compounds have been found to increase or induce resistance of banana plants to diseases such as menadione sodium bisulphite (Borges et al., 2004) and silicon (Guntzer et al., 2012; Kablan et al., 2012). Furthermore, the action of some soil microorganisms has been found to enhance plant resistance and defence to pathogens (Zhang et al., 2011).

Suppressing the pathogen in the soil relies on the action of antagonistic soil organisms. This may include the production of metabolites which have antibiotic compounds

(Saravanan et al., 2003), competition within the soil and hyper-parasitism of the fungus. The addition of biocontrol organisms has been successful in pot trials, but has not been demonstrated to be as effective in the field, possibly due to competition from indigenous microorganisms (Jie et al., 2009; Zhang et al., 2011). An alternative view which has developed is the enhancement of indigenous antagonistic organisms in order to suppress pathogens through the design of agriculture production systems (Doornbos et al., 2012; Dore et al., 2011; Malezieux, 2012; Ratnadass et al., 2012). The use of cover crops and increasing plant species diversity has been suggested as one method of altering soil organisms and increasing pathogen antagonists (Djigal et al., 2012; Ratnadass et al., 2012; Ratnadass et al., 2006; Van der Putten et al., 2006; Wardle et al., 2004).

The aim of this experiment was to develop an integrated management system for the suppression of Fusarium wilt on susceptible banana cultivars, which could suppress disease symptoms and allow banana production to continue in an infected field.

Materials and methods

Site description

A field trial was established on a commercial farm near East Palmerston (S17^{o} 35.095' and E 145^{o} 51.372', 183 masl) approximately 15 km west of Innisfail in north Queensland. The soil is described as a clay loam ferosol, having poor drainage but high permeability of soil water. A range of other physical characteristics of the soil have been determined (data not shown).

Treatment description

The site was managed under commercial practices for irrigation, nutrient application (approximately 220 kg N/ ha /yr), fungicide application, de-leafing and bunch management. Differences in the management practices in the four different systems were as follows:

• 'A', an aspirational system using all management practices available regardless of expense and availability. Practices may be commercially viable in the future. This treatment included the use of unregistered chemicals that had been show to have some affect against *Foc* in published and unpublished work. It also included management practices that could be implemented in the B treatment.

• 'B', best practice system using only those management practices that could be adopted by growers immediately, not using any unregistered products. It is based on regular de-suckering, interplant vegetation, monthly application of potassium silicate to the soil, foliar fertiliser application during cooler night temperatures,

• 'C', conventional system using current grower practices which included use of herbicides to maintain bare soil around banana plants.

• 'D', demonstrating detrimental practice, those practices that are believed to increase disease severity, which may be common practices used by banana growers without Fusarium, which included a 25% N application as pelletised chicken manure.

Each treatment was replicated 6 times in a randomised plot design, with 12 banana plants in each plot.

Table 16: Treatment schedule for integrated management trial of *Foc* on Ducasse bananas

Practice	Treatment			
	A aspirational	**B best**	**C conventional**	**D detrimental**
De-suckering (monthly)	X	X		
Interplant ground cover	X	X		
Bare interplant space			X	X
Potassium silica	X	X		
Foliar fertilising (May-August)	X	X		
Plant defence activation chemical	X			
Plant growth regulator	X			
Additional N as chicken manure				X

Plant growth and development

Plant measurements have been made monthly for plant growth stage and expression of symptoms. The plant growth stages were determined using a growth stage rating scale (1-9) (Table 16). Additionally the proportion of plants to produce a bunch and the proportion of bunches to make it through to commercial harvest are being recorded.

Disease progress and development

The symptoms for *Foc* were determined using the expression of visual symptoms based on the yellowing of leaves and death of the plant (1-6 scale) (Table 16). Additionally, the proportion of plants to show any symptoms of Fusarium wilt were recorded. Further, a rating based on pseudostem discolouration (1-6) at 1.5 m above ground level at harvest of the bunch or removal of the mother plant was made (Table 16).

Sampling description

Soil was initially sampled in July 2010 before the trail was established. A second set of measurements was made in May 2011 and third set of measurement in September 2011. Soil samples were taken from the top 15 cm of soil in each plot.

Soil physical measurements were made including bulk density. Soil chemical measurements were done be a commercial laboratory (IncitecPivot) and included OM, OC, pH, EC, NO_3, P, PBI, K, Ca, Mg, Na, Cu, Fe, Mn, Zn, SO_4, sand, silt and clay. Biochemical analysis of soil samples were conducted at the DAFF Centre for Wet Tropics Agriculture and included soil enzymes, labile C and soil nematode community analysis (pH, EC, NO3, FDA, β- glucosidase, labile C, nematode trophic groups (parasites, fungivores, bacterivores, omnivores, predators), diversity, enrichment, structure and channel indices).

Table 17: Rating for bananas for external and internal symptoms of Fusarium oxysporum f.sp. cubense on banana.

Rating	Banana growth and development stages	External symptom description	Internal symptom description
1	Bits	Plant showing no symptoms or yellowing	No vascular discolouration in pseudostem
2	Emergence	Plant showing slight streaking and /or yellowing of lower leaves	Isolated points of discolouration in vascular tissue of the pseudostem
3	15 leaf stage	Plant showing streaking and/or yellowing of majority of lower leaves and/or some symptoms on younger leaves	Discolouration of up to one-third of vascular tissue of the pseudostem
4	25 leaf stage	Plant showing extensive streaking and/or yellowing of most or all of the leaves.	Discolouration of between one-third and two thirds of the vascular tissue of the pseudostem
5	Bunch emergence	Mother plant dead sucker alive	Discolouration of greater than two-thirds and of vascular tissue of the pseudostem
6	Bract fall	Mother plant and sucker dead	Total discolouration of vascular tissue of the pseudostem
7	½ mature bunch		
8	Mature bunch		

Results

The experiment was terminated in April 2013 after two years from when treatments were imposed. There were no significant differences in bunch weights between treatments, although the mean bunch weight had declined in the second year relative to the first (Figure 5). The proportion of bananas that were harvested appeared greater in the first year relative to the second year of the experiment. The greatest proportion of plants producing a bunch that were harvested was in the A treatment (Figure 6). Further analysis of the types of bunch losses is proposed, as Fusarium wilt was not always the cause of bunch losses in the experiment.

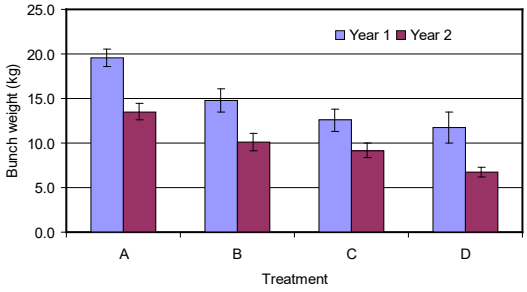


Figure 5: Bunch weights of bananas over two successive years of Ducasse bananas under four different management treatments.

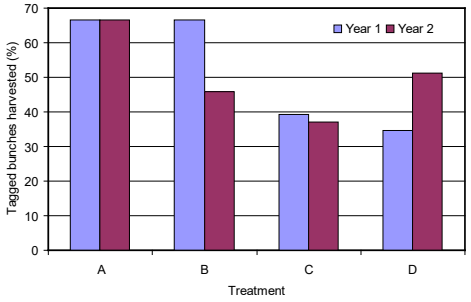


Figure 6 Proportion of bananas harvested over two successive years of Ducasse bananas under four different management treatments.

The incidence of disease, calculated from the proportion of plants showing symptoms was found to fit a double Fourier model, which suggested that there were two peaks of disease incidence around the 4 and 9 months of the year (Figure 7). The lines of best fit determined from the A value in the model were significantly different between treatments, which moved the curves on the Y axis. Further, analysis of the peaks from the curves with seasonal conditions is currently underway which it is hoped to explain why peaks are seen at the two different times of the year. It is hypothesized the curves follow changes temperature and water availability during the year. Furthermore, the movement of the curves along the Y axis is due to soil factors affecting disease incidence and expression which are still to be determined, although treatments with bare soil were not significantly different from one another but were significantly higher on the Y axis relative to the treatments with vegetated soil cover.

The amount of pseudostem discolouration tended to be greater in treatments with bare soil in both years of the experiment (Figure 8). There did not appear to be any significant differences between the years among the treatments. A final pseudostem rating for all plants in the trail was conducted at the conclusion of the experiment and deta is still to be analysed and presented.

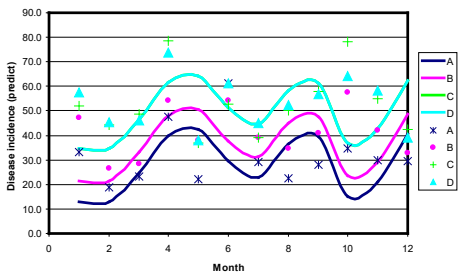


Figure 7: Model for the seasonal incidence of Fusarium wilt in Ducasse bananas based on a double Fourier curve (A + B*SIN(2pi(X-E)/W) + C*SIN(4pi(X-F)/W)) with different A values for each treatment.

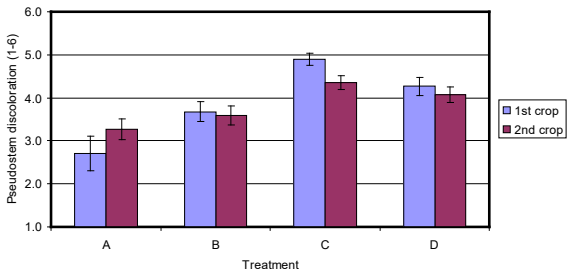


Figure 8: Ratings of internal symptom of *Foc* by pseudostem discolouration in four different *Foc* management treatments in two successive banana crops.

Values for soil parameters measured are give in table 17 for chemical properties and 18 for biochemical and nematode indices. Significant differences between treatments at each

sampling time are presented in Table 19. Further analysis of the soil parameters are required using a split plot over time analysis to determine significant changes in soil properties over the course of the experiment. The measurement of the final soil properties is currently underway. Changes in soil properties will be correlated with changes in disease incidence and severity and production of bananas. The April 2013 sampling is still being analysed and further microbial analysis using Biolog ecoplotes will also be conducted to match data collected in September 2012.

Table 18: Mean values of soil chemical analysis of soils from a *Foc* experimental site at four sampling periods.

Sampling dates		Jul-10	May-11	Sep-11	May-12	Sep-12	Apr-13
Organic Matter	%	4.6	4.5	4.5	4.8	4.8	
Organic Carbon	%	2.7	2.6	2.6	2.8	2.8	
pH	(1:5 w)	7.1	7.0	6.7	6.3	6.6	
pH	(1:5 $CaCl_2$)	6.5	6.4	6.2	5.9	6.1	
Elect. Conductivity	dS/m	0.08	0.10	0.22	0.38	0.25	
Chloride	mg/kg	17	10	25	21	24	
Nitrate Nitrogen (NO3)	mg/kg	11	27	18	95	27	
Phosphorus (Colwell)	mg/kg	58	98	66	120	117	
Phosphorus Buffer Index (PBI-Col)		590	585	513	505	512	
Available Potassium	mg/kg	92	140	470	612	487	
Cation Exch. Cap.	Meq/100g	10.6	10.8	10.9	11.9	12.8	
Calcium (Amm-acet.)	Meq/100g	9.0	7.8	7.4	7.9	9.6	
Potassium (Amm-acet.)	Meq/100g	0.24	0.37	1.2	1.5	1.2	
Magnesium (Amm-acet.)	Meq/100g	1.3	2.6	2.2	2.4	1.9	
Sodium (Amm-acet.)	Meq/100g	0.04	0.02	0.07	0.07	0.05	
Calcium/Magnesium Ratio		6.9	3.1	3.3	3.3	5.1	
Copper (DTPA)	mg/kg	2.1	2.5	2.1	2.3	2.3	
Iron (DTPA)	mg/kg	22	29	27	29	30	
Manganese (DTPA)	mg/kg	48	58	48	57	61	
Zinc (DTPA)	mg/kg	1.7	2.6	5	6	6.5	
Sulfate Sulfur (MCP)	mg/kg	26	12	109	152	125	
Calcium	%	85	72	68	66	73	
Magnesium	%	12	24	21	20	15	
Potassium	%	2.3	3.4	11	13	10	
Sodium	%	0.0	0.0	0.0	0.4	0	
Potassium to Magnesium Ratio		0.2	0.2	0.6	0.6	0.7	

Table 19: Nematode community and biochemical changes in soils from a *Foc* experimental site at four sampling periods

Sampling dates		Jul-10	May-11	Sep-11	May-12	Sep-12	Mar-12
Total nematodes	100 g soil	1291	920	1450	839	1043	
Parasites	100 g soil	975	582	875	411	567	
Parasites	%	76	62	60	54	54	
R. reniformis	100 g soil	693	269	673	267	384	
H. dihystera	100 g soil	22	48	64	147	134	
Meloidogyne spp.	100 g soil	62	17	47	23	14	
Criconema spp.	100 g soil	198	245	90	33	35	
Fungivores	100 g soil	67	48	108	59	77	
Fungivores	%	5	6	8	9	7	
Bacterivores	100 g soil	124	107	366	135	296	
Bacterivores	%	9	11	24	19	28	
Ba1	100 g soil	59	60	200	87	183	
Ba2	100 g soil	43	28	150	43	101	
Ba3	100 g soil	22	17	8	15	12	
Predator & Omnivores	100 g soil	124	93	101	115	103	
Predator & Omnivores	%	10	21	8	17	10	
Ca4	100 g soil	57	67	30	47	43	
Om4	100 g soil	59	90	48	63	37	
Taxa		10.2	12.0	12.5	12.1	11.8	
Diversity H'		1.55	1.88	1.80	1.98	1.92	
B/(B+F)						0.80	
Enrichment index		67	72	74	76	79	
Structure index		76	88	58	80	68	
Channel index		37	28	14	20	11	
Detrital channel		22	21	44	35	48	
Predation channel		26	42	17	33	19	
Root channel		52	37	39	32	32	
Organic carbon	(%)	2.7	2.6	2.6	2.8	2.8	
Organic matter	(%)	4.6	4.5	4.5	4.8	4.8	
Labile carbon	(g kg^{-1})	0.47	0.39	0.50	0.51	0.49	
Fluorescein diacetate (FDA)	(mg FDA hydrolysed kg^{-1} soil hr^{-1})	37	42.2	44.9	28.0	48.7	
β-glucosidase	(mg nitrophenol kg^{-1} soil h^{-1})	12.7	23.7	31.1	3.30	39.3	

Table 20: May 2011 significant differences in biological soil parameters between treatments to manage *Foc* in bananas.

Parameter	Units	Treatment				LSD
May 2011		**A**	**B**	**C**	**D**	
Detritus channel	%	18 b	13 b	36 a	16 b	14.7
Predation channel	%	44 a	48 a	27 b	51 a	13.4
Enrichment index	%	73 ab	64 b	86 a	68 b	14.8
Om4	100 g soil	90 a (4.51)	90 a (4.51)	47 b (3.87)	93 a (4.55)	(0.50)
September 2011		**A**	**B**	**C**	**D**	
Total nematodes	100 g soil	1574 a (7.36)	2125 a (7.66)	880 b (6.78)	908 b (6.81)	(0.48)
Bacterivores	100 g soil	343 ab (5.84)	615 a (6.42)	190 b (5.25)	189 b (5.25)	(0.68)
Ba1	100 g soil	194 ab (5.27)	350 a (5.86)	62 c (4.14)	108 bc (4.69)	(0.75)
Fungivores	100 g soil	132 a (4.89)	152 a (5.03)	43 b (3.78)	54 b (4.01)	(0.71)
Parasites	100 g soil	970 a (6.88)	1152 a (7.05)	546 b (6.30)	550 b (550)	(0.56)
Pp2	100 g soil	926 a (6.83)	1113 a (7.02)	419 b (6.04)	424 b (6.05)	(0.55)
R. reniformis	100 g soil	787 a (6.67)	914 a (6.82)	380 b (5.94)	348 b (5.86)	(0.56)
H. dihystera	100 g soil	57 ab (4.07)	95 a (4.57)	15 c (2.75)	15 bc (2.80)	(1.30)
Criconema spp.	100 g soil	8 b (2.24)	26 ab (3.31)	84 a (4.44)	106 a (4.67)	(1.72)
Fluorescein diacetate (FDA)	(mg FDA hydrolysed kg^{-1} soil hr^{-1})	45 b	53 a	38 c	44 bc	7.5
β-glucosidase	(mg nitrophenol kg^{-1} soil h^{-1})	37.0 a	38.1 a	26.3 b	22.9 b	3.72
May 2012		**A**	**B**	**C**	**D**	
Total nematodes	100 g soil	1061 b	1000 b	665 ab	479 a	
B/(B+F)		0.56 a	0.63 ab	0.75 b	0.76 b	0.14
Fungivores	100 g soil	129 b	87 b	32 a	33 a	
Fungivores	%	13 b	9 ab	6 b	8 b	
Pp2	100 g soil	574 b	558 b	391 ab	229 a	
H. dihystera	100 g soil	291 b	191 ab	58 a	48 a	
Predators + Omnivores	100 g soil	174 b	208 b	65 a	73 a	
Ca4	100 g soil	65 b	73 b	29 a	21 a	
Om4		97 b	99 b	21 a	21 a	
Fluorescein diacetate (FDA)	(mg FDA hydrolysed kg^{-1} soil hr^{-1})	32.3 c	30.5 bc	25.8 a	23.2 ab	
β-glucosidase	(mg nitrophenol kg^{-1} soil h^{-1})	4.13 b	3.86 b	2.75 a	2.47 a	
September 2012		**A**	**B**	**C**	**D**	
Fungivores	100 g soil	41 b	28 ab	15 a	15 a	
Criconema	100 g soil	2 a	1 a	55 b	53 b	
Taxa		11.8 ab	10.5 a	12.8 b	12.2 c	
B/(B+F)		0.68 a	0.77 ab	0.86 bc	0.88 c	
Channel index		18 b	12 ab	8 a	7 a	
Fluorescein diacetate (FDA)	(mg FDA hydrolysed kg^{-1} soil hr^{-1})	58.8 bc	59.1 b	39.8 a	37.3 a	
β-glucosidase	(mg nitrophenol kg^{-1} soil h^{-1})	50.0 b	47.2 b	34.0 a	26.1 a	
pH		7.2 b	7.0 b	6.2 a	6.3 a	
NO3-N	mg/L	5.5 a	7.0 a	19.7 ab	32.2 b	

* numbers in parenthesis are transformed means with which the analysis of variance was conducted on. Means in columns followed by the same subscript are not significantly different from one another at P=0.05

It appears that vegetative ground cover can significantly reduce the incidence and severity of disease but doe not change when symptoms will occur during the year. Further, analysis of factors contributing to the possible suppression and enhancement of disease symptoms is required including climatic data as well as the soil data which is associated with suppression.