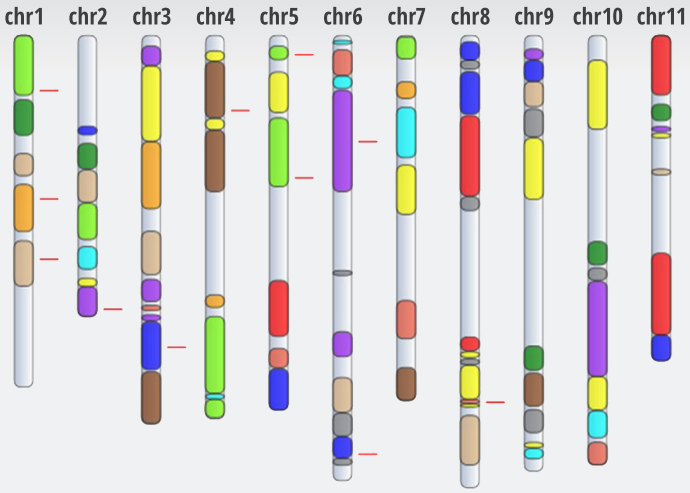
RESEARCH PROGRAM ON
Roots, Tubers and Bananas

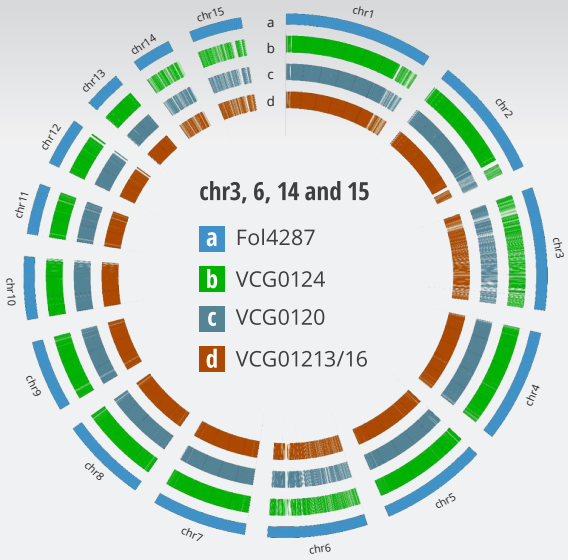
CGIAR

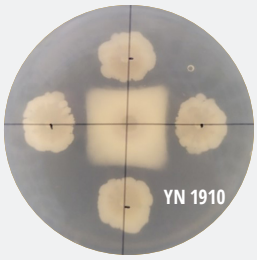
ASPECTS OF CURRENT RESEARCH TO COMBAT

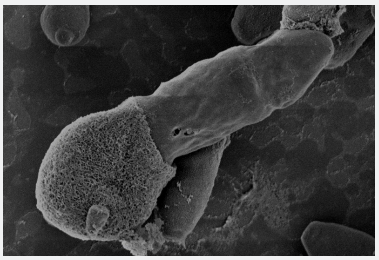
Fusarium wilt of banana with a special focus on TR4

Brief proceedings of an RTB virtual mini-symposium
FP3 Resilient Crops - Cluster BA3.3 Fungal and Bacterial Wilts
16-17 December 2020









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Preface by
James Legg

Alliance

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The Alliance of Bioversity International and the International Center for Tropical Agriculture (CIAT) delivers research-based solutions that address the global crises of malnutrition, climate change, biodiversity loss, and environmental degradation.

The Alliance focuses on the nexus of agriculture, nutrition and environment. We work with local, national, and multinational partners across Africa, Asia, and Latin America and the Caribbean, and with the public and private sectors and civil society. With novel partnerships, the Alliance generates evidence and mainstreams innovations to transform food systems and landscapes so that they sustain the planet, drive prosperity, and nourish people in a climate crisis.

The Alliance is part of CGIAR, the world's largest agricultural research and innovation partnership for a food-secure future dedicated to reducing poverty, enhancing food and nutrition security, and improving natural resources.

The International Institute of Tropical Agriculture (IITA) is a non-profit institution that generates agricultural innovations to meet Africa's most pressing challenges of hunger, malnutrition, poverty, and natural resource degradation. Working with various partners across sub-Saharan Africa, we improve livelihoods, enhance food and nutrition security, increase employment, and preserve natural resource integrity.

The **CGIAR Research Program on Roots, Tubers and Bananas (RTB)** is a partnership collaboration led by the International Potato Center implemented jointly with the Alliance of Bioversity International and the International Center for Tropical Agriculture (CIAT), the International Institute of Tropical Agriculture (IITA), and the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), that includes a growing number of research and development partners. RTB brings together research on its mandate crops: bananas and plantains, cassava, potato, sweetpotato, yams, and minor roots and tubers, to improve nutrition and food security and foster greater gender equity especially among some of the world's poorest and most vulnerable populations.

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Alliance of Bioversity International and CIAT, Rome, Italy

Credit for cover illustrations: Fabiola Alfaro and team; Reuben Ssali and team; Huacai Fan; Dr. R. Thangavelu; Evans Were and team; and Miguel Dita. Frontispiece layout by Daniel Gutiérrez, front-matter coordination by Vicky Rengifo, Alliance of Bioversity International and CIAT Communications team.

Disclaimer:

This document has been developed by researchers in Fusarium Wilt of Banana. The Alliance of Bioversity International and CIAT and IITA consider that they provide an informed and realistic foundation for prioritising Banana Fusarium Wilt research and linked development. The goal is to use this research to control Banana Fusarium Wilt in a sustained manner, while protecting food security, by encouraging partnerships that increase the impact of research and adoption of technological innovations. The Alliance of Bioversity International and CIAT, and IITA encourage international, regional and national public research organizations, development agencies, NGOs, the private sector and other stakeholders to use the information set out herein to guide their activities and investment decisions. All views and opinions expressed herein are those of the contributors but do not necessarily reflect the views and opinions of their individual institutes. While every effort was made to ensure the accuracy of the information reported in this publication, The Alliance of Bioversity International and CIAT, IITA, and any contributing authors will not/cannot accept any responsibility for the consequences of the use of this information.

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The team acknowledges the research contributions from the 21 presenting research teams listed in Annex 1, whose work will collectively help win the fight against Fusarium Wilt of Banana (FWB), especially Tropical Race 4 (TR4), and whose findings will also prove more broadly useful for controlling plant diseases, and helping to strengthen food security and agricultural livelihoods, and reduce poverty across the world.

We would also like to acknowledge the individual members of the organising team behind this symposium, Dr Guy Blomme (The Alliance of Bioversity and CIAT), Dr Miguel Dita (The Alliance of Bioversity and CIAT), Dr George Mahuku (IITA) and Anne Vezina (the Alliance of Bioversity and CIAT), without whose initiative this important collaborative and knowledge-sharing event would not have occurred.

We would like to thank Dr James Legg of IITA for his review and for providing the Preface.

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Preface

James Legg, IITA

TR4 symptomatic banana plant (credit George Mahuku, IITA)

Banana wilt stem xylem blockage (credit Miguel Dita, Bioversity-CIAT Alliance)

The banana is one of a handful of truly global fruits. Although it is almost entirely produced in the tropics, it has become an essential part of the fruit baskets of people from all corners of the world. As well as being a plantation-produced, globally-traded commercial crop, bananas are also a staple food for hundreds of millions of smallholder farmers.

It is because of this planet-wide strategic importance of bananas, that epidemics of damaging pathogens pose such a threat. In the 1950s and '60s, the Gros Michel variety of dessert banana that dominated commercial production systems was devastated by a fungal disease that was dubbed 'Panama disease'. The causal agent was a highly pathogenic and 'hard-to-control' strain (Race 1) of *Fusarium oxysporum f. sp. cubense* (*Foc*). The problem was temporarily solved through the expansion in production of an alternative dessert variety – Cavendish – which was resistant to this strain of *Foc*. Fast-forward to the 1990s, and the scenario was repeated as a new resistance breaking Fusarium strain – Foc Tropical Race 4 (*Foc* TR4) – began to 'take down' plantations of Cavendish in Asia. Inevitably, new introductions and outbreaks were subsequently reported from Africa, the Middle-East and Latin America, and banana producers, traders, and processors are looking once again to the international research community for solutions to the potential catastrophe.

Bananas are one of the focus crops of the Roots, Tubers and Bananas (RTB) research programme of the CGIAR, and in view of the grave threat to banana production posed by *Foc* TR4, research into solutions to tackle this problem is one of RTB's top priorities. Most of the pest and disease management work of the Programme is housed within one of its five Flagship Projects – Flagship Project 3 (FP3) – which addresses 'Resilient Cropping Systems'. One of the six 'clusters' of FP3 (BA3.3) focuses specifically on banana fungal and bacterial diseases, and research teams from IITA and the Alliance of Bioversity and CIAT involved in BA3.3 are working with a diverse set of partners from both national and international institutions to improve understanding of how Foc TR4 spreads, as well as developing and deploying effective management strategies.

Sharing both recent research results and plans for future studies are vital components of RTB's strategy to contribute to tackling the threat posed by *Foc* TR4, and it was for this reason that researchers from BA3.3 convened a mini symposium entitled '*Aspects of current research to combat Fusarium Wilt of banana with a special focus onTR4*'. This brought

together more than 60 researchers from across the world for a two-day virtual exchange of cutting-edge research presentations and discussion. Importantly, the results presented are to be published through a special issue of the *Journal of Fungi*.

The work presented in this booklet, which summarizes the proceedings of the mini symposium, constitutes cutting-edge research to address the challenges posed by *Foc* TR4 that threatens Cavendish as well as other key susceptible cultivars. The research reveals new insights into the spread of *Foc* TR4 in southern Africa, the Greater Mekong Delta, and Latin America; evaluating biocontrol agents; the survival of Fusarium spores in water; the effects of nematodes and weevils in pathogen spread and infection; and in breeding for resistance.

There are great opportunities to harness potential synergies from the many complementarities revealed in the symposium, and a need to scale the research from *in vitro* to field studies, with the ultimate goal of implementing scalable findings at farm and plantation level. Fusarium wilt will continue to be a challenge for banana producers in the near future. However, as control technologies described here are scaled out, there is real hope that production of what is one of humanity's most treasured fruits will be restored and that livelihoods of all those that depend on it will be enhanced.

Fusarium sample taking, Mozambique, (credit Guy Blomme, Bioversity-CIAT Alliance)

1 Introduction

Approximately 131 million MT (84%) of bananas are annually produced by smallholders for domestic markets, and 25 million MT (16%) internationally traded, these latter harvested from almost exclusively "Cavendish" cultivar monocultures. Cavendish is also important in domestic markets and altogether represents approximately 50% of global banana production. Fusarium wilt of banana (FWB), caused by the soilborne fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*), is devastating crops. Cavendish bananas are resistant to the widely distributed strain, *Foc* Race 1, but susceptible to more recently emerging tropical race 4 (TR4), which now seriously threatens global production, and for which there is no known comprehensive cure (Kema et al., 2021[1])

The Alliance of Bioversity International and CIAT (the Alliance), and IITA held a 2-day virtual mini-symposium 16 – 17 December 2020 on current research into ways of combating FWB with a special focus on TR4, with the support of the CGIAR research programme on Roots, Tubers and Bananas (RTB). The scope of research falls under RTB Flagship Project 3 (FP3) on resilient RTB crops. FP3 aims to close RTB crop yield gaps arising from (a)biotic threats and develop more resilient RTB production systems that will enhance food security and natural resource quality.

Seventy-five scientists registered for the event and 67 attended one or more of four sessions (average two sessions per attendee). There were 21 presenters (see Annex 1) and three supporting attendees (moderator and organising team). Forty-four (65%) delegates were non-presenters. Levels of engagement were very high (over 80% on average) (Table 1; see Annex 1 for full list). One possible advantage of this virtual platform is that scientists could more easily attend the sessions of specific interest.

Table 1: Symposium participant statistics

Session	Registered	Attended	Average attendance (%)
1	50	43	78.3
2	44	36	78.1
3	36	31	77.6
4	40	37	86.5
Average/session	43	37	80.1
Total	75	67	80.1
Presenters		21	
Other organising team/ moderator		3	
Other delegates		44	

Despite the constraints of working across time zones, faltering connectivity, individual PC problems, understanding softly-spoken or heavily-accented participants, issues with pre-recorded presentations, and variable fluency with virtual conferencing tools, the symposium achieved its objectives of delivering and discussing all presentations within the planned time frame.

Complementary to this virtual mini-symposium, the organizers also held a Masterclass on FWB, including a series of discussions with experts that took place between 24 and 26 of November 2020. The videos of the presentations prepared by the experts in advance of the discussions are available at the masterclass' YouTube channel https://bit.ly/FWBMasterclass. The recordings of the live events have also been uploaded to the channel.

[1] https://www.frontiersin.org/articles/10.3389/fpls.2020.628888/full

2 Abstracts Synopsis

The symposium provided a platform for 21 research teams from across the globe to present their latest research, summarised in this booklet. Key papers will also be published in a special issue of the *Journal of Fungi* in 2021 (see special issue on Fusarium wilt of bananas).

2.1 TR4 geographical spread and aspects of tolerance/resistance

Abstracts covered: i) TR4 distribution in Colombia, India, Lao PDR, Mozambique, Tanzania, & Vietnam; ii) Efforts to improve germplasm (e.g. mutagenic breeding); and iii) Examples of *Foc* surveillance & control methods.

Chittarhat *et al*.'s 11-province survey has identified an alarming spread of FWB in 'Pisang Awak' and 'Cavendish' plantations in northern, central and southern **Laos**. There are additional significant risks of Foc TR4 spreading to Cavendish and local cultivars in southern Laos exacerbated by farmers' lack of knowledge on Panama disease and its management.

Chung Huy et al., are finalising the analyses of samples collected in an 11-province survey in **Vietnam**, whereTR4 is already widespread.

In response to TR4 incursions being recorded in **Mozambique** in 2015, Mahuku *et al*. confirmed TR4 presence in Cavendish and Bluggoe in surveyed smallholder banana farms situated up- and down-stream from the two commercial Cavendish plantations with first reporting *Foc* TR4. They also conducted extensive surveys in **southern Tanzania** and **Zanzibar**. Samples are being analysed and will inform regional mitigation strategies for protecting smallholder banana production systems against *Foc* TR4 incursion.

Otuba *et al*. reported on FWB surveys in three-major banana producing provinces (Manica, Maputo and Nampula) in **Mozambique**, characterising the vegetative compatibility groups (VCGs) responsible, and demonstrating promising management potential of selected fungicides, biocontrol agents and phenolic compounds *in vitro.* Field studies are needed to corroborate *in vitro* results.

Betancourt *et al* reviewed the current state of TR4 in **Colombia**, country responses and lessons learnt. TR4 was first officially reported in August 2019, and remains contained in La Guajira province, with affected farms having increased from two to ten. A regional multi-stakeholder innovation platform is fundamental to TR4 containment and guaranteeing the sustainability and competitiveness of banana agri-food systems in Latin America and the Caribbean.

Saraswathi *et al*.'s **India**n research team reported significant progress in mutation breeding for FWB resistance (*Foc* race 1), having mutagenically developed 35 FWB-resistant lines, one of which (NRCBRM15) has good growth characteristics. Annotation and proteomic studies revealed that nine genes are involved in the resistance mechanism.

Amorim *et al*. presented a review of initiatives to strengthen FWB resistance. Information obtained from sequencing the *Musa spp*. genome helps reveal sources of resistance, especially by the evaluation of banana transcriptome data after infection with *Foc*, but published data on TR4-resistant hybrids from crossbreeding are still scarce. A transgenics approach has been more frequently adopted, and the tools for symptomatological assessments are still reliable methods for evaluating TR4 resistance (see section II).

Discussion on Geographical Spread

Participants raised questions on drivers to *Foc* spread and made comparisons with recent prior FAO-led *Foc* surveys in Laos and Vietnam. Speakers highlighted new areas where TR4 was

detected, and suggested some evidence of spread by rivers, as well as the more common route of planting material from nurseries, and via soil on people and equipment. It was noted that the situation differs in the three regions of Asia, Africa and LAC, each with regionally specific contexts. In Africa (Mozambique), movement of people on and off land is a major driver, even if spread events are less frequent and slower, on account of the extensive smallholder production systems compared with the more intensive and commercially oriented plantations in Asia. A major issue which needs addressing is how to regulate the behaviour of investment companies in some situations, who hire equipment, machinery and vehicles from contractors and do not implement effective biosecurity, even where tissue-cultured planting material is used. Such companies may hire land only for 2-3 years, and then move on, leaving TR4 infestations in their wake, with no incentive to implement appropriate and expensive biosecurity. Clearly a policy and legal issue.

It was also noted that the Colombians are applying lessons learned from existing contingency plans, including the one developed by OIRSA and Australia to contain TR4. The discussion highlighted the involvement of expert agencies in monitoring the disease, where new tools can discriminate between living and dead cells (see next section). Diagnostic tools could confirm *Foc* TR4 absence from areas where disease has been reportedly eradicated and assess the viability of re-establishing bananas in such *Foc*-TR4-free areas. This may only happen once the tools and sampling protocols have been validated.

2.2 Diagnostics and molecular work

Presentations included *Foc* survival / treatment in water, *Foc* detection, comparative *Foc* genome sequence analyses & pathogenicity studies; and studies on genetic markers linked to TR4 biological control.

Water acts as an effective *Foc* inoculum dissemination channel. Mostert *et al*. explored factors influencing *Foc* survival and treatment in water. Their study revealed that *Foc* chlamydospores can survive for longer than 6 months, but viability is reduced in stagnant water. *Foc* spores survive for longer in water with soil than without, especially in running water with a higher oxygen content. Chlorine, ozone, UV and peracetic acid treatment efficacies were reduced in the presence of soil, with peracetic acid treatment being the most practical treatment.

Matthews *et al.* assessed the potential of propidium monoazide (PMA) combined with quantitative PCR to detect viable *Foc* inoculum from environmental samples. PMA-qPCR can distinguish *Foc* TR4 from other *Foc* isolates and could be used to compare the efficacy of management strategies and investigate TR4 epidemiology in different matrices.

Using bulk segregant analysis, Ssali *et al*. have identified DArT markers suitable for the TWB-resistance gene, pd1, a tool that can be used to help develop *Foc*-resistant banana cultivars.

Thangavelu *et al*. reported on comparative whole genome sequence analyses of VCGs from the three *Foc* strains infecting Cavendish in India, emphasising their pathogenicity mechanisms. Analyses revealed a variation in genome assembly organisation and virulence-associated genes, when compared to the reference genome. The findings inform our understanding and provides new designs for effective *Foc* management practices for different pathogen races.

Shu Li *et al*. reported on a real-time fluorescent reverse transcription quantitative PCR (RT-qPCR) assay for rapid detection of the expression of genetic markers associated with TR4 biological control activities in *Bacillus velezensis* and *Bacillus amyloliquefaciens.* Five effectively biocontrolling *B. velezensis* and *B. amyloliquefaciens* strains were found to have different TR4 biocontrol mechanisms

Ping He *et al*. reported on their successful construction and validation of a fluorescence transformation system based on *B. velezensis* and *B. amyloliquefaciens* for monitoring TR4 biological control. Monitoring its interaction with TR4 and its biocontrol mechanism is under further study.

2.3 Pathogen and disease control

Presentation on this theme included work on antagonists to FWB and biological control, e.g. *Trichoderma virens;* the effect of herbicides application on *Foc* inoculum; biological control, including use of suppressive mushroom compost and ground-cover root flavonoids & phenolic acids; the influence of nematodes and weevils, and soil disinfectants.

Complementing the Chinese work reported at the end of section II, Huacai Fan et al. identified two *Bacillus* strains. By morphological and molecular identification, the isolate YN0904 was identified as *B. amyloliquefaciens*. Another isolate YN1910 was identified as *B. velezensis*. These strains were effectively antagonistic to FWB and their biological control effects were elucidated.

East *et al*. provided an update on the well-known *Trichoderma virens* as an effective FWB inoculum management biocontrol agent. *T. virens* inoculations resulted in a 60% reduction in rhizome necrosis, with a corresponding reduction in *Foc* R1 detected using the qPCR assay. Chlamydospore production was reduced by two-thirds. Treated pseudostems exhibited rapid decomposition, which potentially limit *Foc* proliferation and chlamydospore development within decaying banana pseudostems. The ability of *T. virens* to reside in the banana rhizome and pseudostem indicates it acts as an endophyte, exhibiting niche competition with *Foc* R1. Anderson and Aitken presented research on the effects of treating Cavendish banana with herbicides and fungicides, on Foc subTR4 colonisation and sporulation during infected stem/ plant removal. Herbicide treatments hastened colonisation of banana tissues and the production of micro- and macroconidia. The use of a fungicide did not prevent sporulation.

Ocimati *et al*. examined the potential of edible mushroom (*Pleurotus ostreatus [Po]*) spent substrate for managing FWB. Their findings suggest that *Po* could be used to effectively manage Foc race 1. Studies to understand suppression of FWB by Po in natural soils and mechanisms of Po suppression of Foc are recommended.

Were *et al*. examined the suppressive effect of legume root-exuded phenolics on proliferation and biosynthesis of virulence factors in *Foc TR4.* The team used leguminous cover-crop species, *Desmodium uncinatum* and *Mucuna pruriens* in which three common phenolic acids were identified, low concentrations of which inhibited chlamydospore germination, production of macro- and micro-conidia, and synthesis of fusaric acid. Their results highlight a mechanism that may underlie direct suppression of the earliest stages of pathogen development in intercropping systems.

Alfaro *et al*. demonstrated how the burrowing nematode (*Radopholus similis [Rs]*)) acts as a predisposing factor for FWB. Heck *et al*. presented a research demonstrating the extent to which the banana *weevil borer (Cosmopolites sordidus)* affects the spatio-temporal dynamics of FWB. Interactions between *Rs,* weevils and *Foc* demonstrated with Race 1 must be considered for integrated management approaches of FWB, including TR4.

Biosecurity measures aiming at *Foc* containment strongly rely on disinfectant efficacy. Soto-Suárez *et al*. evaluated the biocide efficacy on both reproductive structures (micro- and macroconidia) and survival spores (chlamydospores) of *Foc* TR4 of ten commercial products available in Colombia. Disinfectants showed differential biocidal efficacy in the absence/presence of soil in reducing microconidia, macroconidia and chlamydospores. Comparative results of each product were presented and discussed.

Discussion on epidemiology and disease management

Participants discussed Foc spore survival in water and soil. Before translating the reported findings into practical management approaches (e.g. for irrigation dams/ basins), field water and soil sampling protocols need to be refined and validated.

There was some discussion about the apparent polymorphism displayed by TR4 isolates from India. The results of their whole genome-sequencing are available and can be further anlayzed. There was also talk of differential pathogenicity of the same strains in and beyond India, as well as a discussion on the possibility of mutations of some strains belonging to VGCs other than 01213 (TR4). The Indian team was unsure if the VCGs affected all Cavendish group cultivars, only dwarf cavendish or only those in India, so more research is needed to characterise their range of pathogenicity. A concern was also expressed regarding whether non-pathogenic VCGs to a given cultivar could become pathogenic, especially to Cavendish.

Use of disinfectants as part of a control strategy needs to be considered in the light of cost, efficacy, availability and the trade-offs between disease control and adverse effects on soil microbiomes. Also, a 98% efficacy level still leaves 2% risk of inoculum transfer, which may be too risky. Normal levels of TR4 soil inoculum are around 10^3, whereas the trials were implemented with a much higher inoculum pressure of 10^6. So, it is possible that trialling the products for longer and at lower inoculum levels may result in 100% disinfection. Researchers need to work with national plant protection organizations (NPPOs), as contexts vary across countries and regions.

There was interest regarding using ozone for TR4 management and mention of ozone being assessed for direct use on soils in Ecuador for Black Sigatoka control, and for stagnant water disinfection. However, efficacy, cost-benefits, persistence and application protocols are yet to be evaluated.

Although *in vitro* studies indicate effective activity against TR4 of some fungicides (e.g. Prochloraz) they have not proved effective *in vivo* (stem injections, soil drenching) and are therefore not considered as a viable option for TR4 control

In considering TR4 cover-crop suppressive root exudates, a next step will be to explore through metabolomic studies the potential of using identified molecular markers to select new TR4 suppressive crop species (e.g. different legumes) or even crop cultivars for use in crop rotations. When studying mechanisms in the rhizosphere, the nature of the mechanism depends on genotypes, so researchers must first elucidate mechanisms, then examine how to effectively deploy suppressive species and cultivars in field. Research also needs to understand temporal aspects of species and rotations. More work is needed on soil type and efficacy of suppression, especially organic matter and carbon levels. Consideration should be given to how or whether the range of biocontrol agents discussed may be scaled and integrated into a broader control strategy that harnesses synergistic combinations.

Pathologists and epidemiologists also need to examine more closely disease complex interactions with other pests and biocontrol agents, as exemplified by the work on interactions between *R. similis, weevils* and *Foc*.

3 Abstracts

3.1 Geographical spread of Fusarium in the Lao People's Democratic Republic.

Khonesavane Chittarhat[1], Pinkham Vongpachane[1], Souliya Sayapattha[1], Zheng Sijun[2,3], Miguel Dita, [4] Altus Viljoen[5], Diane Mostert[5], George Mahuku[6], Walter Ocimati[7], and Guy Blomme [8]

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Fusarium wilt of banana (FWB), caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*), cause a high negative impact on banana yields. Present in most banana-producing countries, including in North and Central Laos, FWB threatens local varieties. Cavendish (Hom) cultivars are resistant to the *Foc* Race 1 populations present in Laos, but very susceptible to the devastating *Foc* Tropical Race 4 (TR4), which has been found in Laos recently. Accurate data on the spatial distribution of TR4 as well of the impact on local cultivars in Laos is needed to better implement containment strategies. In this work we present accurate data on TR4 surveillance conducted in 11 Laotian provinces: Attapue, Borlikhamxai, Champasak, Laungphabang, Salavanh, Savannaket, Sekong, Udomxay, Vientiane, Vientiane Capital, and Xiengkaoung. A surveillance system based on specific survey for FWB collected samples from suspicious plants with photos, date of collection, the variety of the host plant, location and GPS coordinates. Samples were analysed for molecular identification to confirm TR4 presence and epidemiological analysis to verify the spatial distribution of TR4 and associated banana production system. The survey has identified an alarming spread of FWB in Pisang Awak (ABB) and Cavendish (AAA) plantations in the Northern, Central and Southern Laos. It has also confirmed TR4 infections in Cavendish plantations in Borkeo, Laung Nam Tha, LaungPhaBang, Udomxay, Vientiane Province, Vientiane Capital and Xaiyabouly. There are significant risks of TR4 spreading to Cavendish and local cultivars in Southern Laos exacerbated by farmers 'lack of knowledge on FWB epidemiology and management.

Keywords: Banana cultivar, *Fusarium oxysporum,* soil-borne

3.2 Current knowledge on Fusarium spread in Vietnam

Chung Huy Nguyen[1], Hung Tien Nguyen[1], Tho Thi Nguyen[1], Trang Thi Le et al. [1], Zheng Sijun[2,3], Miguel Dita[4], Altus Viljoen[5], Diane Mostert[5], George Mahuku[6] , Walter Ocimati[7] and Guy Blomme [8]

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Banana (*Musa spp*.) is a major crop in Vietnam, planted throughout the country. Fusarium wilt of banana (FWB), caused by the fungus *Fusarium oxysporum* f. sp *cubense (Foc),* is amongst the most serious banana diseases in Vietnam. Pisang Awak (ABB), a local banana cultivar widely distributed and used in Vietnam, is seriously affected by *Foc* race 1 (R1). Cavendish (AAA) banana cultivars, resistant to *Foc* R1, have been used in Vietnam in R1-affected areas. However, a the tropical race 4 (TR4) of *Foc*, which causes severe damages to Cavendish and other banana cultivars, was already identified in Vietnam and poses a serious threat to the Vietnamese banana industry. Surveys conducted in 2018 detected TR4 in six Vietnamese provinces: Lao Cai, Lai Chau, Ha Noi, Hung Yen, Long An, and Tay Ninh. However, systematic data on TR4 spread is needed to better perform containment and TR4 management strategies. Aiming to understand the current extent of TR4 spread in Vietnam, a survey conducted in the frame of CGIAR research Programme, Roots Tubers and Bananas was carried out. Following a standard survey protocol for FWB a total of 121 samples were collected from tree varieties (Pisang Awak, Pisan Mas and Cavendish) in 11 provinces in northern, central and southern Vietnam. Samples are under analysis for molecular identification to confirm TR4 presence and epidemiological analysis to verify the spatial distribution of TR4 and associated banana production system. However, assuming that samples collected on Cavendish correspond to TR4, we can anticipate that TR4 has spread to Hai Phong, Phu Tho, and Vinh Phuc in the North and Dong Thap in the South of Vietnam. The impact of the TR4 spreading in Vietnam and management strategies in place are further discussed.

Keywords: Banana, Fusarium Wilt, *Fusarium oxysporum*, TR4,

3.3 Improvement of Silk group of bananas for Fusarium wilt resistance (Foc race 1) through mutation breeding

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Building banana disease resistance is needed to combat emerging diseases such as Fusarium wilt (FW), which seriously threaten global banana production. Race 1 of Fusarium wilt poses a significant threat to Rasthali (AAB, Silk) - a popular cultivar traditionally grown in southern India. Resistant gene introgression into such susceptible cultivars through conventional breeding has several limitations especially male and female sterility and the long generation time. Under these circumstances, induced mutagenesis combined with toxin-based *in-vitro* screening offers a more attractive alternative for the development of FW-resistant Rasthali. Different explant sources, including shoot tips, proliferating buds and embryogenic cell suspension (ECS) were used to generate explants for mutagenesis using physical or chemical mutagens or both. After mutagenesis, the plants were screened *in vitro* for FW resistance using new generation toxins like fusaric acid, culture filtrate and beauvericin. This was followed by pot screening through challenge inoculation with *Foc* race 1 which has led to the identification of 35 resistant lines, all of which have been derived from EMS treated shoot tips, proliferating buds and ECS. Results suggest that induced mutagenesis could be used as a viable tool for the development of fusarium wilt resistance in banana.

Among the 35 resistant mutants, one mutant line NRCBRM15 with good growth parameters (like pseudostem girth, number of leaves and root biomass) was selected for global proteomic analysis of its responses to *Foc* race 1 infection, along with its wild type. Out of 37 proteins obtained, 20 showed differential expression. Of these, 19 proteins were significantly abundant, inclusive of four unique proteins in NRCBRM15, whereas one protein was significantly abundant in wild Rasthali. A total of nine genes were further investigated using quantitative real time polymerase chain reaction (qRT-PCR) to determine whether the observed proteome changes are associated with changes in the mRNA levels or due to post transcriptional regulation. The annotation results revealed that they these regulatory genes are involved in diverse functions such as carbohydrate metabolism, energy production, electron carrier, response to wounding, binding proteins, cytoskeleton organization, extracellular region, structural molecule and defence.

* Authors contributed equally.

Keywords: Banana, ECS, EMS, Fusarium wilt, gene expression, screening

3.4 Distribution of Fusarium oxysporum f. sp. cubense in Mozambique and management potential of select fungicides, biocontrol agents and phenolic compounds in vitro

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Fusarium oxysporum f. sp. *cubense* (*Foc*), the pathogenic fungus responsible for Panama disease in banana is among the most devastating plant pathogens in the world, that are difficult to eradicate once introduced into an area. The objective of this study was to establish the occurrence and distribution of *Foc* in Mozambique and to evaluate the effect of phenolic compounds, biological control agents, and fungicides on mycelial growth of identified *Foc* isolates from Mozambique *in vitro*. To determine occurrence and distribution of Panama disease in Mozambique, samples from symptomatic banana plants were collected from three-major banana producing provinces of Manica, Maputo and Nampula. Tropical race 4 (TR4) was found in the northern province of Nampula and race 1 in both Nampula and Maputo. Panama disease was not found in the leading banana producing province of Manica. The identified *Foc* races were found to fall within vegetative compatibility groups (*VCGs*) 01213/16, 01213, 01216, 0125, 01220 and 0124. In the *in-vitro* evaluation for antagonism against *Foc* isolates from Mozambique, Trans-ferulic acid at 2.5 mM concentration was the most suppressive phenolic compound (51.2%). Sinapic acid promoted growth of *Foc* isolates at all concentrations (0.5 mM, 1.5 mM and 2.5 mM). Prochloraz was the best fungicide that completely inhibited *Foc* mycelia growth on both the inoculation plug and amended PDA at concentration 1 ppm. Propiconazole at 100 ppm was also completely suppressive to *Foc* isolates on amended PDA. Sporekill, a disinfectant, was effective against the *Foc* isolates. *Bacillus subtilis* was the most effective biological control product that formed a clear inhibitory zone against the *Foc* isolates. This study formed a platform for management of Panama disease in Mozambique and further field studies on how to integrate effective *Foc*-suppressive phenolic compounds, fungicides and biocontrol agents into sustainable Panama disease management strategies.

Keywords: *Fusarium oxysporum* f. sp. *Cubense, in-vitro,* Mozambique, vegetative compatibility groups

3.5 Surveillance of banana Fusarium wilt caused by Fusarium oxysporum f.sp. cubense in Tanzania and Mozambique.

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Fusarium wilt, caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*), is a major threat to banana production globally. In 2013, a new race of *Foc* called tropical race 4 (TR4) was detected on a Cavendish banana plantation in Nampula province, northern Mozambique. The fungus was then detected within a second commercial plantation 200 km from the first farm in 2014, and on a smallholding in 2015. To understand the status of *Foc* TR4 spread in smallholder banana farming systems, surveys were conducted in northern Mozambique (2017) and southern Tanzania (considered at high risk from TR4 incursion) in 2019. In Mozambique, smallholder banana farms situated up- and down-stream from the two commercial Cavendish plantations with *Foc* TR4 were surveyed. Samples (150) from Fusarium wilt symptomatic plants were collected mainly from two banana cultivars, Bluggoe (98%) and Cavendish (2%). Molecular analysis with species-specific primers identified 26 samples to be potentially *Foc* TR4. Of these, 24 were confirmed as *Foc* Lineage 6, while two were *Foc* TR4. The results reveal the presence of *Foc* TR4 in smallholder farmers' fields and in another banana variety, Bluggoe. In Tanzania, surveys were conducted in three districts (Ruvuma, Mbeya and Mtwara) sharing the border with Mozambique. None of the 182 samples collected tested positive for *Foc* TR4 with specific PCR markers. All pathogenic Fusarium samples belonged to *Foc* Lineage 6. *Foc* TR4 has recently been detected in Mayotte, an island in the Indian Ocean, East of Mozambique. To establish the curent status of the disease, a more extensive survey was conducted in 2020, covering southern Tanzania and Zanzibar. Two hundred samples were collected from banana plants with fusarium wilt symptoms. The samples are currently being analyzed and will inform on mitigation strategies to adopt for protecting smallholder banana production systems against *Foc* TR4 incursion in Africa.

Keywords: *Fusarium oxysporum* f. sp. *Cubense,* Banana, Foc surveys

3.6 The epidemic of Fusarium wilt, Tropical race 4 in Colombia

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Banana and Plantains (*Musa spp.*) unite Latin American and the Caribbean (LAC) countries from Mexico to Argentina. Synonymous with family farming, food security and income generation, these crops currently face various challenges and threats. Officially first reported in Colombia in August 2019, Fusarium wilt of banana (FWB), tropical race 4 (TR4) now poses the major threat. However, suspected infested farms had already been under quarantine since June 2019. More than one year later TR4 is still contained in the province of La Guajira, although the number of affected farms has increased from two to ten. In this paper the current state of TR4 in Colombia, country responses and lessons learnt are presented and discussed in the context of spreading risks for other banana producing countries in LAC. There is a need for proactive and concerted efforts at the regional level, for creating legal frameworks and for developing capacities to prevent the further dispersion of TR4 in Colombia and to other LAC countries. Surveillance efforts may include the rapid detection and containment of eventual outbreaks. In parallel, research capacities must be developed or strengthened to generate solutions in the short, medium and long term. The creation of a regional multi-stakeholder innovation platform bringing together public and private sectors is seen as a fundamental step in catalysing these processes and guaranteeing the sustainability and competitiveness of banana agri-food systems in Latin America and the Caribbean.

Keywords: Banana, *Foc* TR4, legal frameworks, plantain.

3.7 The survival and treatment of Fusarium oxysporum f. sp. cubense in water

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Fusarium oxysporum f. sp. *cubense* (*Foc*) is one of the most devastating constraints to banana production worldwide. Due to the persistence of the pathogen in the soil, and the perennial production system of the host, the disease is difficult to manage. *Foc* can spread from infested to non-infested fields in water and soil attached to shoes, field equipment and vehicles. Prevention of spread is a major challenge, particularly in an environmentally safe and cost-effective way. The objectives of this study were to investigate the survival of *Foc* in water, and to test the effectiveness of four different irrigation water treatments. The survival of *Foc* was determined in water with and without soil, which was agitated and not agitated, over a period of 6 months. Chlamydospores were first produced in liquid culture and added to the water in 20-L buckets to obtain a final concentration of 10^2 spores/ml. To determine how long the chlamydospores survive, five 1-ml samples were collected from the top, middle and bottom of water in the buckets, and plated onto potato dextrose agar. Chlamydospores were able to survive for longer than 6 months. Their viability was reduced in stagnant water, probably due to anaerobic conditions when spores sinks to the bottom of the buckets. *Foc* spores survived longer in water with soil than in water without soil. *Foc*-infected water with and without soil was then treated with chlorine, ozone, UV and peracetic acid. The efficacy all treatments were reduced in the presence of soil, indicating that soil would need to be removed before treatment. The peracetic acid treatment would be recommended to treat *Foc*-contaminated water, as it does not require any installation costs and is safe for use. The efficacy of these products should however be further tested for treatment potential of irrigation and flood waters on-farm, and the practicality and affordability determined.

Keywords: chlorine, Fusarium wilt, ozone, peracetic acid, survival in water, UV

3.8 The potential of propidium monoazide combined with quantitative PCR to detect viable Fusarium oxysporum f. sp. cubense inoculum from environmental samples

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Fusarium oxysporum f. sp. *cubense* (*Foc*) causes Fusarium wilt (FW) of banana, is considered to be one of the most devastating constraints to banana production. *Foc* produces highly resistant chlamydospores that spread with infected plant, water, soil and field equipment. The detection of *Foc* spores in infected plants, water and soil, therefore, is an important activity in managing banana FW. Quantitative PCR (qPCR) is the preferred method of pathogen detection in environmental samples. A major drawback of DNA-based diagnostic techniques, however, is the inability to distinguish between viable and non-viable cells. The dye propidium monoazide (PMA) coupled with qPCR provides a novel technique to estimate cell viability, as PMA-bound DNA cannot be amplified. In this study the PMA-qPCR methodology was optimized, validated and applied to quantify viable cells of *Foc* Tropical Race 4 (TR4) in artificially and naturally infected plant, water and soil samples. Additionally, PMA-qPCR and counting colony forming units (CFUs) were used to compare the efficiency of two different commercial sanitisers on *Foc* TR4 spore suspensions. The optimised PMA-qPCRs were able to distinguish viable, mixed and non-viable at 10^6 spores/mL in all matrices, but sensitivity was reduced at lower spore concentrations (10^5 and 10^4 spores/mL). Significant differences were observed between *Foc* TR4 inoculum estimated with qPCR and PMA-qPCR from naturally infected soil and plant samples. This indicated that an overestimation of viable inoculum does occur without PMA treatment prior to qPCR. *Foc* TR4 spores estimated with PMA-qPCR and CFUs after sanitiser treatments were well correlated and indicated that Sporekill was more effective than Farmcleanse and Chlorine. Compared to CFUs the advantage of PMA-qPCR is that it can distinguish *Foc* TR4 from other *F. oxysporum* isolates. The optimised PMA-qPCRs could be used to compare the efficacy of management strategies and investigate *Foc* TR4 epidemiology in different matrices.

Keywords: *Fusarium oxysporum* f. sp. *cubense* tropical race 4; Fusarium wilt of banana; Quantitative PCR; Propidium monoazide; PMA-qPCR

3.9 Identification of DArT markers for the Fusarium wilt resistance gene, pd1, in Musa by bulk segregant analysis

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Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*), is a serious constraint to the production of bananas globally. A major recessive gene, *pd1,* was identified as conferring resistance to *Foc* race 1 in a segregating F_2 population from a cross between a susceptible cultivar 'SukaliNdiizi' (AAB) and a resistant genotype 'TMB2X8075-7' (AA). In the current study, quantitative bulk segregant analysis (BSA) on the Diversity Arrays Technology (DArT) platform was used to identify markers associated with resistance in banana to *Foc* race 1. DNA of 40 resistant and 40 susceptible F_2 progenies were pooled for BSA-DArT analysis, along with DNA from the resistant and the susceptible parents. A total of 14,354 DArT markers were polymorphic between the parents and the resistant and susceptible bulks and were used to estimate the genetic similarity between the parents and bulks. The genetic similarity was highest between the resistant and susceptible bulks (0.61) and lowest between the *Foc* race 1-susceptible parent 'SukaliNdiizi' and the two phenotypic bulks (0.03). One hundred and one DArT markers were in qualitative linkage disequilibrium, with 13 markers linked to resistance and 88 markers to susceptibility. Putative functions have been assigned to the DArTs that were mapped to coding sequences of the banana reference genome through *in-silico* database analysis of DArT clone sequences. DArTs closely associated with resistance/susceptibility can be used to help develop banana cultivars with resistance to *Foc* race 1.

Keywords: banana breeding, linkage disequilibrium, Musa reference genome

*3.10 Comparative whole genome sequence analyses of Fusarium wilt pathogen (*Foc *R1, STR4 and TR4) infecting Cavendish (AAA) bananas in India- a special emphasis on pathogenicity mechanisms*

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Fusarium wilt is caused by the fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*), the most serious disease affecting banana (*Musa* spp.) classified into *Foc* race 1 (R1), *Foc* race 2, and *Foc* race 4 based on the host specificity. *Foc* tropical race 4 (TR4) and R1 in Cavendish banana has spread rapidly during recent years in India and other regions, threatening the global banana industry, attracting international attention regarding future food security in banana-producing countries. Although Fusarium wilt incidence caused by *Foc* TR4 in India initially ranged from 2 to 26.6%, a recent survey revealed that the incidence was more than 50% in both Bihar and Uttar Pradesh where Cavendish banana is being cultivated in more than one lakh (100,000) hectares. As the rate of spread and ranges of devastation of the *Foc* races is higher than the centre of banana's origin even in non-targeted cultivars, there could be a chance of variation in virulence-associated genes. Therefore, the present study explores the genome assembly of Indian *Foc* races belongs to vegetative compatibility group (VCG) 0120 (subtropical race 4), 0124 (race 1) and 01213/16 (tropical race 4) infecting Cavendish (AAA) group of banana in India. The study also examined virulence-associated genes, specifically effector genes, and explored insights regarding race-specific molecular mechanisms of infection based on the presence of unique genes besides the GO, SSRs, SNPs and InDels. The results of the analyses revealed that there is a variation in the organisation of genome assembly and virulence-associated genes, specifically secreted in xylem (SIX) genes when compared to the reference genome. The findings help to understand and will help to design effective *Foc* management practices for different *Foc* races in India and beyond.

Keywords: Banana, Fusarium wilt, pathogenicity genes, SIX genes, whole genome sequencing

3.11 Construction and verification of a fluorescence transformation system of Bacillus velezensis and B. amyloliquefaciens for monitoring TR4 biological control

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Bacillus velezensis and *B. amyloliquefaciens* are effective biocontrol agents for controlling *Fusarium wilt* (TR4). In order to explore the colonization of *B. velezensis* and *B. amyloliquefaciens* and the antagonistic mechanism of TR4 biocontrol in banana plants, we screened four strains of *B. velezensis* and two of *B. amyloliquefaciens* from Yunnan and Guangxi provinces that can significantly inhibit TR4 *in vitro*. We successfully constructed an optimized fluorescent electro-transformation system of TR4-inhibitory *Bacillus* sp. strains (OD_{600} = 0.7, plasmid concentration = 50ng/μL, volume = 2μL, voltage = 1.8kV, capacitance = 400Ω). The red fluorescent protein (RFP) labelled strains have high stability where the plasmid retained frequency is above 98%, and its growth rate and inhibition of TR4 are not affected by fluorescent plasmid insertion. *In vivo* colonizing observation by scanning electron microscopy (SEM) showed that *Bacillus spp*. can colonize the internal cells of banana plantlet roots. Fluorescent colonizing observation by laser scanning confocal microscopy (LSCM) showed these RFP-labelled bacteria are clearly gathered around the hyphae of the green fluorescent (GFP)-labelled TR4 pathogen in banana plants. We can conclude that *B. velezensis* and *B. amyloliquefaciens* can successfully colonize banana plants and interact with TR4. Monitoring their interactions with TR4 and biocontrol mechanism is under further study.

Keywords: *Bacillus velezensis*; *B. amyloliquefaciens;* biocontrol; electro-transformation; RFP-labelled *Bacillus;* TR4 interaction

3.12 A real-time fluorescent reverse transcription quantitative PCR (RT-qPCR) assay for rapid detection of the expression of genetic markers associated with TR4 biological control activities in Bacillus

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Banana (*Musa spp*.) is a globally important tropical and subtropical crop. Fusarium wilt of banana caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*) Tropical Race 4 (TR4) seriously threatens the global banana industry worldwide. To date, there are no single effective measures that prevent or control this disease. Using biocontrol agents to control TR4 shows great promise. Certain Bacillus strains secrete a range of antibiotics considered as an important group of biocontrol agents against plant disease. The research team found that five laboratory-stored *Bacillus* strains.: WBN-06, HN-4, YN1282-2, N67, G9R-3 from Yunnan and Guangxi displayed strong antibiotic activity against TR4 both *in vitro* and *vivo*. In order to elucidate the biocontrol mechanisms of these five strains, conventional PCR with 13 pairs of specific primers was used to detect genes related to their biocontrol activities and accurately identify their expression in each strain using quantitative real-time PCR (qRT-PCR). Additionally, in order to get high quality RNA templates for qRT-PCR detection, strain-culturing and its RNA isolation methods were optimized. Results showed that, all five strains are negative for *sboA* (involved in synthase of subtilisin), and that *srfAA*, *fend, ituC, yngG, yndJ* genes are present, which were responsible for syntheses of five non-ribosomal peptide synthetase (NRPS) metabolites. Three genes: *bae*, *dfn*, *bac* were found to be involved in the syntheses of three polyketide synthetase (PKS) metabolites in all five strains, but the macrolactin synthase gene *mln* was only detected in WBN-06 and YN1282-2. All five Bacillus strains have the genes *dhbA*, and *bioA*, essential for synthesis of bacillibactin and biotin. The expression of those genes showed that different biocontrol *Bacillus* have different mechanisms for biocontrol of TR4. The outcome of our study offers a foundation for revealing the biocontrol mechanisms of functional genes in above strains.

Keywords: *Bacillus velezensis*; *B. amyloliquefaciens*; biocontrol; gene expression; genetic markers associated with TR4 biological control; qRT-PCR detection

3.13 Identifying strains of Bacillus antagonistic to Fusarium wilt of banana and elucidating their biological control effects

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Two antagonistic isolates-YN0904 and YN1910, were screened for *Fusarium oxysporum* f. sp. *cubense* (*Foc*) Tropical Race 4 (TR4) inhibition by primary and secondary selections in our laboratory. The inhibition rate to *Foc* TR4 of YN0904 and YN1910 was 79.56% and 81.37%, respectively. By molecular identification, the isolate YN0904 was related to *Bacillus amyloliquefaciens*. Another isolate YN1910 was related to *Bacillus velezensis*. The control effects of YN0904 and YN1910 to TR4 was 82.62% and 91.30% in greenhouse tests, respectively. The two antagonistic isolates significantly promoted plant growth (banana plant height). We conclude that these two antagonistic strains could be used as biocontrol agents for management of Fusarium wilt of banana in field in our next step research.

Keywords: antagonistic isolates; *Bacillus velezensis*; *B. amyloliquefaciens;* biocontrol; colonization; TR4-inhibitory

3.14 Trichoderma virens: a potential biocontrol agent for Fusarium wilt in banana

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Banana Fusarium wilt (FW) is a worldwide economically important disease, caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*). Inoculum management techniques to limit disease expression are vital to reduce production losses within *Foc*-infected banana plantations. Organisms that are antagonistic to FW can reduce crop losses as part of an integrated disease management system. Twenty *Trichoderma* species isolates, recovered from banana farm soils, were screened for antagonistic activity against *Foc* Race1 (*Foc* R1). A strain of *Trichoderma virens* native to Far North Queensland was identified as having the greatest potential as a biocontrol agent, using a dual plate culture assay. To validate the efficacy of *T. virens* observed in the laboratory against Banana FW, an *in vivo* bioassay using Ducasse (*Musa* ABB synonym Pisang Awak) banana plants was undertaken. The glasshouse experiment was conducted in a completely randomized design with six replicates. Three months after inoculation with *Foc* R1, the plants were scored for external and internal disease symptoms. Quantification of *T. virens* and *Foc* R1 was conducted for different banana compartments using a qPCR assay. The application of *T. virens* resulted in a 60% reduction in rhizome necrosis, which corresponded with a reduction in *Foc* R1 detected using the qPCR assay. Furthermore, there was a significant ($p<0.05$) reduction in *Foc* R1, specifically in the rhizome and pseudostem of bananas when *T. virens* was present. Moreover, when the *T. virens* isolate was added to banana pseudostem tissue infected with *Foc* R1, chlamydospore production was reduced by two-thirds. Further studies investigated the efficacy of *T. virens* suppression in the field, by injecting *T. virens* isolates directly into banana pseudostem of recently harvested banana plants. The initial results indicated that treated pseudostems exhibited rapid decomposition, which potentially limit *Foc* proliferation and chlamydospore development within decaying banana pseudostems. The ability of *T. virens* to reside in the banana rhizome and pseudostem indicates it acts as an endophyte, exhibiting niche competition with *Foc* R1.

Keywords: banana, biocontrol, Fusarium wilt, *Trichoderma*.

3.15 Effect of in planta treatment of 'Cavendish' banana with herbicides and fungicides on the colonisation and sporulation by Fusarium oxysporum f.sp. cubense Subtropical Race 4

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Fusarium wilt caused by the soil-borne fungus *Fusarium oxysporum* f.sp. *cubense* (*Foc*) is a significant constraint to banana production world-wide, with recent expansion of banana growing regions impacted by Fusarium wilt caused by *Foc* Tropical Race 4 (TR4). The lack of commercially acceptable cultivars with *Foc* resistance means the only current means of effective disease control is through strict quarantine and inoculum management. One method of control that is currently advocated includes the removal of infected plants which have been killed using herbicide injections. The aim of this work was to examine the effect of herbicide and fungicide treatments on sporulation of the fungus. In glasshouse studies using a green fluorescent transformed *Foc* Subtropical Race 4 isolate, we found treatments with herbicide hastened colonisation of the banana tissue and the production of micro- and macroconidia. The use of a fungicide did not prevent sporulation of the fungus in such tissue. This study demonstrates that herbicide treated plants are a source of potential inoculum for infection of nearby plants.

Keywords: biosecurity, *Fusarium oxysporum*, herbicide, Tropical Race 4 (TR4),

3.16 Substrate of the edible mushroom Pleurotus ostreatus has potential for managing Fusarium wilt of banana

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Fusarium wilt of banana (FWB), caused by *Fusarium oxysporum f. sp. cubense (Foc)* race 1, has severely hampered dessert banana production in Uganda and globally. Effective disease control has been elusive due to the pathogen's diverse modes of spread, and survival capacity, as well as a lack of commercial resistant varieties. A range of basidiomycetes including edible *mushrooms* have been reported to suppress phytopathogens including *Fusarium* spp. in different crops, though not investigated for banana. With the current increase in the production and consumption of mushrooms in Ugandan banana-based systems, through regenerative farming, the spent mushroom substrates (SMS) could potentially be used to manage FWB. This study determined the potential of *spent substrate of Pleurotus ostreatus* (*Po*), the most widely cultivated mushroom in Uganda, to inhibit *Foc in vitro* and in potted plants. *In-vitro* studies of the effect of *Po* on *Foc* were conducted through a co-culture assay and culturing *Foc* on media amended with different concentrations (0% *w/v* to 50% *w/v* of sterile water) of either sterilized or unsterilized SMS filtrates. For the pot experiment, a *Foc* Race 1-resistant East African Highland banana cultivar 'Mbwazirume' (*Musa* AAA) and a susceptible dessert cultivar 'Ndizi' (*Musa* AAB) were planted in soils inoculated with i) 50 g of *Foc*-colonized millet grains (~7.6 million *Foc* colony-forming units), ii) 50 g *Foc* and 80 g of *Po* substrate (applied two weeks later), iii) 80 g of *Po* substrate, and iv) a *Foc/Po*-free control. *Po* mycelia thickened at point of contact with *Foc* and significantly suppressed the growth of *Foc* in the co-cultures. All concentrations of the un-sterilized SMS filtrate significantly suppressed *Foc* growth whereas *Foc* growth in the sterile SMS filtrate was comparable to the control with 0% SMS. Corm damage scores due to FWB (on a scale of 0 to 5) were low (0.25) for 'Mbwazirume' compared to 3.75 for 'Ndizi' in the *Foc*- inoculated soils. No corm damage occurred in the *Foc* + *Po* treated soils in the resistant cultivar. Plants of 'Ndizi', treated with *Po* showed significantly lower corm damage values (1.25) compared to 3.75 in the *Foc* only treatment. No corm damage occurred in the *Po* only and the *Foc*-free controls. These findings suggest that *Po* could be used to effectively manage *Foc* race 1. Studies to understand suppression of FWB by *Po* in natural soils and mechanisms of *Po* suppression of *Foc* are recommended.

Keywords: *basidiomycetes,* biocontrol, corm damage, *Fusarium oxysporum f. sp. cubense,* spent mushroom substrates, susceptible.

3.17 Effect of legume root-exuded phenolics on proliferation and biosynthesis of virulence factors in Fusarium oxysporum f. sp. cubense TR4

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Banana *Fusarium wilt (FW) is a devastating disease caused by the root-infecting fungus, Fusarium oxysporum f. sp. cubense (Foc). FW suppression in banana intercropped systems has been linked to* root-exuded metabolites that influence host-pathogen and root-soil-microbiome interactions. Yet, less is known about the specific metabolites and the underlying mechanisms. *This study, through hydroponic culture and metabolite profiling investigated the effects of root-exuded* phenolic acids and flavonoids *of Desmodium uncinatum* and *Mucuna pruriens* on *Foc* tropical race 4 (*Foc* TR4). Out of 12 metabolites, three phenolic acids (benzoic, *t*-cinnamic, *p*-hydroxybenzoic) were common in root exudates of *D. uncinatum* and *M. pruriens* while *p*-coumaric and vanillin were only detected in *M. pruriens*. Only the flavonoid quercetin, was detected in *M. pruriens*. Bioassays with benzoic-, *t*-cinnamic-, or *p*-hydroxybenzoic acid, or a combination thereof showed a concentration-dependent suppressive effect on *Foc* TR4. Low concentrations (0.01 and 0.1 mM) of phenolic acids inhibited chlamydospore germination, production of macro- and micro-conidia, and synthesis of fusaric acid, whereas radial mycelial growth and synthesis of beauvaricin was promoted in *Foc* TR4. Mycelial growth of *Foc* TR4 was only inhibited at high concentrations (1 mM) of benzoic acid, *t*-cinnamic acid, and the combination. Our results highlight a mechanism that may underlie direct suppression of the earliest stages of pathogen development in intercropping systems.

Keywords: benzoic, *Desmodium uncinatum,* Foc TR4, *Mucuna pruriens*, , *p*-hydroxybenzoic, *t*-cinnamic

3.18 Radopholus similis as a predisposing factor for Fusarium wilt of bananas

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Fusarium oxysporum f. sp. *cubense* (*Foc*) and the burrowing nematode *Radopholus similis* (*Rs*) are aggressive soil-borne pathogens of banana. For adequate management of these pathogens, especially *Foc*, a more comprehensive understanding of their interactions is needed. In this work the interaction between both pathogens were evaluated under greenhouse conditions using Gros Michel (AAA) *in vitro* plantlets and two *Foc* race 1 (R1) strains. Treatments included plants inoculated only with *Foc*, co-inoculated with *Rs* (*Foc*+*Rs*) and without inoculation (Control). Six weeks after planting, 500 *Rs* females were inoculated on the base of plants and 4 days later 10 ml of a suspension of 1 x 10^5 conidia/ml of *Foc* R1 was inoculated by drench.

Incubation period, incidence, severity of external symptoms at different times during the experiment and severity of internal symptoms in the corm were evaluated. Independently of the *Foc* strain, plants inoculated only with *Foc* showed significantly longer incubation periods (62.5 days) than plants co-inoculated with *Rs* (38 days). In addition, plants co-inoculated (*Foc*+*Rs*) showed significantly higher values both of incidence of internal symptoms (100 %) and severity of external symptoms (77 %) of Fusarium wilt (FW) than those only inoculated with *Foc*, which showed 50% and 33.5%, respectively. Our results suggest that *Rs* predisposes banana plants to *Foc* infection and increases both FW symptoms expression and severity. The interaction between *Rs* and *Foc* must be considered for integrated management approaches of FW, including *Foc* tropical race 4.

Keywords: burrowing nematode, *Fusarium oxysporum* f. sp. c*ubense*, Fusarium wilt management.

3.19 Weevil borers affect the spatio-temporal dynamics of banana Fusarium wilt.

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Dispersal of pathogen propagules can affect the development of epidemics. Previous studies suggested that insect pests play a role in the development of Fusarium wilt (FW) epidemics in banana. We provide complementary evidence for the involvement of two insect pests of banana, weevil borer (WB; *Cosmopolites sordidus* L.) and false weevil borer (FWB; *Metamasius hemipterus* L.), in the dispersal of *Fusarium oxysporum* f. sp. *cubense* (*Foc*) using two approaches: a comparative epidemiology study under field conditions and association analysis between *Fusarium* spp. and the insects. Epidemics of FW were studied in two banana fields: one managed with *Beauveria bassiana* to reduce the WB population, and the other without *B. bassiana* applications (control). The number of WB and FWB and the incidence of FW were periodically assessed for two years. Both final FW incidence (6.7%) and disease progress rate (r = 0.002) were significantly lower in the managed field than in the control field (13.0%, r = 0.006). Aggregation of FW was significantly higher in the field with WB management. The association analysis revealed a significantly greater number (7,933) of colony-forming units (CFU) of *Fusarium* spp. associated with WB (n = 115 insects) compared to FWB (1,414 CFU; n = 102 insects). *Fusarium oxysporum* was found in WB and FWB's exoskeleton by morphological and molecular methods. WB affects the spatial and temporal dynamics of FW epidemics under field conditions, and *Fusarium* spp. was associated with both insect pests.

Keywords: *Cosmopolites sordidus*, epidemiology, *Metamasius hemipterus*.

3.20 Efficacy of disinfectants against Fusarium oxysporum f. sp. cubense, tropical race 4 in Colombia

Luisa F. Izquierdo-García [1], Sandra L. Carmona [1], A. Paola Zuluaga [1], Miguel Dita [2], Gustavo Rodríguez[1], Mónica Betancourt[1], **Mauricio Soto-Suárez**[1]*.
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Banana, the main export fruit from Colombia is threatened by *Fusarium oxysporum* f. sp *cubense*, tropical race 4 (*Foc* TR4). Biosecurity measures aiming at pathogen containment strongly rely on disinfectant efficacy. In this study the biocide efficacy of ten commercial products based on quaternary ammonium compounds (QACs) were evaluated on both reproductive structures (microconida and macroconidia) and survival spores (chlamydospores) of *Foc* TR4. Treatments included different exposure time-points to the disinfectants (15 seconds and 15 minutes), and with and without presence of soil. Disinfectants showed differential biocidal efficacy in the absence/presence of soil in reducing microconidia, macroconidia and chlamydospores. In the absence of soil, all the disinfectants evaluated showed a 100% biocidal effect on chlamydospores, micro and macroconidia at two evaluated time-points. However, in the presence of soil, the biocidal efficacy of 4/10 products was reduced in a range of 2 to 8%. An increase in the exposure time was necessary to attain a more homogeneous biocidal effect, 5/10 products showed biocidal efficacy higher than 98% at 15 sec. and 100% at 15 min. As a result, there was a product with 100% of biocidal efficacy at both 15 sec. and 15 min. Comparative results of each product is presented and discussed.

Keywords: banana, chlamydospores, disinfectants, Fusarium wilt, Quaternary ammonium

3.21 Strengthening Fusarium wilt resistance in banana species: A systematic review of methods and perspectives

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The production of bananas and plantains (*Musa* spp.), important commodities associated with food and nutrition security in many places worldwide, is currently under threat. The fungus *Fusarium oxysporum* f. sp. *cubense* (*Foc*), which causes Fusarium wilt, has threatened the production and export trade of this fruit. A specific cause of concern is a highly virulent strain, namely the tropical race 4 (*Foc* TR4). Currently, in infested fields, biological, chemical, or cultivation measures are not economically viable to effectively control the spread of the disease; hence, the use of resistant cultivars is the safest and most efficient strategy known for producers to challenge this disease. This statement is valid both for wilt epidemics caused by race 1 (*Foc* R1), endemic to some regions, or the *Foc* TR4, which causes pandemics. Therefore, great efforts have focused on strategies for achieving plants with resistance to the disease. This article presents the first systematic review of studies conducted in the last 10 years on the resistance of *Musa* spp. to Fusarium wilt, focusing on methods and tools. Remarkably, when analyzing the systematized bibliographic dataset, we observed that the information obtained from sequencing the *Musa* spp. genome certainly helps reveal sources of resistance, especially by the evaluation of banana transcriptome data after infection with *Foc*. Exploring the knowledge of resistance sources in *Musa* germplasm allows the development, acquisition and selection of resistant hybrids from crossbreeding, although published data are still scarce. In contrast, a transgenics approach has been frequently adopted. Moreover, the tools for symptomatological assessments in different environments, using scales, are still reliable methods for evaluating the evolution of this disease to resistance.

Keywords: Musa spp., Fusarium oxysporum f. sp. cubense; genetic improvement; resistance

4 Conclusions and Recommendations

Building crop resilience is at the heart of the RTB flagship project 3, and the research cluster BA3.3 specifically aims to combat banana wilt diseases. The work presented in this booklet constitutes cutting-edge research to address the challenges posed by Fusarium Wilt of Banana (FWB), especially that by *Foc* Tropical Race 4 (TR4), that threatens Cavendish amongst other key susceptible cultivars. The research reveals new insights into Fusarium TR4 spread in southern Africa, the Greater Mekong Delta, and Latin America; evaluating biocontrol agents; the survival of Fusarium spores in water, the effects of nematodes and weevils in pathogen spread and infection, and in breeding for resistance.

In efforts to protect worldwide banana productivity for at least an annual harvest of 60 million MT, and to boost associated livelihoods and food security, there is great potential for harnessing potential synergies from the range of technologies and complementarities revealed by this symposium. There is also a need to scale the research from *in vitro* to field studies and to implement scalable findings at farm and plantation level.

TR4 needs to be differentially managed according to each specific food-system context, and governments need to work closely with the private sector to regulate production practices, including the behaviour of private investment companies. Researchers need to work with national plant protection associations, as contexts vary across countries and regions. The symposium highlights some new diagnostic / molecular tools that, once validated will improve diagnoses. These tools can also help better understand the nature of pathogenicity and virulence, allowing more effective control measures to be developed and implemented.

The symposium research findings can support more effective FWB inoculum management (e.g. for irrigation dams/ basins), but water and soil sampling protocols first need refinement and validation. Fungicidal and disinfection cost-benefits, persistence, application protocols, and penetration rates must be comprehensively evaluated.

Finally, the symposium has offered new insights into TR4 suppression using a range of biocontrol agents including microbes, cover crops and soil amendments. Consideration should be given to how or whether the range of biocontrol agents discussed may be scaled and integrated into existing broader control strategies that harness synergistic combinations. Pathologists and epidemiologists also need to examine more closely disease complex interactions with other pests and biocontrol agents, as exemplified by the work on interactions between *R. similis* and *Foc*.

5 Annexes

Annex 1: Attendee list

	Last Name	First Name	Email Address	attended				registered				total attended	total registered but not attended
				1	2	3	4	1	2	3	4		
1	Acosta	Jorge Eliécer Vargas	jve0583@gmail.com				1					1	0
2	Aitken	Elizabeth	e.aitken@uq.edu.au				1					1	0
3	Alfaro Alvarado	Fabiola	falfaro@corbana.co.cr					1				0	1
4	Amisse	Jamisse	jamisse.amisse@gmail.com							1		0	1
5	Amorim	Édson	edson.amorim@embrapa.br				1					1	0
6	Amugoli	Otuba	mozes.otuba@gmail.com	1								1	0
7	Anderson	Jay	jayanderson@edu.au	1		1		1		1		2	2
8	Anthony	Tazuba	tazubatony@gmail.com				1		1			1	1
9	Aragón Caballero	Liliana	Lili@lamolina.edu.pe	1	1	1	1					4	0
10	Arinaitwe	Ivan Kabiita	ivankabiita@gmail.com	1								1	0
11	Bai	Ting-ting	baiting8797@126.com					1		1		0	2
12	Bedoya	Eliana Torres	et475@exeter.ac.uk									0	0
13	Blomme	Guy	G.Blomme@CGIAR.org	1	1	1	1					4	0
14	Carr	Claudiana	claudianacarr@gmail.com								1	0	1
15	Chittarath	Khonesavanh	chittarhat_2005@yahoo.com	1					1			1	1
16	Coyne	Danny	d.coyne@cgiar.org				1					1	0
17	David	Mónica	monicadavid053@gmail.com				1					1	0
18	Dinh	Hoan	xuanhoan2008@gmail.com	1	1	1						3	0
19	Dita	Miguel	m.dita@cgiar.org		1							1	0
20	Drenth	Andre	a.drenth@uq.edu.au	1								1	0
21	Du	Xia	duxiadaisy@163.com			1						1	0
22	Dusunceli	Fazil	Fazil.Dusunceli@fao.org		1			1				1	1
23	East	David	david.east@daf.qld.gov.au			1						1	0
24	Esack	Edwinraj	edwinrht@gmail.com			1					1	1	1
25	Fan	Huacai	hcfan325@126.com	1						1		1	1

	Last Name	First Name	Email Address	attended				registered				total attended	total registered but not attended
				1	2	3	4	1	2	3	4		
26	Godwin	Rosie	Rosie@abgc.org.au	1								1	0
27	GuangDong	Zhou	2084639117@qq.com	1								1	0
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29	He	Ping	heping_superv@163.com	1		1						2	0
30	Heck	Daniel	dwinterheck@gmail.com		1		1	1		1		2	2
31	Henry	Buregyeya	henrymanrb@gmail.com		1	1	1					3	0
32	Hung	Nguyen	hungnguyen1218@gmail.com		1		1					2	0
33	JOHNSON	VINCENT	v.johnson@cgiar.org	1	1	1	1					4	0
34	Karamura	Georgina	georginakaramura@gmail.com	1	1	1						3	0
35	Kaushal	Manoj	M.Kaushal@cgiar.org	1	1	1	1					4	0
36	Kimunye	Janet	j.kimunye@cgiar.org			1						1	0
37	Kolombia	Yao	Y.Kolombia@cgiar.org	1	1	1	1					4	0
38	Legg	James	j.legg@cgiar.org	1								1	0
39	Li	Xundong	xundonglee@sina.com	1								1	0
40	Lombard	Paul-Henri	phlombard@sun.ac.za		1							1	0
41	Magdama	Freddy	frearmag@espol.edu.ec		1		1	1				2	1
42	Mahuku	George	g.mahuku@cgiar.org	1	1	1	1					4	0
43	Martínez	Gustavo	martinezgve@yahoo.es		1	1	1					3	0
44	Matthews	Megan	megs.c.dt@gmail.com		1		1					2	0
45	mduma	hassan	h.mduma@cgiar.org	1	1	1						3	0
46	Milton	Ali	aliilton100@gmail.com	1					1			1	1
47	Mostert	Diane	diane@sun.ac.za	1	1	1	1					4	0
48	Nakato	Gloria Valentine	V.Nakato@cgiar.org	1	1	1	1					4	0
49	Nguyen	Chung	hchungvasi@yahoo.com	1	1	1	1					4	0
50	Nsubuga	Julius	joeljuliusnsubuga@yahoo.com					1	1			0	2
51	Ocimati	Walter	w.ocimati@cgiar.org	1	1	1	1					4	0
52	Omondi	Bonaventure	b.a.omondi@cgiar.org						1			0	1
53	Pallangyo	Beatrice	beatricepallangyo91@gmail.com								1	0	1

	Last Name	First Name	Email Address	attended				registered				total attended	total registered but not attended
				1	2	3	4	1	2	3	4		
54	Peralta	Esther L.	esther.peralta@fao.org		1			1				1	1
55	Pérez	Gloria	FITOSANIDAD@AUGURA.COM.CO				1					1	0
56	Raman	Thangavelu	rtbanana@gmail.com	1	1							2	0
57	Rasche	Frank	frank.rasche@uni-hohenheim.de	1	1		1					3	0
58	Roux	Nicolas	n.roux@cgiar.org	1	1	1	1					4	0
59	Sánchez Valverde	Marylin	msanchez@corbana.co.cr				1					1	0
60	Sandoval	Jorge	jsandoval@corbana.co.cr		1		1					2	0
61	Saraswathi	Marimuthu Somasundaram	saraswathimse@gmail.com	1		1			1		1	2	2
62	Soto-Suárez	Mauricio	msoto@agrosavia.co		1		1					2	0
63	SSALI	Reuben	rtendo@gmail.com	1	1							2	0
64	Ssekandi	Joseph	jose.ssekandi@gmail.com	1	1	1	1					4	0
65	Swennen	Rony	Rony.Swennen@kuleuven.be	1		1						2	0
66	Tazuba	Anthony	tazubatony@gmail.com	1			l		l			2	1
67	Tian	libo	1924498257@qq.com	1								1	0
68	Tindamanyire	Jimmy	tindajm@gmail.com	1		1	1		1			3	1
69	Tinzaara	William	w.tinzaara@cgiar.org	1								1	0
70	Torres Bedoya	Eliana	et475@exeter.ac.uk		1			1				1	1
71	Uwimana	Brigitte	B.Uwimana@cgiar.org	1	1		1					3	0
72	Vezina	Anne	a.vezina@cgiar.org	1	1	1	1					4	0
73	Viljoen	Altus	altus@sun.ac.za	1	1	1						3	0
74	Wang	Yongfen	158338801@qq.com	1					1	1		1	2
75	Were	Evans	e.were@uni-hohenheim.de	1	1	1	1					4	0
76	Zheng	Sijun	s.zheng@cgiar.org	1	1	1	1					4	0
				43	37	30	35	9	8	9	4	142	27

5.2 Annex 2: List of papers and presenters

TR4 geographical spread & aspects of tolerance/resistance

No.	Corresponding Author	Paper draft title	contact email
1	Khonesavanh Chittarath	Current knowledge on Banana Fusarium Wilt spread in Lao PDR.	chittarhat_2005@yahoo.com
2	Chung Huy	Current knowledge on Banana Fusarium Wilt spread in Vietnam.	hchungvasi@yahoo.com
3	Marimuthu Somasundaram Saraswathi	Improvement of Silk type bananas for Banana Fusarium Wilt resistance (Foc race 1) through mutation breeding.	saraswathimse@gmail.com
4	Moses Amugoli Otuba	Distribution of *Fusarium oxysporum f. sp. cubense* in Mozambique and management potential of select fungicides, biocontrol agents and phenolic compounds in vitro.	mozes.otuba@gmail.com
5	George Mahuku	Surveillance of *Fusarium oxysporum f. sp. cubense* in Tanzania and Mozambique.	G.Mahuku@cgiar.org
6	Betancourt Vasquez	The epidemic of Fusarium oxysporum f. sp. cubense TR4 in Colombia.	mbetancourtv@agrosavia.co

TR4 diagnostics & molecular work

No.	Corresponding Author	Paper draft title	contact email
7	Edson Amorim	Improvements in the resistance of the banana species to Banana Fusarium Wilt: A systematic review of methods and perspectives.	edson.amorim@embrapa.br
8	Diane Mostert	The survival and treatment of *Fusarium oxysporum f. sp. cubense* in water.	diane@sun.ac.za
9	Megan Matthews	The potential of propidium monoazide combined with quantitative polymerase chain reactions to detect viable *Fusarium oxysporum f. sp. cubense* inoculum from environmental samples	megandutoit@sun.ac.za
10	Reuben Tendo Ssali	Identification of DArT markers for the Banana Fusarium Wilt resistance gene, pd1, in Musa by bulk segregant analysis.	R.Ssali@cgiar.org
11	Raman Thangavelu	Comparative whole genome sequence analyses of Banana Fusarium Wilt pathogens (Foc R1, STR4 and TR4) infecting cavendish (AAA) bananas in India a special emphasis on pathogenicity mechanisms.	rtbanana@gmail.com
12	Shu LI	A real-time fluorescent quantitative PCR assay or rapid detection of genetic markers associated with TR4 biological control activities in *Bacillus velezensis*.	s.zheng@cgiar.org

No.	Corresponding Author	Paper draft title	contact email
13	Ping He	Construction and verification of fluorescence transformation system of *Bacillus velezensis* for monitoring TR4 biological control.	s.zheng@cgiar.org

TR4 disease control (key focus on biological control)

No.	Corresponding Author	Paper draft title	contact email
14	Huacai Fan	Screening and identification of an antagonistic strain to Fusarium wilt of banana and its biological control effect.	s.zheng@cgiar.org
15	David East	*Trichoderma virens*: a potential biocontrol agent for Fusarium wilt in banana.	David.EAST@daf.qld.gov.au
16	Elizabeth Aitken	Effect of herbicides application on Fusarium inoculum.	e.aitken@uq.edu.au
17	Walter Ocimati	Using the substrate of the edible fungus *Pleurotus ostreatus* in the biocontrol of *Fusarium oxysporum f. sp. cubense race* 1.	W.Ocimati@cgiar.org
18	Evans Were	The effect of ground cover root flavonoids and phenolic acids on the germination, growth, conidiation and virulence of *Fusarium oxysporum f. sp. cubense* TR4.	e.were@unihohenheim.de
19	Fabiola Alfaro	*Radopholous similis* as a predisposing factor for Fusarium wilt of bananas.	mguzman@corbana.co.cr
20	Daniel Heck	Weevil borers affects the spatiotemporal dynamics of banana Fusarium wilt.	dwinterheck@gmail.com
21	Mauricio Soto Suárez	Disinfectants against TR4.	msoto@agrosavia.co

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