Fusarium wilt of bananas: Research advances, needs and regional initiatives

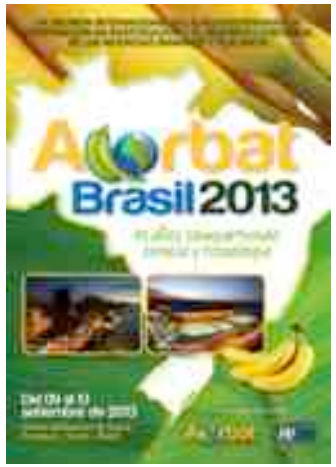
Miguel Angel Dita Rodriguez

miguel.dita@embrapa.br

What countries are doing Research on Fusarium wilt in LAC?

- ✓ Brazil (exports is limited, but national market rely on susceptible genotypes)
- ✓ Cuba (no export - it has been active because susceptible varieties and national interest)
- ✓ Venezuela (Almost no export)
- ✓ In countries with more export tradition Fusarium wilt has not been a priority in national research agenda in the last 60 years:
  - Ecuador
  - Costa Rica* (re-starting)
  - Colombia (at the beginning)

Little attention to a Foc in LAC in the last 10 years, even with the TR4 threat

2004	2006	2008	2010	2013
				
Sigatoka = 22 Nematodes = 4 Others = 6 Fusarium= 1	Sigatoka = 15 Nematodes = 4 Others = 13 Fusarium = 1	Fusarium 3 Sigatoka 20 Nematodes 5 Other 95 Total 123	Fusarium 6 Sigatoka 24 Nematodes 0 Others 59 Total 89	Fusarium: 11 Total: 145

Low number of indexed publications

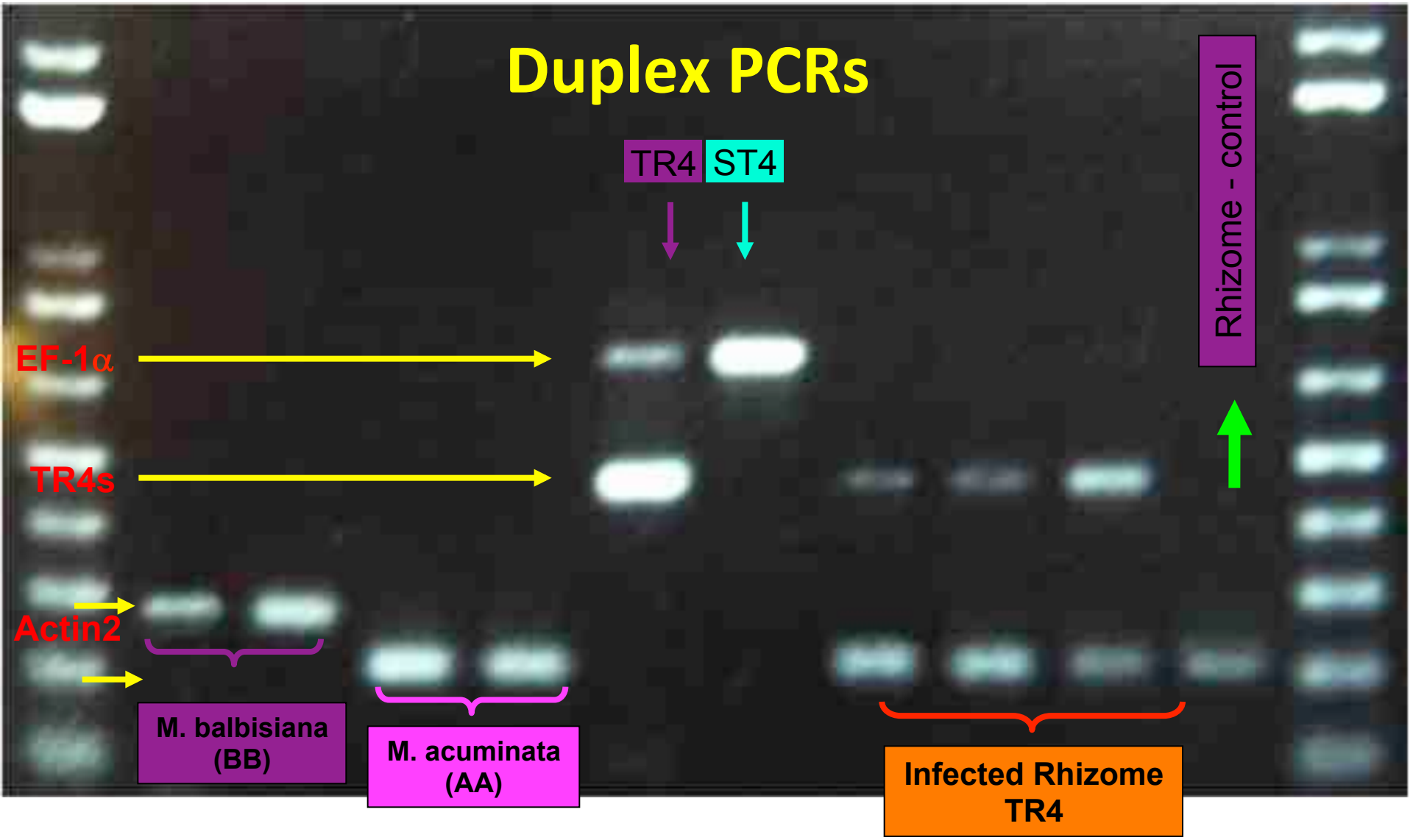
Research Targets on Foc Tropical Race 4

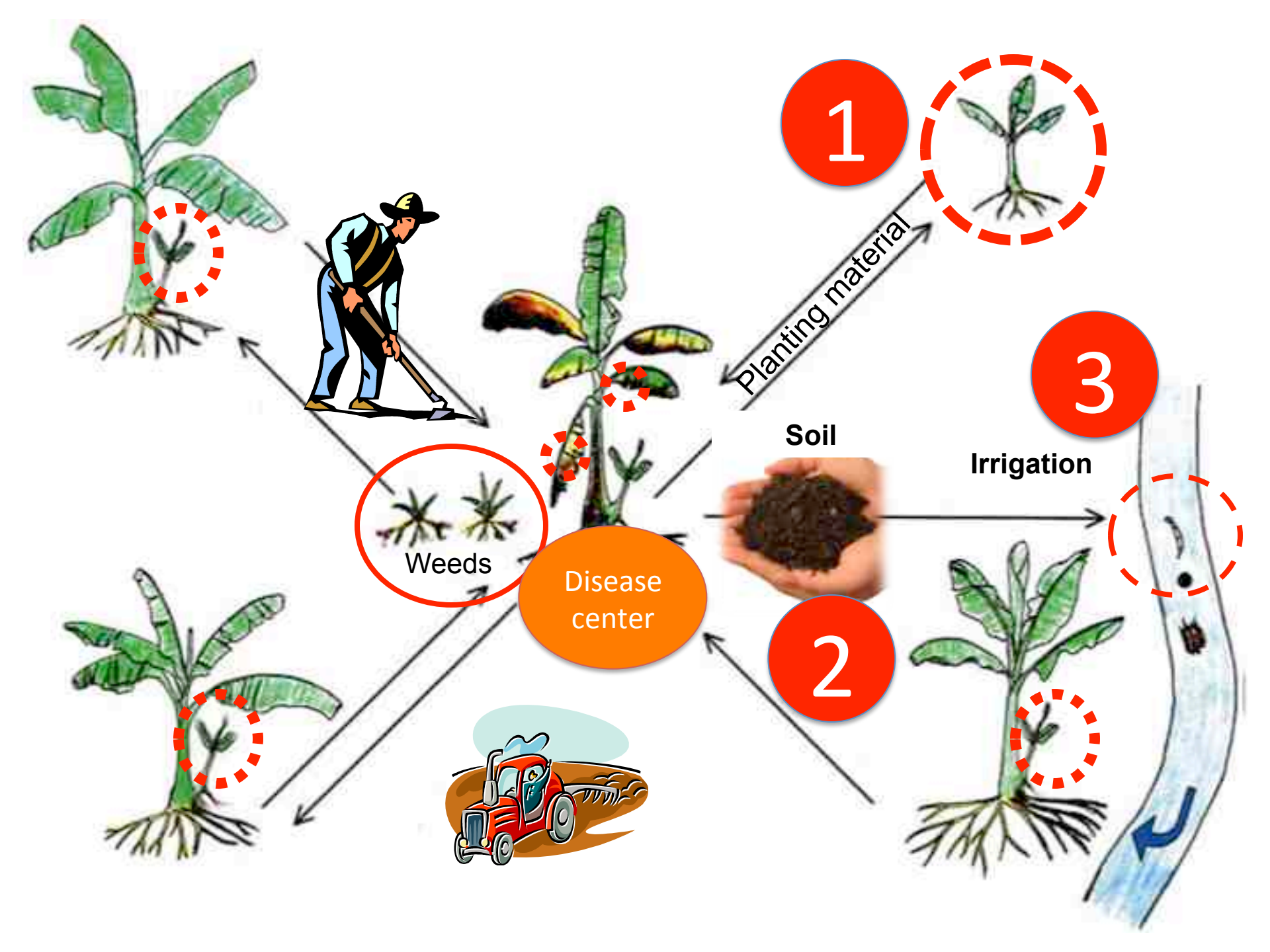
1. Prevention - early detection
2. Use of resistant varieties
3. Disease management

a. Diagnostic tools for Foc TR4 and other important races/strains of Foc;
b. Fast and reliable bioassays for high throughput phenotyping
c. Characterize genotypes of Musa for TR4 resistance/ Identify Resistant sources
d. Understand Foc- banana interaction at genetic, cytological and molecular level;
e. Epidemiology for integrated management strategies - Eradication

Foc TR4 – Diagnostic

EF +FocTR4 // Actin2 + FocTR4

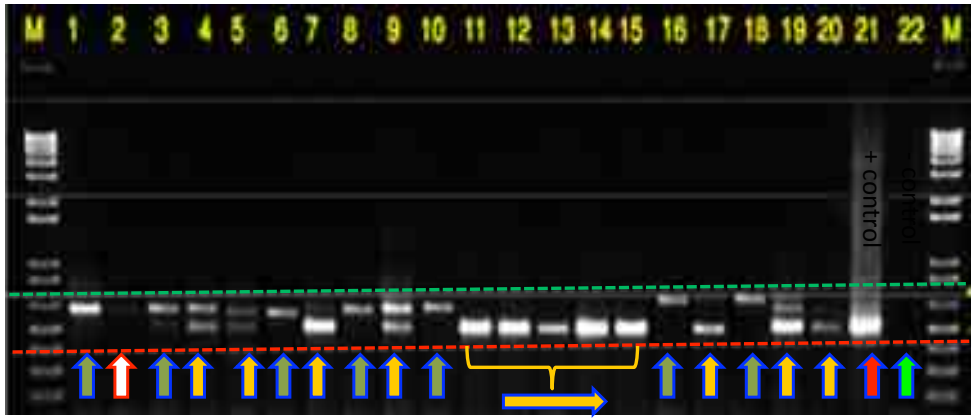



1
Planting material
3
Soil
Irrigation
Weeds
Disease center
2

Detecting Foc TR4 in soil and symptomless tissues

DNA extraction

Sample ID	Cultivar [a]	Plant stage	Location	Source	Sample processed	Single PCR	Nested PCR
AuD1	Cavendish	Symptomatic	Darwin	Australia	Plant	+	nd
AuH1	Cavendish	Symptomless	Darwin	Australia	Plant	+	nd
TS1	Cavendish	Symptomless	Chaojhou	Taiwan	Soil	-	+
					Plant	+	nd
TS3	Cavendish	Symptomatic	Chaojhou	Taiwan	Soil	+	+
					Plant	+	nd
TS6	Cavendish	Symptomless	Wandan	Taiwan	Soil	-	-
					Plant	-	nd
TS7	Cavendish	Symptomatic	Jiuru	Taiwan	Soil	+	+
					Plant	+	nd
TS8	Cavendish	Symptomless	Jiuru	Taiwan	Soil	-	+
					Plant	+	
TS9	Cavendish	Symptomatic	Luye	Taiwan	Soil	+	+
					Plant	+	nd
Phi126C	Gran Naine	Symptomatic	Kapalong	Philippines	Plant	+	nd
Phi39B	Tall William	Symptomatic	Kapalong	Philippines	Plant	-	nd
Phi2SV	Latundan	Symptomatic	Kapalong	Philippines	Plant	-	nd
ChlamyD2	n.a.	n.a.	n.a.	n.a.	Foc-colonized Substrate	+	+



Dita et al 2013

Water is an efficient way for FOC dissemination….

- Can we detect Foc TR4 easily in water samples?
- What would be the app/implications?

Risk Assessment and Surveillance of Foc TR4

Strengthening capacities and increasing preparedness for Foc TR4 in LAC

Bioversity, MUSALAC & National Plant Protection Organizations

▲ **Trained**

△ In the pipeline

RECOMENDACIONES PARA PREVENIR LA ENTRADA DE ENFERMEDADES CUARENTENARIAS DE ALTA IMPORTANCIA ECONÓMICA PARA BANANO Y PIÑA.

Dirigidas a: Empleados de las compañias fruteras, representantes de compañias de agroquímicos, inspectores de Compañías Certificadoras, representantes de ONG's, y cualquier otro visitante a plantaciones de banano en el Sur Este Asiático,

RECOMMENDATIONS TO PREVENT THE ENTRANCE OF QUARANTINE PLANT DISEASES OF MAJOR ECONOMIC IMPORTANCE FOR BANANA AND PINEAPPLE

Aimed at: Employees of the major fruit producing and processing companies, representatives of agrochemical companies and NGOs, certification inspectors, and any other visitor to banana plantations in South East Asia and pineapple plantations in South America.

RECOMMANDATIONS POUR PRÉVENIR L'ENTRÉE DE MALADIES DE QUARANTAINE DE GRANDE IMPORTANCE ÉCONOMIQUE POUR LES BANANIERS ET L'ANANAS.

Cible : Employés de compagnies fruitières, représentants de compagnies phytosanitaires, inspecteurs de Compagnies de Certification, représentants d'ONG, et pour tout visiteur de plantations de banane en Asie du Sud-est, et de plantations d'ananas en Amérique du Sud.

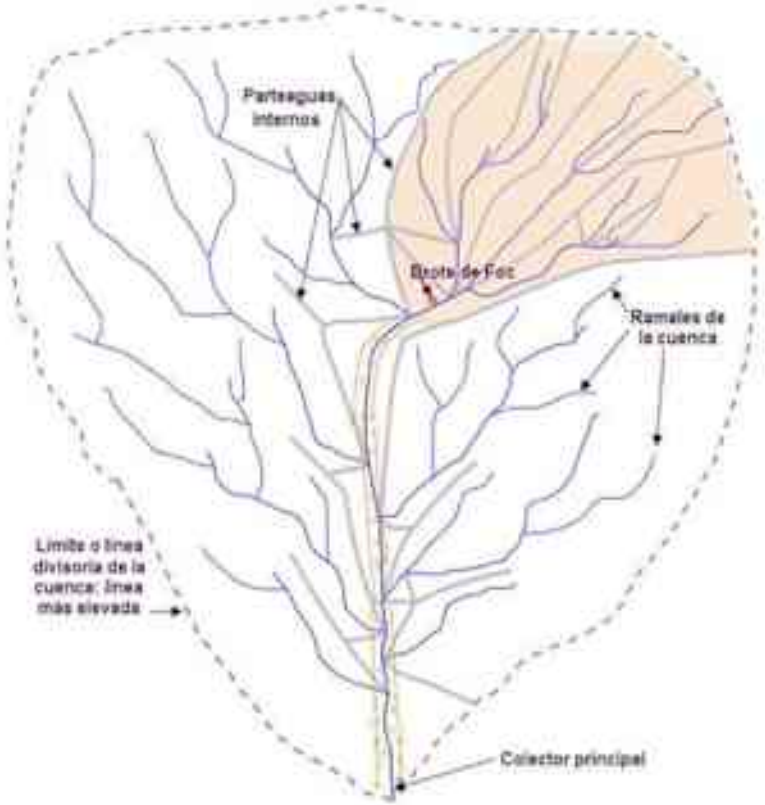
Contingency plan for Foc TR4

ORGANISMO INTERNACIONAL REGIONAL DE SANIDAD AGROPECUARIA
OIRSA

Plan de contingencia ante un brote de la raza 4 tropical de *Fusarium oxysporum* f. sp. *cubense*

En un país de la región del OIRSA

Elaborado por:
Miguel Ángel Dita Rodríguez
Plutarco Elías Echegoyén Ramos
y
Luis Fernando Pérez Vicente



Good example : Caravan for management Helicoverpa and other pests : Embrapa 2014

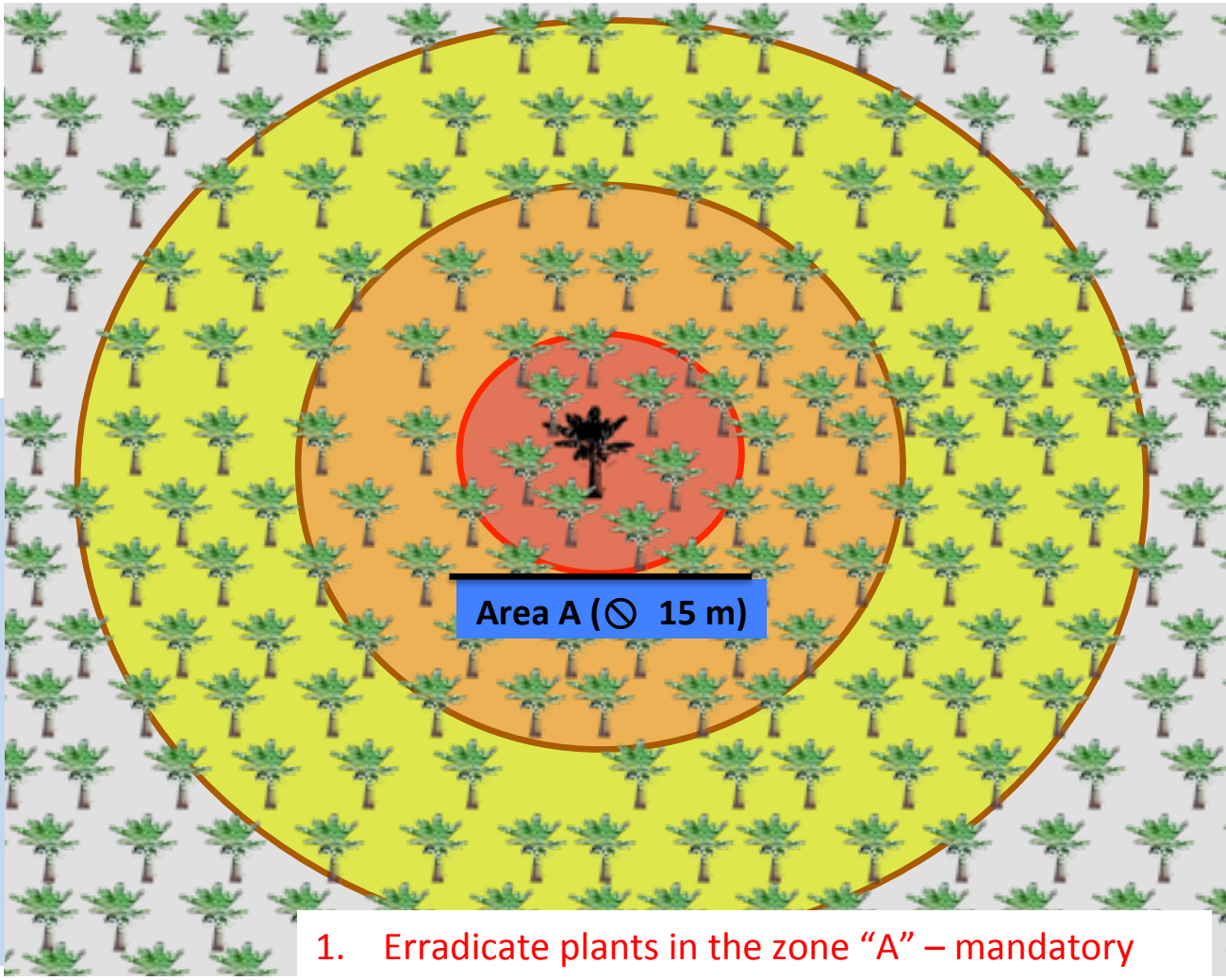
http://www.cnpso.embrapa.br/caravana


It is now too late? How to proceed?
A. Molina

Early diagnostic of Fusarium wilt vs. management

When a plant is detected infected how many 'neighbors' we should destroy around?

Farmer and companies think more on bunches and boxe$... they don't care too much on inoculum pressure, epidemiology ?
....but at the end of the day the scenario jus becomes worst



1. Erradicate plants in the zone "A" – mandatory
2. Erradicate plant in zone B - Recommended

What else needs to be done on the the pathogen side?

Establishment and characterization of national collections

- Population biology studies
- Molecular characterization using informative markers (i.e genes related to pathogenicity/aggressiveness)
- VCG analyses
- Survey using specific primers for TR4
- Characterization for pathogenicity and aggressiveness on reference cultivars
- Identification and characterization of pathogenesis/ aggressiveness -related genes

F. Haddad, Embrapa

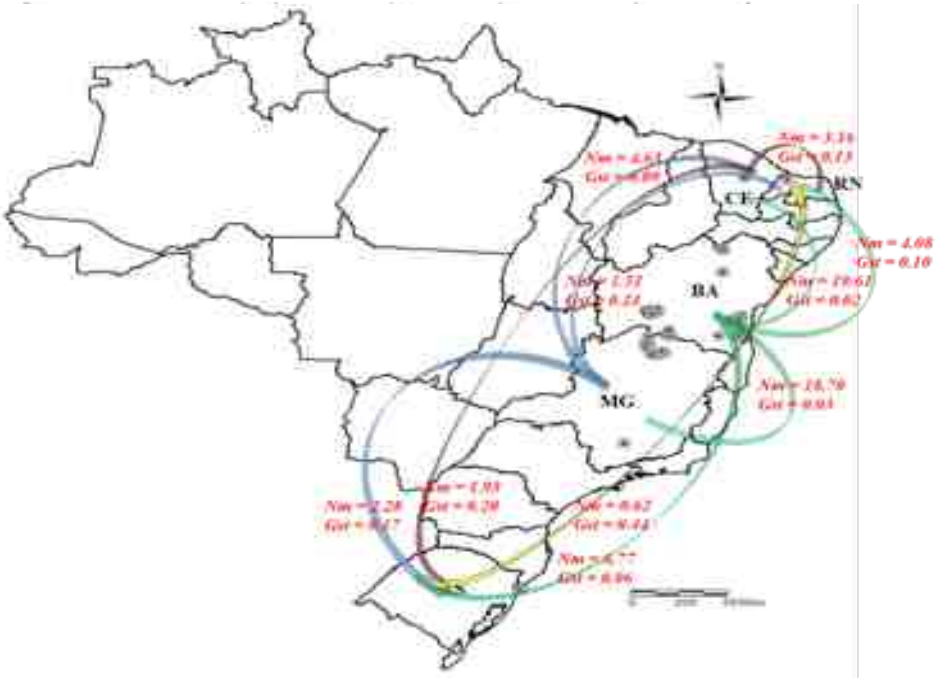
Population biology of Foc in Brazil (Costa et al., 2014)

Plant Pathology (2014) Doi: 10.1111/ppa.12242

Genetic structure of *Fusarium oxysporum* f. sp. *cubense* in different regions from Brazil

S. N. Costa[a†], C. A. D. Bragança[b†], L. R. Ribeiro[a], E. P. Amorim[c], S. A. S. Oliveira[c], M. A. Dita[c], F. F. Laranjeira[c] and F. Haddad[c*]

[a]Federal University of Recôncavo da Bahia; [b]CAPES/Embrapa; and [c]Embrapa Cassava & Fruits, Cruz das Almas 44380-000, Brazil



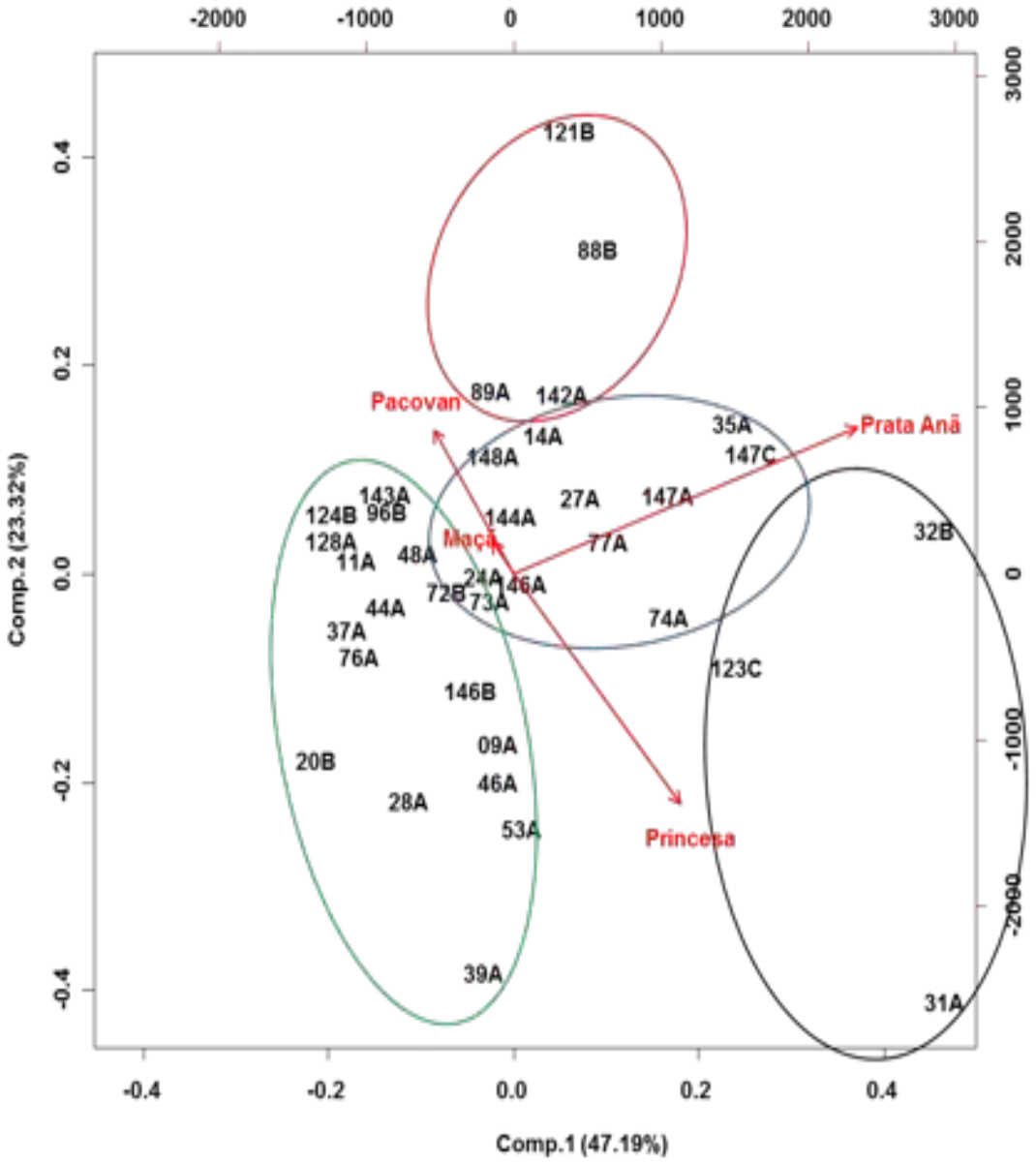
- 05 major regions
- 214 isolates
- 52 haplotypes detected

Migrations are represented by curves. Green curves: migrations between BA and other states; blue curves: migrations between MG and other states; brown curves: migrations between CE and other states; Yellow curve: migration between RN and RS. Nm: Number of migrants.

Population biology of FOC in Brazil (Costa et al., 2014)

Differential Interaction of isolates representing 52 haplotypes with varieties

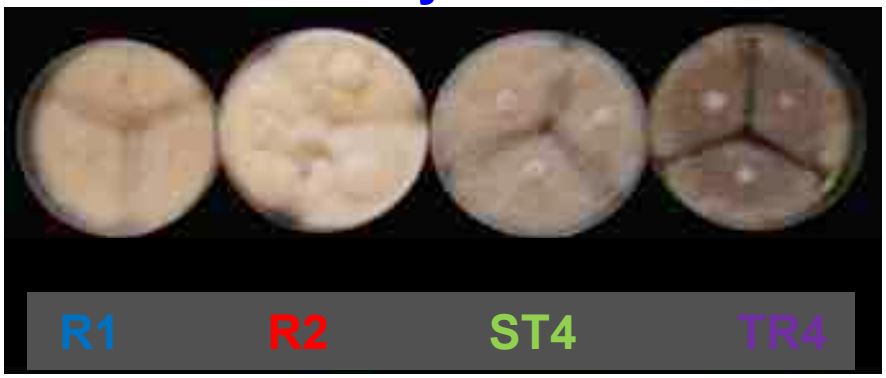
Maça (Silk) = Susceptible
Prata anã = Susceptible
Pacovan : Moderately Resistant
Princesa : Resistant



More info with: Dr. Haddad
fernando.haddad@embrapa.br

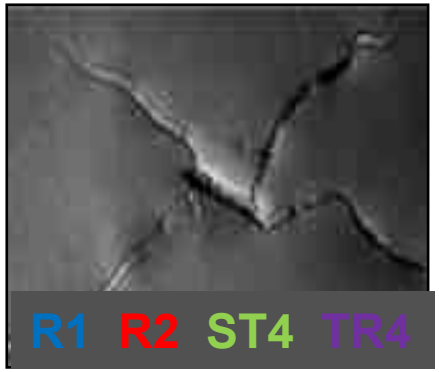
Comparative transcriptomic analysis of Foc [R1, R2, ST4 & TR4]

Mycelia



Conidia

Germinated conidia



RNA extraction

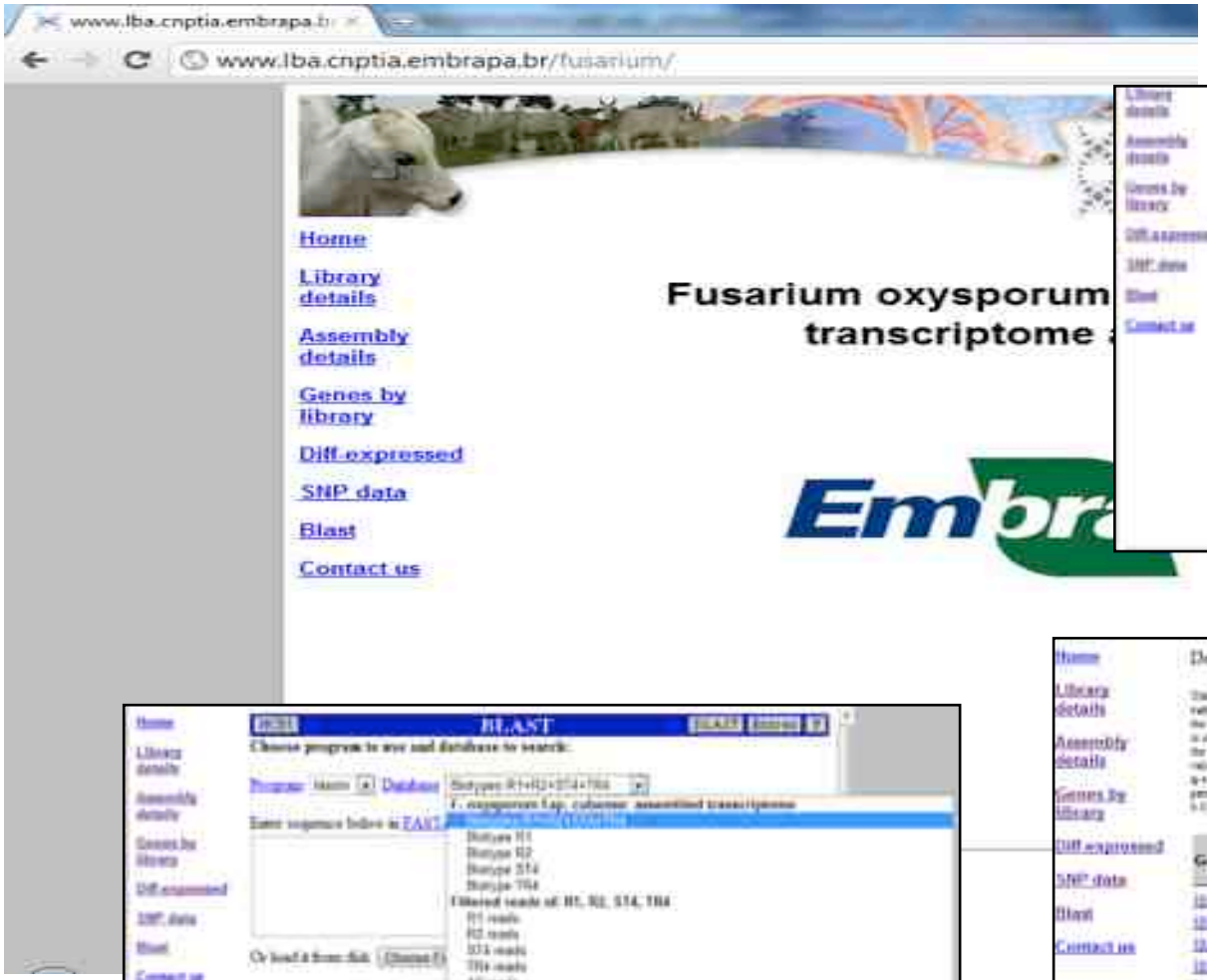
RNA pooling (mycelium + conidia + germinating conidia)

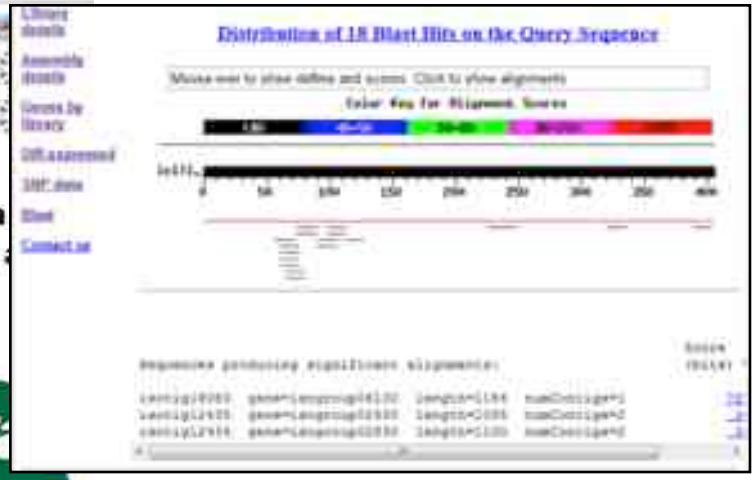
mRNA Isolation

cDNA from R1, R2, ST4, TR4 and 454 sequencing

Comparative EST analyses

Foc web server: software, analysis, interaction



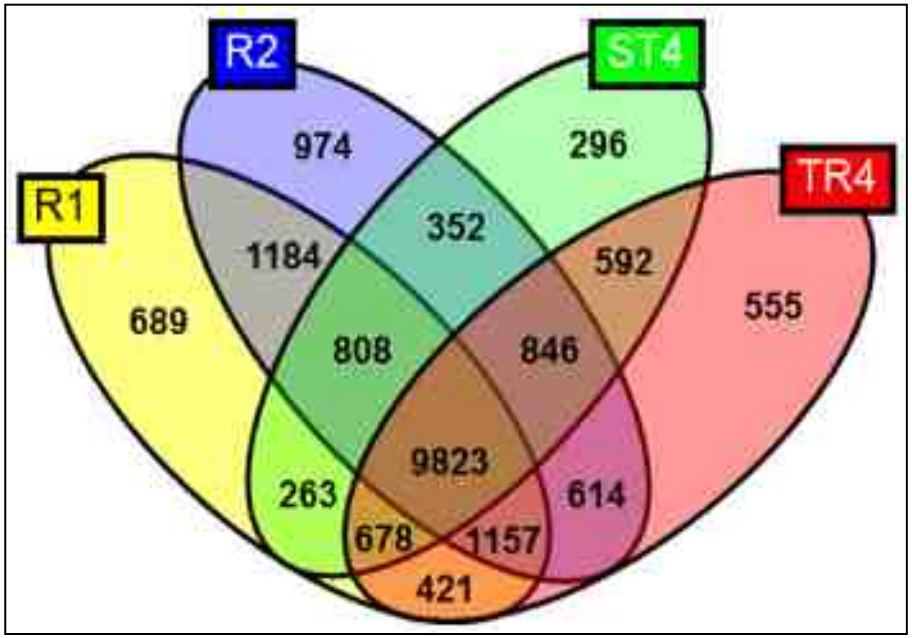


Comparative transcriptomic analysis of Foc [R1, R2, ST4 & TR4]

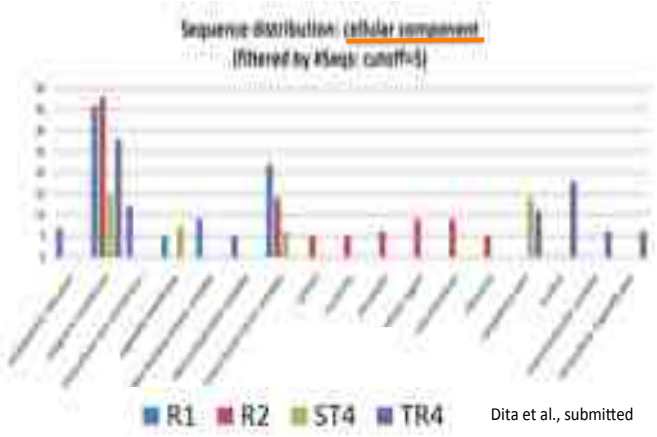
Library statistics

	R1	R2	ST4	TR4	All Libraries
Raw reads	644.444	661.201	633.453	606.533	2.545.631
rDNA	2.988	3.255	1.811	2.746	10.800
Passed reads	637.670	653.748	629.498	601.136	2.522.052
aver. length	396	392	364	371	381
% Singlets	6.2 %	6.3 %	5.5 %	7.2 %	5.3 %
Assembled reads	541.632	558.903	554.771	557.094	2.151.030
# isogroups	8.302	9.059	7.610	8.193	11.058
# isotigs >50nt	9.674	11.114	8.500	10.216	21.445
mean isotig length	1345.8	1440.5	1293.8	1406.3	2161.9
Longest Isotig	8.293	9.732	7.276	7.752	11.432

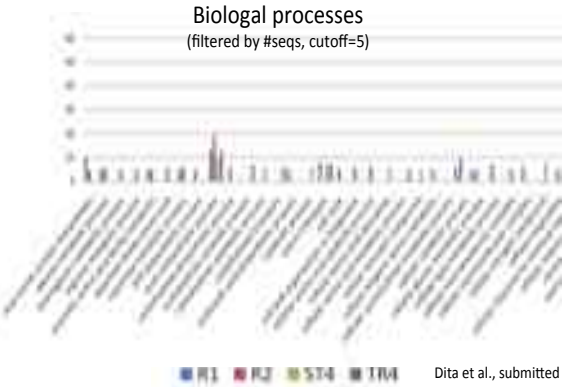
Gene distribution across Foc races



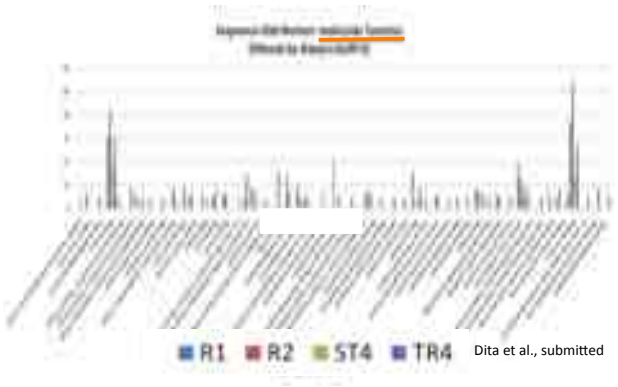
Annotation of UNIQUE genes



Dita et al., submitted



Dita et al., submitted



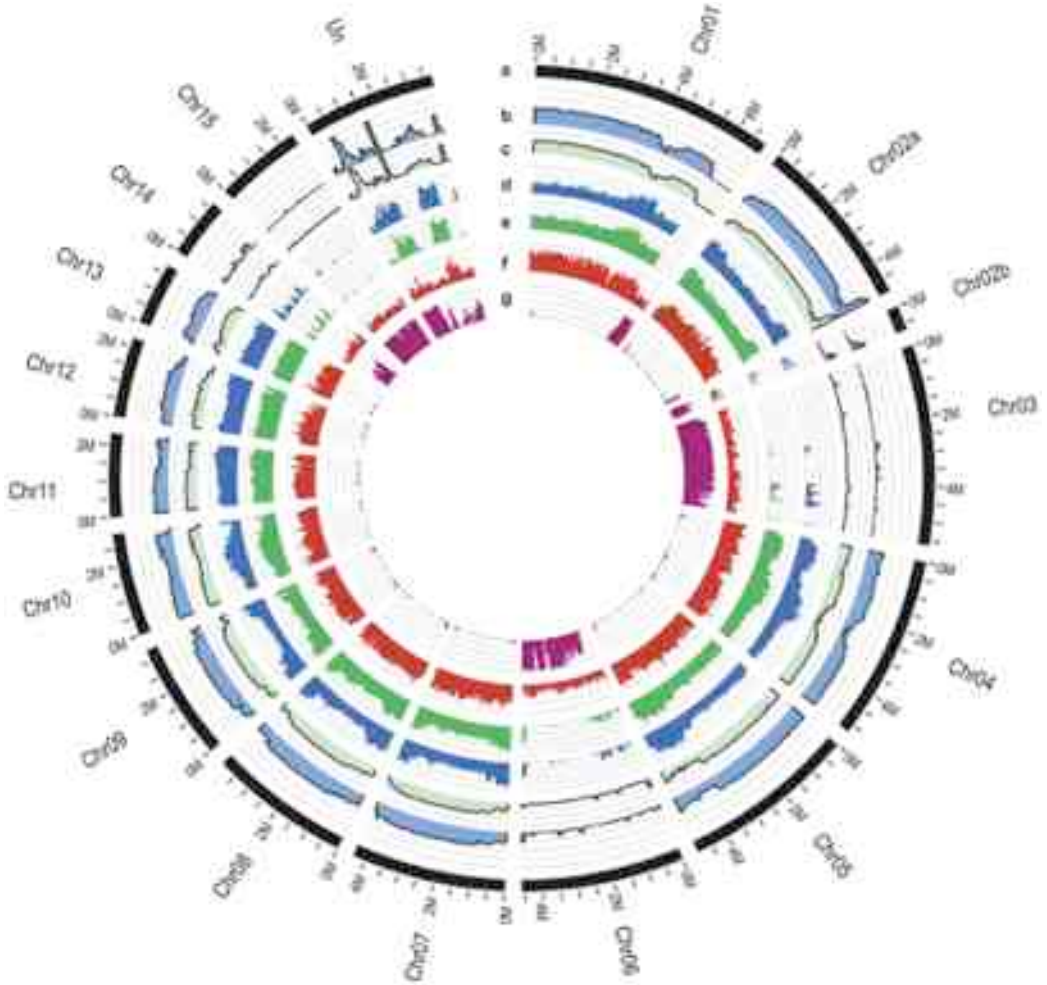
Dita et al., submitted

OPEN ACCESS Freely available online April 2014 | Volume 9 | Issue 4 | e95543 PLOS ONE

Genome and Transcriptome Analysis of the Fungal Pathogen *Fusarium oxysporum* f. sp. *cubense* Causing Banana Vascular Wilt Disease

Lijia Guo[1,2], Lijuan Han[3], Laying Yang[1,2], Huicai Zeng[2], Dingding Fan[3], Yabin Zhu[3], Yue Feng[3], Guofen Wang[1], Chunfang Peng[3], Xuanting Jiang[3], Dajie Zhou[3], Peixiang Ni[3], Changcong Liang[1], Lei Liu[1], Jun Wang[1], Chao Mao[1], Xiaodong Fang[3], Ming Peng[2], Junsheng Huang[1]

1 Key Laboratory of Monitoring and Control of Tropical Agricultural and Forest Invasive Alien Pests, Ministry of Agriculture, Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou, China, 2 Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou, China, 3 BGI-Shenzhen, Shenzhen, China

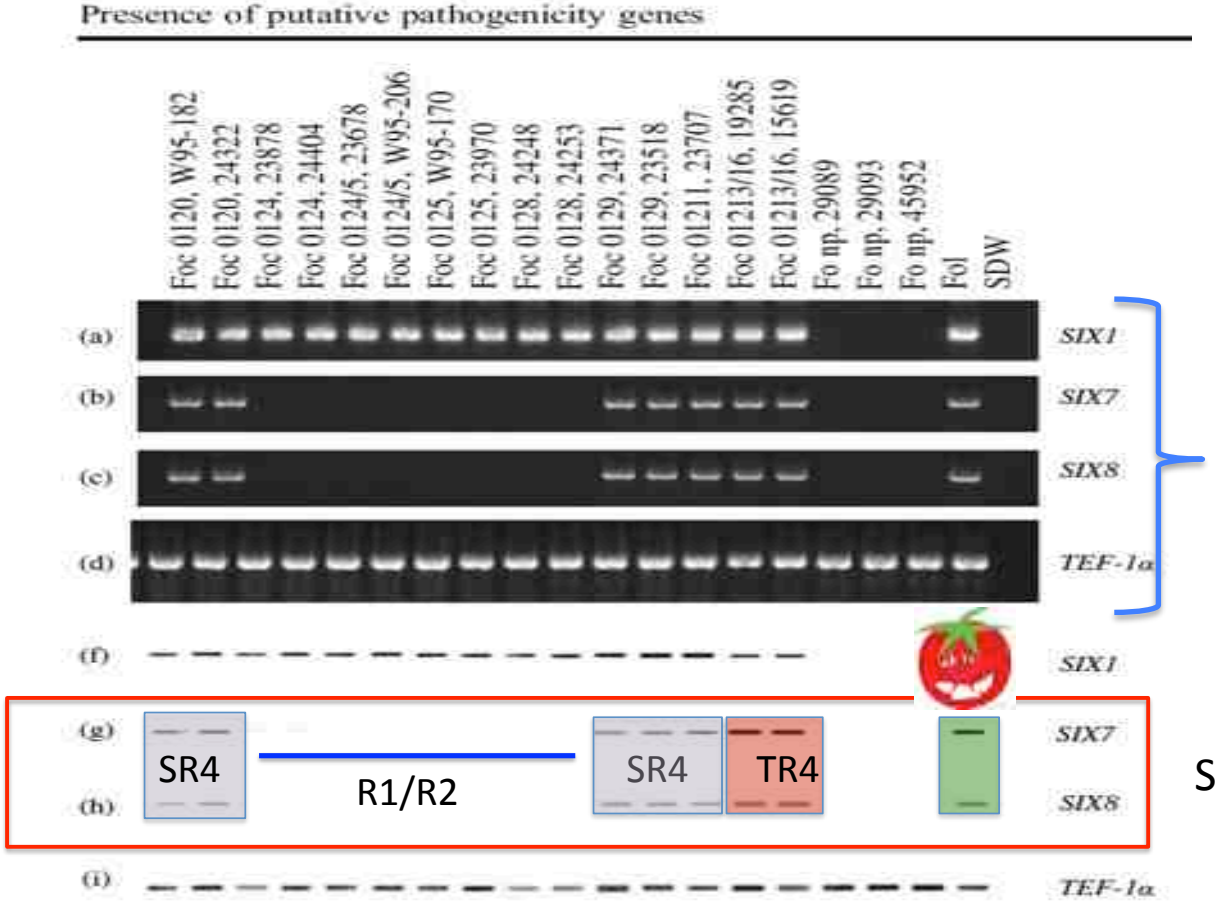


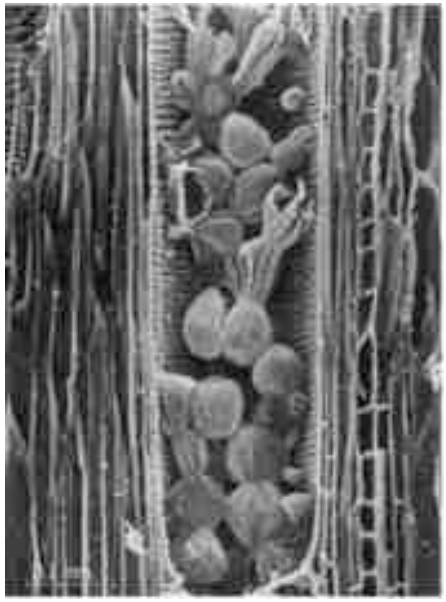
Australasian Plant Pathol
DOI 10.1007/s13313-012-0122-x

Presence of putative pathogenicity genes in isolates of *Fusarium oxysporum* f. sp. *cubense* from Australia

R. A. Meldrum · S. Fraser-Smith · L. T. T. Tran-Nguyen · A. M. Daly · E. A. B. Aitken

Secreted in Xylem Genes – SIX





http://www.accessscience.com/popup.aspx?figID=524000FG0060&id=524000&name=figure

(G. E. VanderMolen
University of Rhode Island)

OPEN ACCESS Freely available online

PLOS | ONE

Genome and Transcriptome Analysis of the Fungal Pathogen *Fusarium oxysporum* f. sp. *cubense* Causing Banana Vascular Wilt Disease

Lijia Guo[1,2], Lijuan Han[3,2], Laying Yang[1,2], Huicai Zeng[2], Dingding Fan[3], Yabin Zhu[3], Yue Feng[3], Guofen Wang[1], Chunfang Peng[3], Xuanting Jiang[3], Dajie Zhou[3], Peixiang Ni[3], Changcong Liang[1], Lei Liu[1], Jun Wang[1], Chao Mao[1], Xiaodong Fang[3], Ming Peng[2], Junsheng Huang[1]

1 Key Laboratory of Monitoring and Control of Tropical Agricultural and Forest Invasive Alien Pests, Ministry of Agriculture, Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou, China, 2 Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences,

Table 2. The orthologs of *SIX*-genes in Foc1 and Foc4.

SIX genes	Foc1 gene ID	RPKM_0h	RPKM_48h	Foc4 gene ID	RPKM_0h	RPKM_48h
SIX1a	Foc1g01632	5.75	39.16	Foc4g00240	6.27	12.77
SIX1b	NA	-	-	Foc4g00324	0.49	0
SIX1c	NA	-	-	Foc4g00575	50.71	113.08
SIX2	NA	-	-	Foc4g07631	NA	NA
SIX6	Foc1g00211	14.32	13.31	Foc4g00351	243.71	192.59
SIX8	NA	-	-	Foc4g00520	0.16	0.63

doi:10.1371/journal.pone.0095543.t002

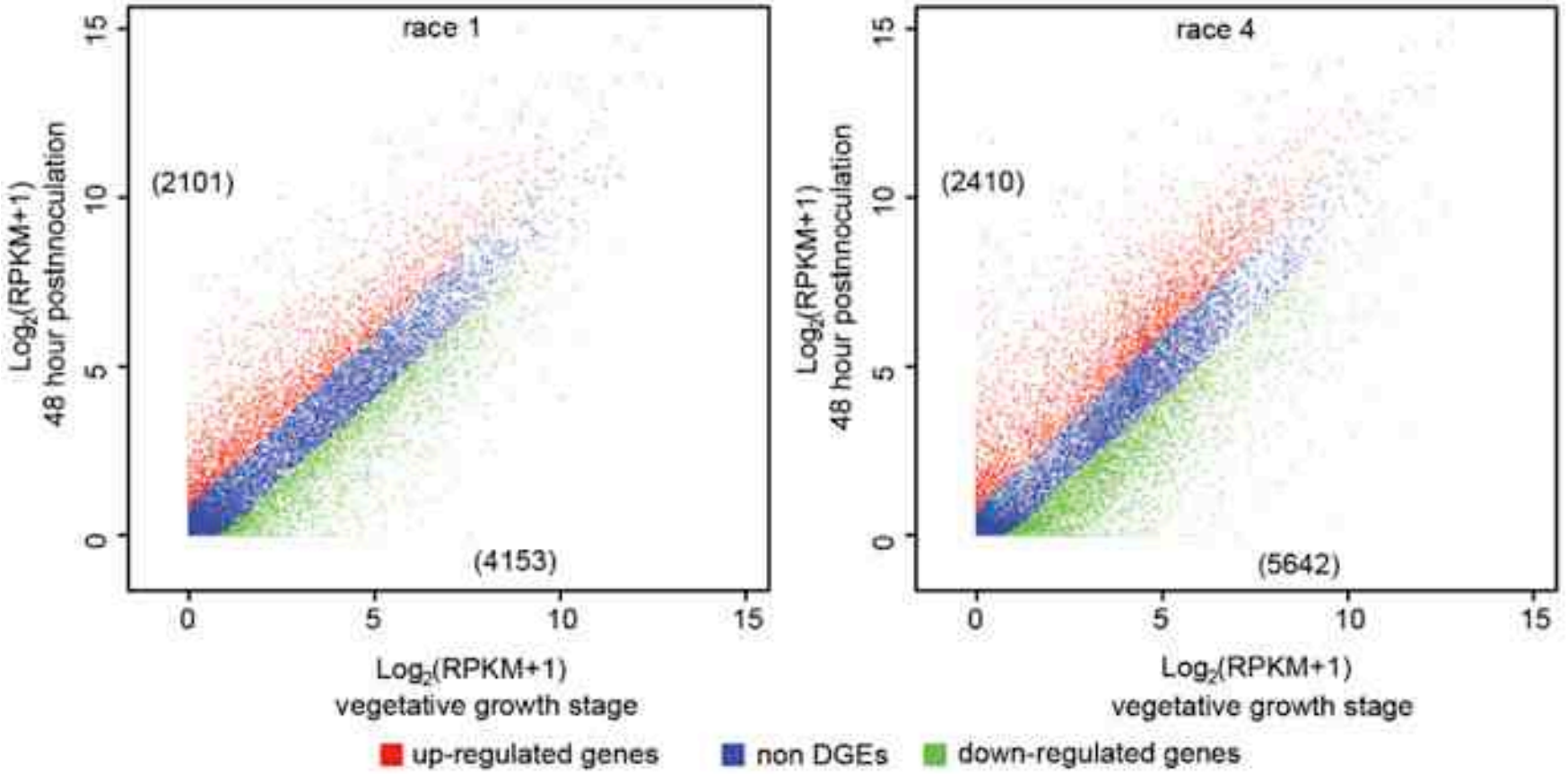
OPEN ACCESS Freely available online

PLOS | ONE

Genome and Transcriptome Analysis of the Fungal Pathogen *Fusarium oxysporum* f. sp. *cubense* Causing Banana Vascular Wilt Disease

Lijia Guo[1,3], Lijuan Han[3], Laying Yang[1,3], Huicai Zeng[2], Dingding Fan[3], Yabin Zhu[3], Yue Feng[3], Guofen Wang[1], Chunfang Peng[3], Xuanting Jiang[3], Dajie Zhou[3], Peixiang Ni[3], Changcong Liang[1], Lei Liu[1], Jun Wang[1], Chao Mao[1], Xiaodong Fang[3], Ming Peng[2], Junsheng Huang[1]

1 Key Laboratory of Monitoring and Control of Tropical Agricultural and Forest Invasive Alien Pests, Ministry of Agriculture, Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou, China, 2 Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou, China, 3 BGI-Shenzhen, Shenzhen, China



Functional analysis in Foc

Agrobacterium-mediated insertional mutagenesis in Foc

(sporulation, pathogenicity, chlamydospore formation)

Flávia da Silva Fernandes

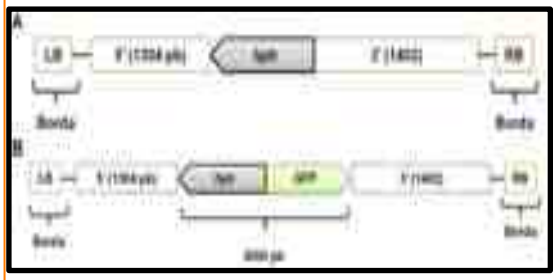
R1 sporulation

R2 sporulation

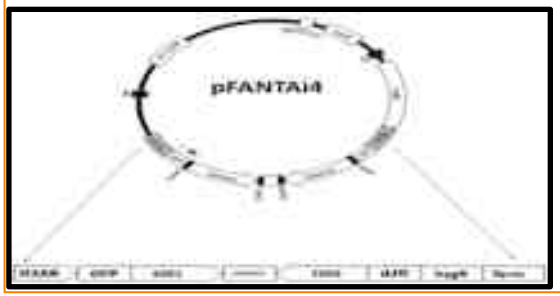
Targeted-gene approach

Joelma dos Santos Fernandes

Knockout



knockdown

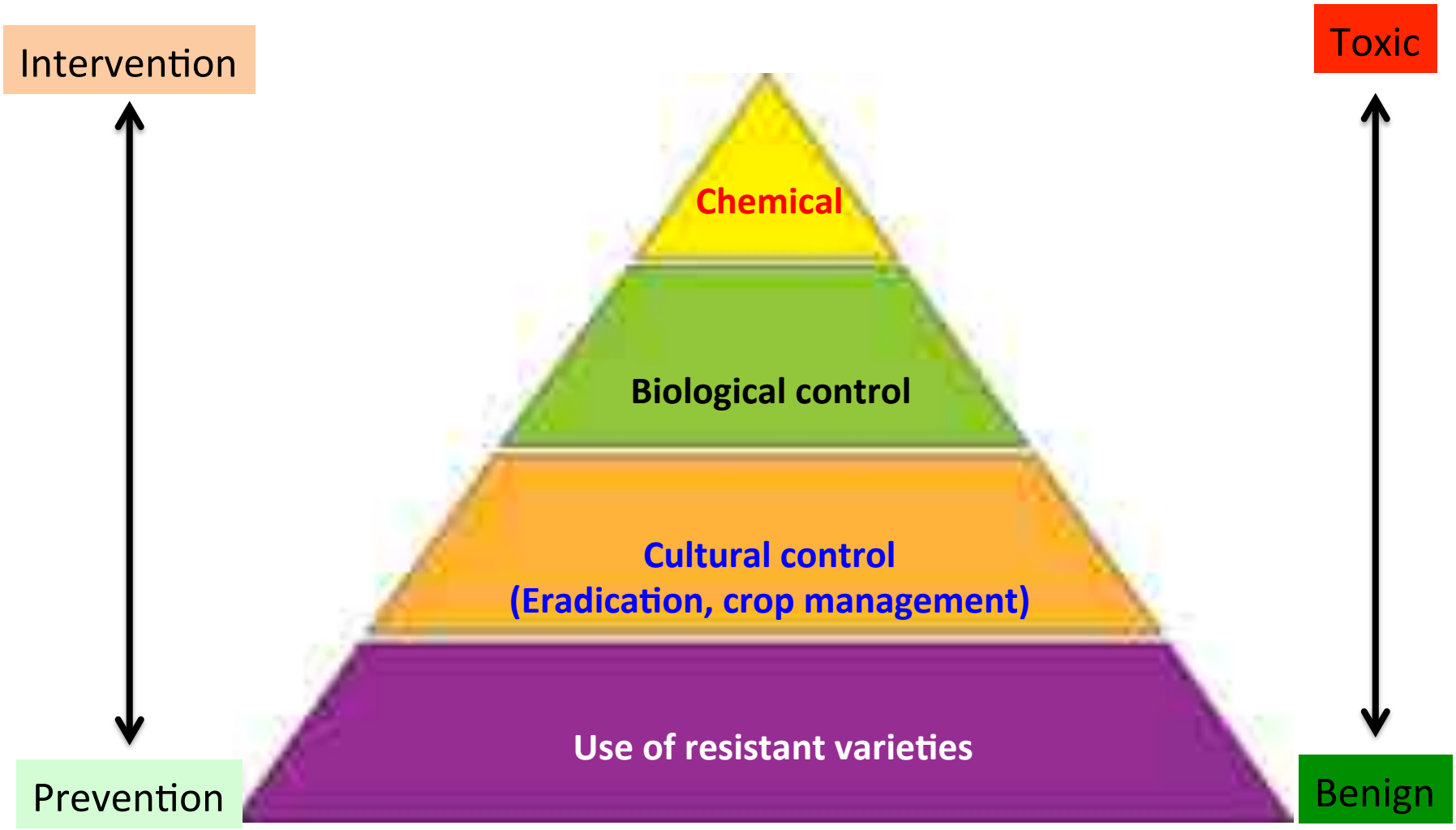


Fow2,
SNF1,
Six1,--14
Sge1,
Frp1,
NEP,
MAPK,
PLS1.

RNAi.
I-Mutation

Work in progress, Race 1 and 2 strains in Brazil

Management options



Resistant varieties vs. Foc TR4?

How many varieties are affected by Foc TR4?

Indonesia

1. Raja (AAB)
2. Raja serai (AAB),
3. Buai (AAA),
4. P.Panjang (AAB),
5. Barangan (AAA)
6. Ambon kuning (AAA),
7. Ambon hijau (AAA)
8. Rejang (Aaw),
9. Jantan (AAB),
10. Kepok (ABB),
11. Mas kirana (AA)

Riska & Hermanto (2012)

Philippines

Variety	1st Crop	2nd
1.Lakatan (AAA),	100	NA
2.Latundan (AAA),	41	100
3.Gran Nanine(AAA),	97	NA
4.Williams (AAA)	94	NA
5.GCTCV 119 (AAA)	1	28
6.GCTCV 218 (AAA)	1	24
7.FHIA 21 (AABB)	81	NA

Molina (2011)

Australia

~ 26 susceptible Varieties (Walduck & Daly, 2007)

http://www.nt.gov.au/d/Primary_Industry/Content/File/horticulture/TAR2006_07/Banana_panama_pg11_15.pdf

~ 80% of bananas produced worldwide are from varieties susceptible to TR4

Banana cultivars reported with resistance to Foc TR4

Variety	Resistance	Market	Sources
FHIA 01	R	Seeded	Walduck & Daly 2007 Bingzhi et al. 2004
FHIA 02	R	Seeded	Bingzhi et al. 2004
FHIA 18	R	Good	Walduck & Daly 2007 Bingzhi et al. 2004
FHIA 25	R	Poor	Walduck & Daly 2007 Bingzhi et al. 2004
Pisan Jai Buya	R	NA	Walduck & Daly 2007 Bingzhi et al. 2004
*GCTV 119	MR	Good	Walduck & Daly 2007 Bingzhi et al. 2004 Molina 2011
GCTV 218	MR	Good	Molina, 2011
*M. accuminata malaccensis***	R	Seeded	Walduck & Daly 2007
ABB varieties (Saba, Tanduk)	R	NA	Molina - unpublished

* There are many GCTV, produced by TBRI, Taiwan, which are continuously replaced for new somaclons with some resistance levels

**Some *M.a. malaccensis* are susceptibles

At least 50 varieties are affected by TR4

~ 80 % of banana produced are from susceptible varieties [Ploetz et al. 2005]

In the Caribbean ~ 63% of **2,856,394 *t*** produced in 2009 were from TR4-susceptible varieties

(Data: Lescot, 2010)

Cavendish Banana Cultivars Resistant to Fusarium Wilt Acquired through Somaclonal Variation in Taiwan

Shin-Chuan Hwang

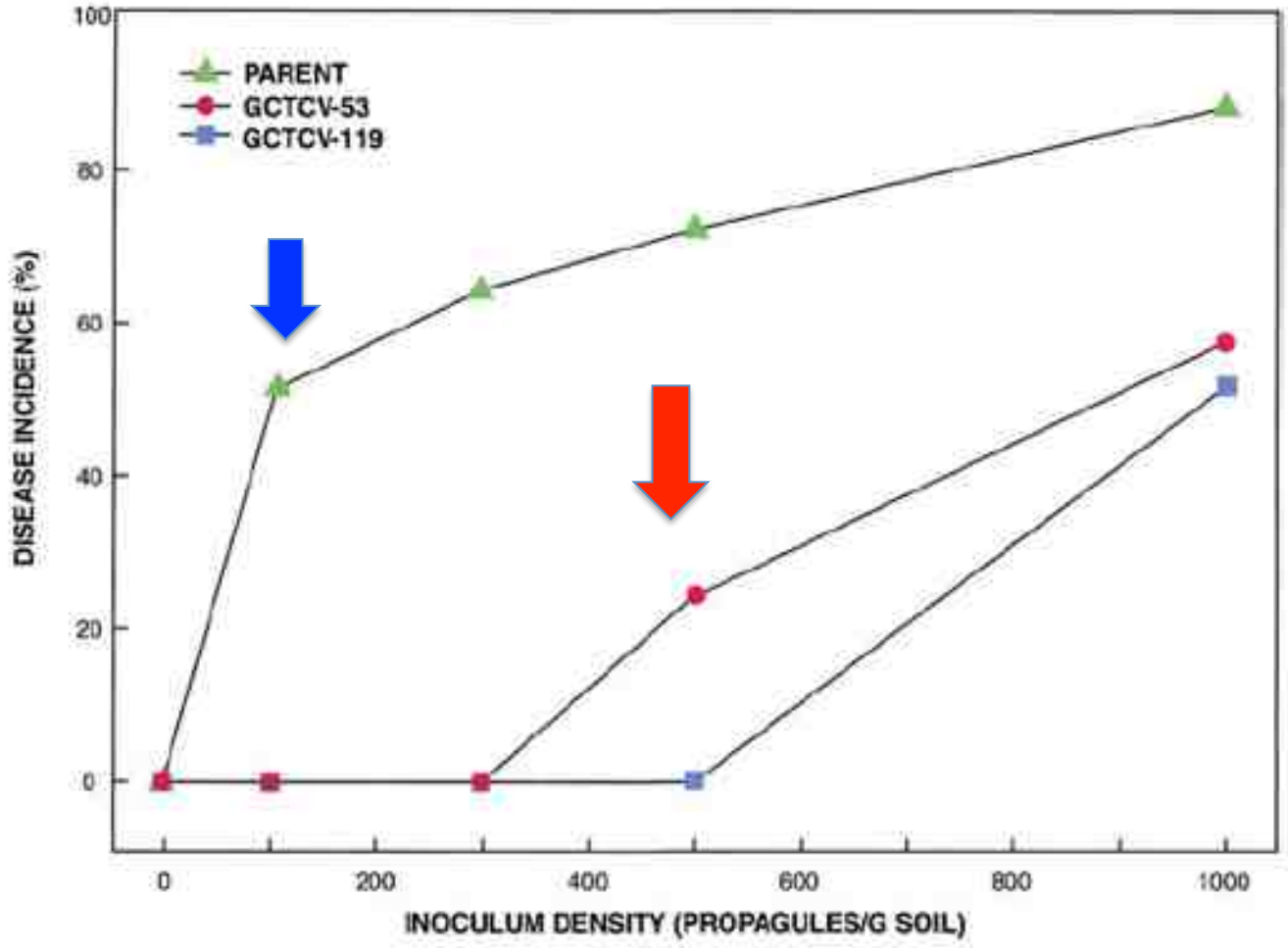
Wen-Hsiung Ko

r. Hwang is a plant pathologist and irector of the Taiwan Banana Re-earch Institute. He received his B.S. egree from National Taiwan Univer-ity and his M.S. and Ph.D. degrees om the University of Hawaii. His esearch interests cover all aspects of anana diseases, with special empha-is on Fusarium wilt. He was elected s one of the Ten Outstanding Young len of the nation in 1982, received he Distinguished Agricultural Re-earch and Extension Award from the ouncil of Agriculture of Taiwan in 988, and was bestowed the Out-tanding Applied Science and Tech-ology Award by the National Science

Dr. Ko is professor of plant patholog at Beaumont Agricultural Researc Center of the University of Hawaii a Manoa. He received his B.S. degre from National Taiwan University an his Ph.D. degree from Michigan Stat University. His research interests in clude ecology and biological control o soilborne diseases of tropical fru crops and biology of *Phytophthora*. H received the Ruth Allen Award in 198 from the American Phytopathologica Society for his contribution in hormo nal regulation of sexual reproductio in *Phytophthora* and is a Fellow of th American Phytopathological Society.

Plant Disease / June 2004

La resistencia de los somaclones es dependiente de la presión de inóculo!



Hwang & Ko 2004, Plant Disease / June 2004

Fig. 5. Disease incidence of resistant somaclone in comparison with the susceptible Giant Cavendish parent in trays with various inoculum densities of race 4 of *Fusarium oxysporum* f. sp. *cubense.*

There are phenotypic variations environmentally-associated

Hwang & Ko 2004, Plant Disease / June 2004

Table 3. Horticultural characteristics of Fusarium wilt resistant clones derived from Giant Cavendish by somaclonal variation

Clone	Resistance[a]	Horticultural characteristics
GCTCV-40	High	Tall and slender pseudostem; weak petiole with narrow and drooping leaves; small bunch
GCTCV-44	High	Short and slender pseudostem; weak petiole and drooping leaves; bunch normal but weak pedicel
GCTCV-46	High	Black spots on pseudostem and leaf sheath; upright leaves; small bunch with short fingers
GCTCV-53	High	Dark green pseudostem; drooping leaves; elongate male bud; small bunch with short fingers
GCTCV-62	Moderate	Pale green pseudostem; fewer suckers; small bunch and fingers
GCTCV-104	High	Pale green pseudostem; fewer fingers; long growth cycle
GCTCV-105	High	Shorter and slender pseudostem; compact bunch with a greater number of short fingers
GCTCV-119	High	Very tall; wavy leaves; short fruit stalk; long growth cycle; fewer hands but large fingers; sweeter fruit
GCTCV-201	Moderate	Robust pseudostem; short fruit stalk; malformed hands
GCTCV-215	High	Tall and slender pseudostem; leaf tip curl and splitting; fewer suckers; normal bunch but slender fingers; long growth cycle
GCTCV-216	High	Very tall; very large and heavy bunch; long growth cycle
GCTCV-217	High	Erect leaves; compact, but heavy bunch; less curved fingers

[a] High is <10% average disease incidence; moderate is 11 to 30% average disease incidence. Disease incidences were determined 1 year after planting.

Cavendish somaclons with resistance levels to Foc TR4, produced by TBRI, promoted by Bioversity-Philippines

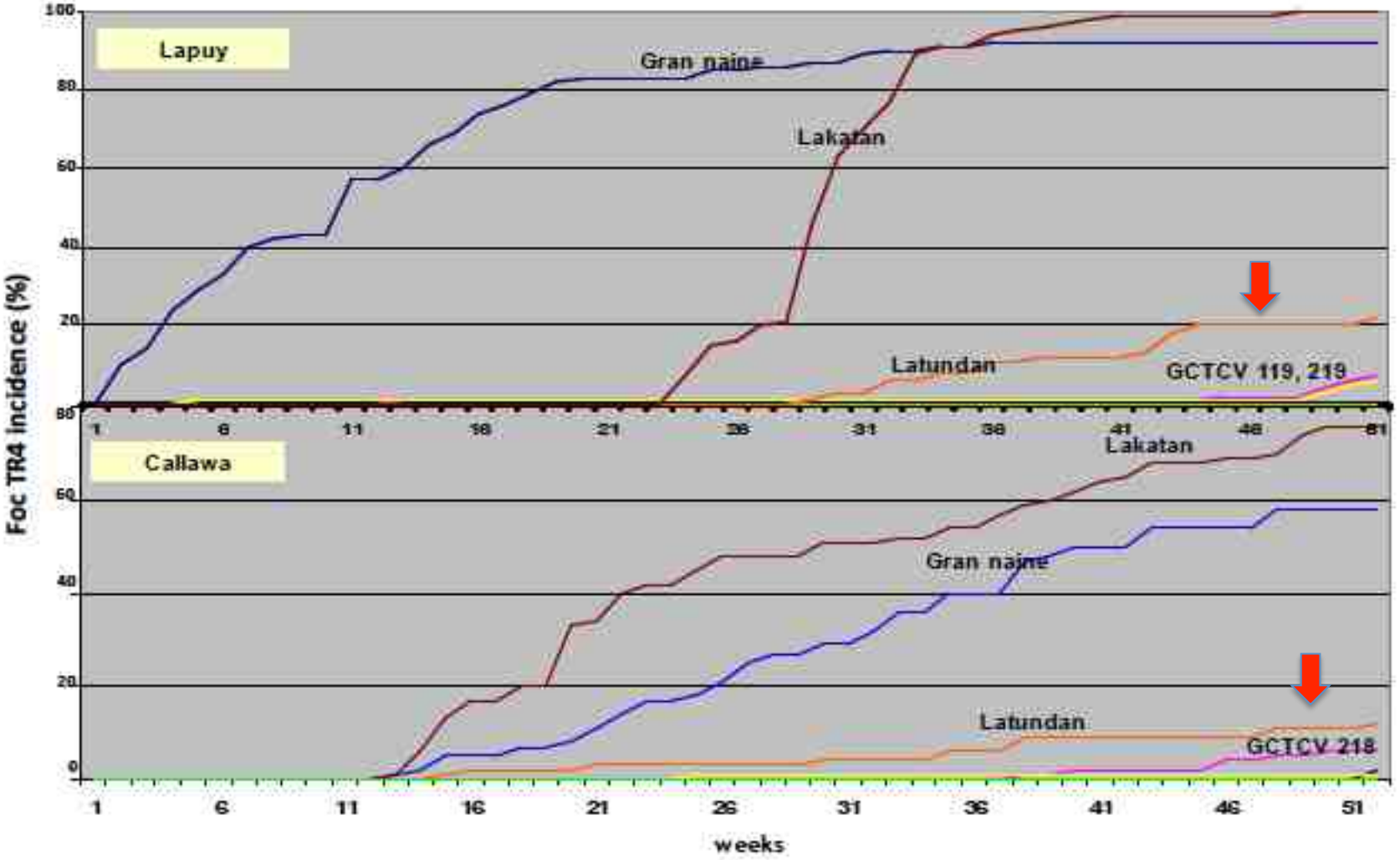


Figure 3. Fusarium incidence of different banana cultivars planted in Lapuy and Callawa, Davao City in 2009 - 2012)

Molina et al 2013

Cavendish somaclons with resistance levels to Foc TR4, produced by TBRI, promoted by Bioversity-Philippines

"No Cavendish types were identified as resistant, although GCTV119 from Taiwan and CJ19 from Indonesia show better survival than other Cavendish types; however, still not enough to be commercially viable (Walduck & Daly 2007)

http://www.nt.gov.au/d/Primary_Industry/Content/File/horticulture/TAR2006_07/Banana_panama_pg11_15.pdf

Plant Pathology (2012) Doi: 10.1111/j.1365-3059.2012.02620.x

Fusarium wilt-resistant lines of Brazil banana (*Musa* spp., AAA) obtained by EMS-induced mutation in a micro-cross-section cultural system

Y. F. Chen, W. Chen, X. Huang, X. Hu, J. T. Zhao, Q. Gong, X. J. Li and X. L. Huang*

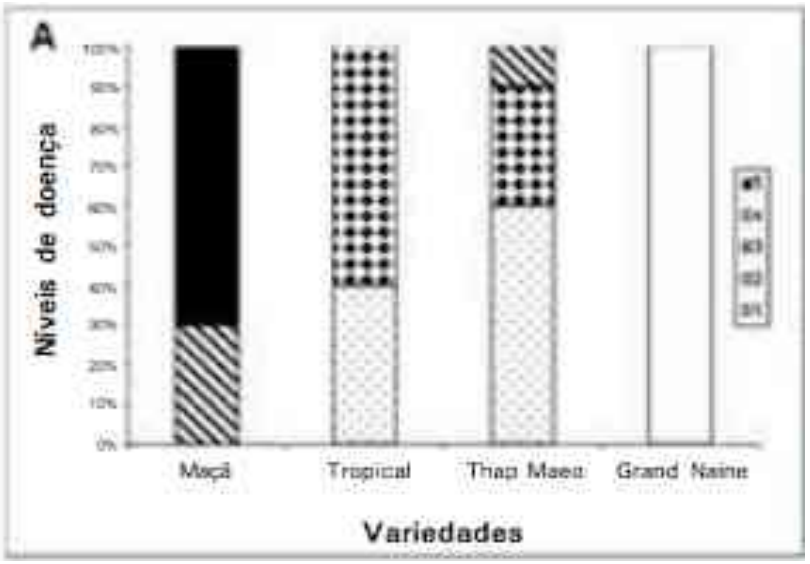
The Key Laboratory of Gene Engineering of the Ministry of Education, School of Life Sciences, Zhongshan (Sun Yat-sen) University, Guangzhou 510275, China

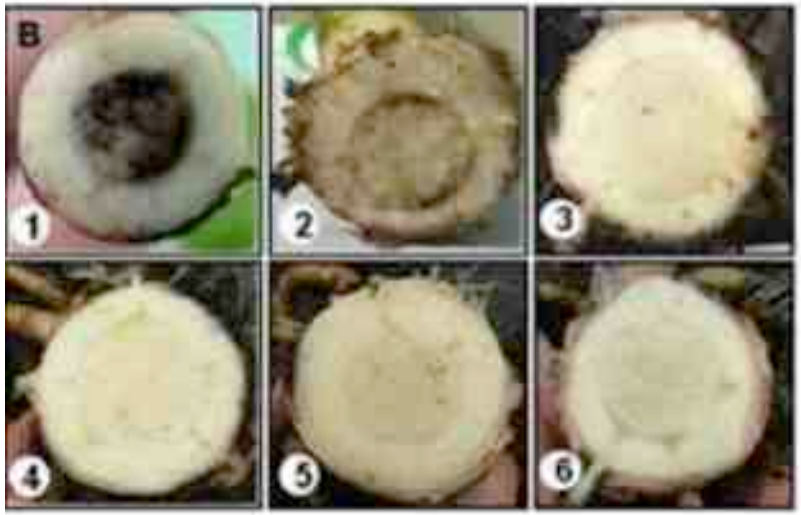
5 of 6 líneas resistentes en invernadero fueron resistentes en campo

Phenotyping for TR4 resistance- high-throughput system

Fast but reliable!

Screening for Fusarium wilt resistance





unicado 150
Técnico
ISSN 1809-502X
Cruz das Almas, BA
Dezembro, 2011

Metodologia para a caracterização de genótipos de bananeira quanto à resistência ao mal-do-Panamá em casa-de-vegetação

Miguel Angel Dita Rodriguez[1]
Lindineia Ribeiro[2]
Edson Perito Amorim[1]
Zilton José Maciel Cordeiro[1]
Sebastião de Oliveira e Silva[2]

Embrapa

Phenotyping for TR4 resistance- high-throughput system

Genotypes vs. Foc TR4

1. Grande Naine (AAA)
2. Gros Michel (AAA)
3. Maçã (Silk) – AAB
4. Prata (AAB)
5. *CIRAD 930 (AA) -*M. a. malaccensis*
6. **Pahang (AA) - M. a. malaccensis*
7. Matavia (Bluggoe) – ABB
8. Banksii (AA)
9. Tuu Gia (AA)

© Miguel Dita

* Parcial resistance

Evaluation of East African Highland Banana (AAA) and Plantain (AAB) genotypes in Asia for Foc TR4 resistance

ITC code	Cultivar	Genome	Subgroup
ITC0081	Igitsiri (Intuntu)	AAA	EAHB
ITC0179	Inkira	AAA	EAHB
ITC0084	Mbwazirume	AAA	EAHB
ITC0166	Ingagara	AAA	EAHB
ITC1354	Enzirabahima	AAA	EAHB
ITC1355	Kazirakwe	AAA	EAHB
ITC1465	Ibwi	AAA	EAHB
ITC0215	Mbi Egome 1	AAB	Plantain - French
ITC0217	Akpakpak	AAB	Plantain - French
	Obubit Ntanga		
ITC0519	green mutant	AAB	Plantain - French
ITC0121	Ihitisim	AAB	Plantain - Horne
ITC0208	Atali Kiogo	AAB	Plantain - False Horne
ITC1165	Curare	AAB	Plantain - False Horne
ITC1325	Orishele	AAB	Plantain - False Horne
	Williams (Bell, South		
ITC0570	Johnstone)	AAA	Cavendish
	GCTCV-119	AAA	Cavendish SC variant
	Grand Nain	AAA	Cavendish
	Lakatan	AAA	(dessert type)
	Latundan	AAB	Silk
	Cardaba	ABB/BBB	Saba
	Baxi	AAA	Cavendish
	Guangfen No.1	ABB	Pisang Awak

Bioversity International – China, Filipinas

Banana Varieties resistant to Race 1 and 2
Embrapa's breeding program

Caprichosa
Type: Prata

YS : RESISTANT
BLS: RESISTANT
FW: RESISTANT

Garantida
Type : Prata

YS : RESISTANT
BLS: RESISTANT
FW: RESISTANT

Tropical
Type- Silk

YS : RESISTANT
BLS: SUSCEPTIBLE
FW: RESISTANT

Princesa
Type : Silk

YS : RESISTANT
BLS: SUSCEPTIBLE
FW: RESISTANT

We don't know yet their reaction for TR4

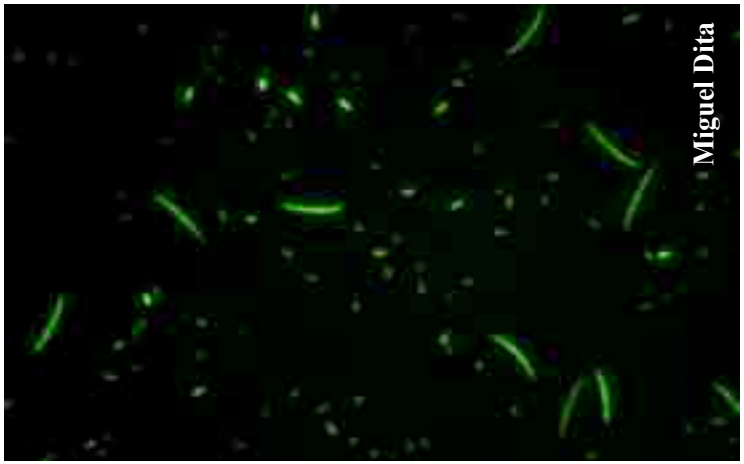
Banana Varieties resistant to Race 1 and 2
Embrapa's breeding program

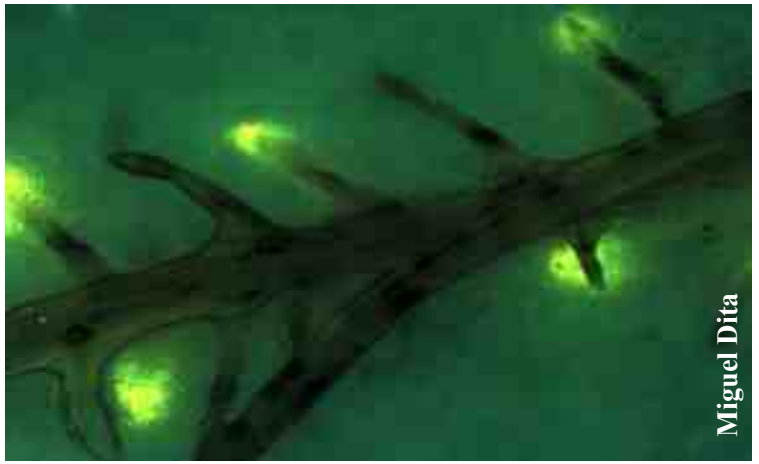
We don't know yet the reaction for TR4

More information with Dr. E. Amorim
edson.amorim@embrapa.br

Understanding genetic and molecular basis of resistance

Comparative transcriptome analyses of compatible and incompatible Musa- FOC interactions





Dita et al. 2010

X

Immune

Partial resistant

Susceptible

454- seq

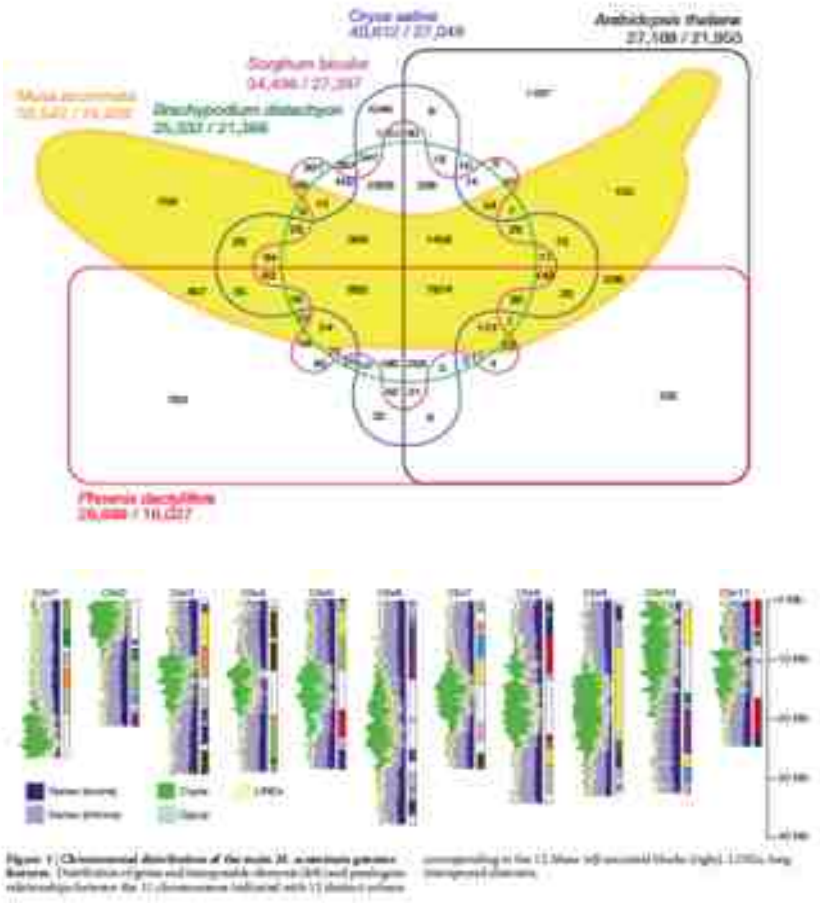
EST data

Some old questions:

- When and where is Foc stopped in Cavendish during Race 1 infection?
- Which gene(s) are involved? At what level?
- Which mechanisms are responsible for resistance (effective >60 years)

Banana genome vs. Foc genome: 1st Insights

Banana genome



D'Hont et al. Nature 488, 2012)

Foc TR4 In vitro

Berg et al. 2012
Li-Jun Ma Team

BLAST Match Characteristics	Number of Proteins	Number of Predicted Secreted Proteins	Number of Predicted Secreted Proteins < 200 AA
Conserved	15238	1526	294
Lineage Specific (to Fo4287)	140	7	2
Unique to TR4	451	40	12

Foc TR4 and R1 in planta

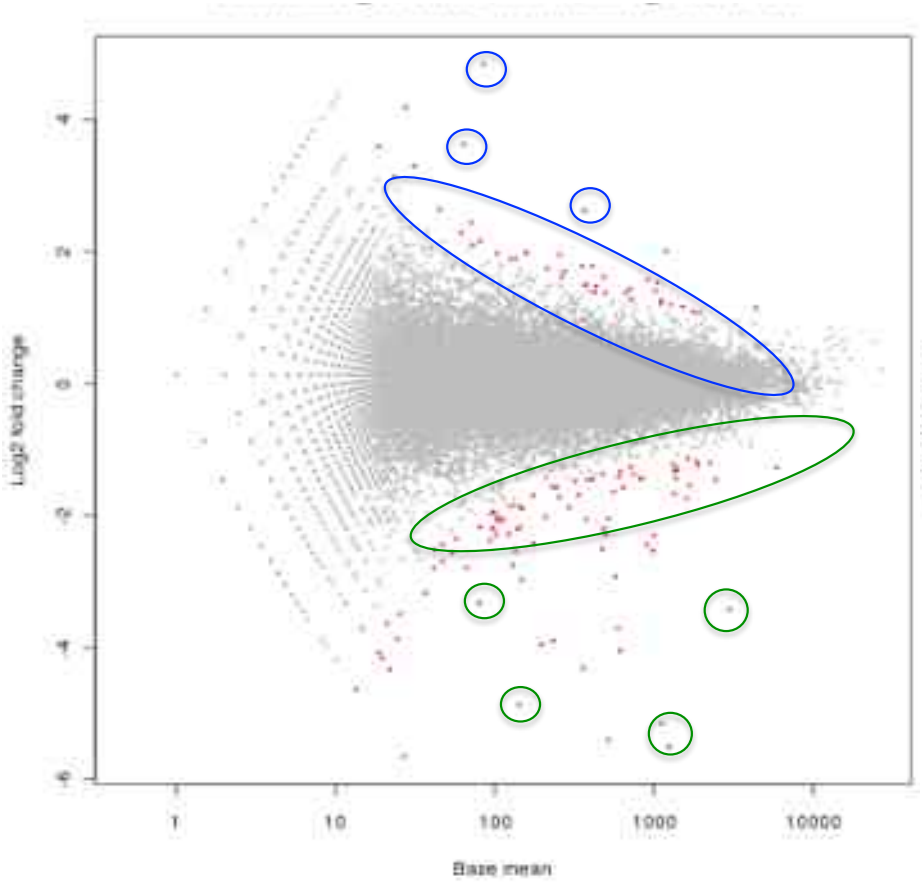
Cellular localization	Gran Naine- TR4	Pahang-TR4
cytoskeleton	198	54
cytosol/mitochondria	26	10
endoplasmic reticulum	6	0
extracellular	**858**	**177**
golgi apparatus	3	1
mitochondria	1332	487
mitochondria/nucleus	9	2
nucleus	2747	672
peroxisome	24	2
plasma membrane	1223	264
Total	8397	2290

Aligned against *Foc (draft)* -software TopHat [Dita un published]

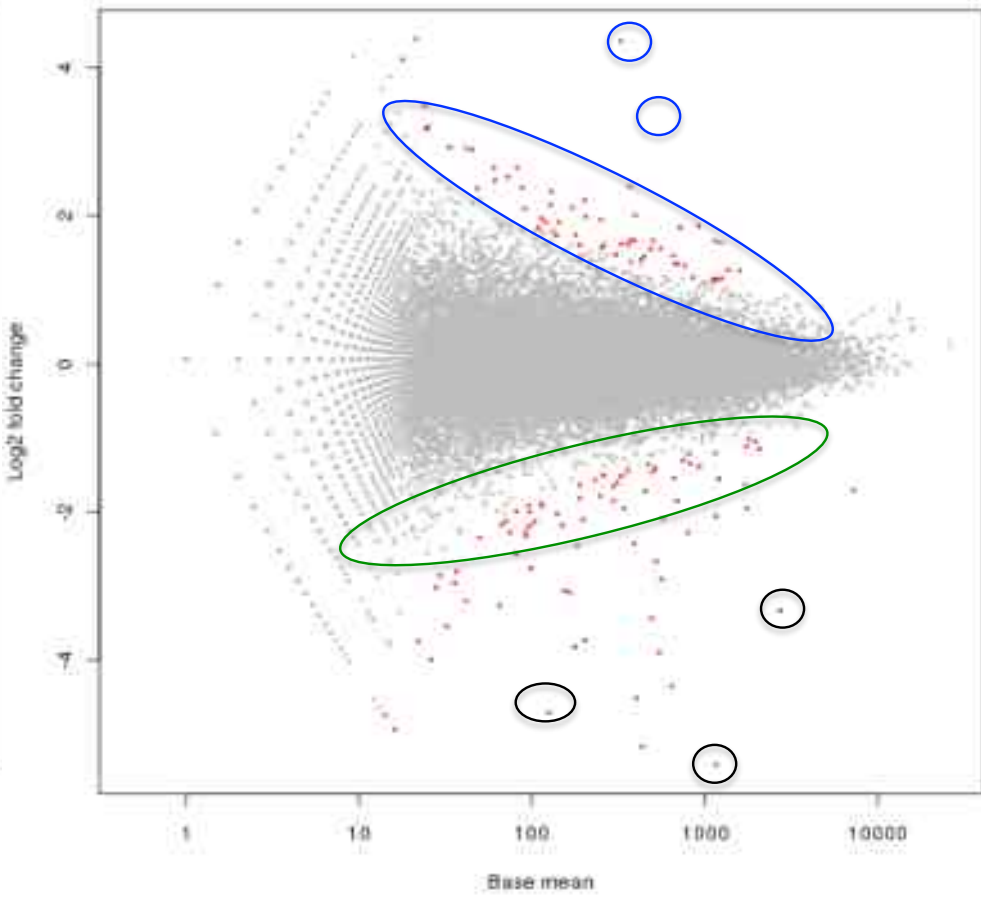
Comparative transcriptome analyses of compatible and incompatible Musa- FOC interactions

Musa Genes

G. Naine **Control** vs. G. Naine **Foc R1**



Gran Naine **Control** vs. Gran Naine **Foc TR4**



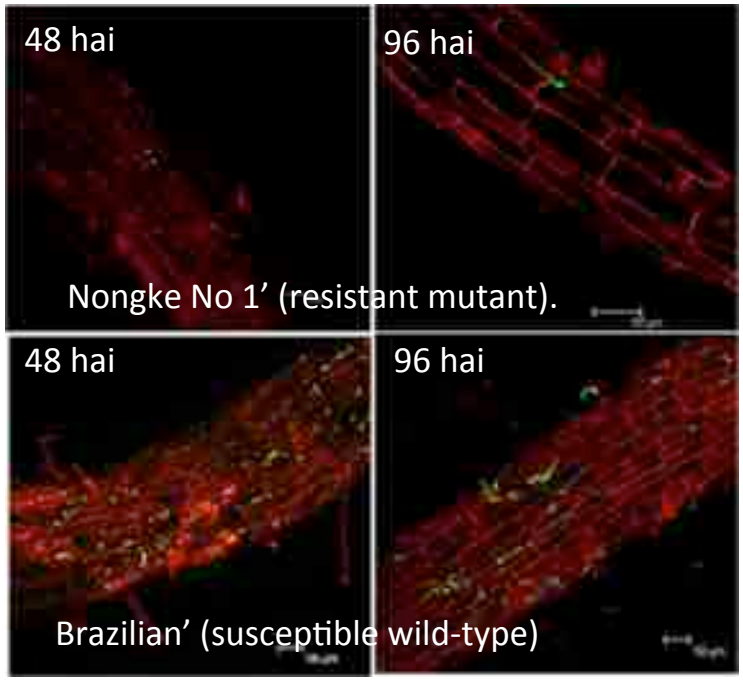
Li et al. BMC Genomics 2012, 13:374
http://www.biomedcentral.com/1471-2164/13/374

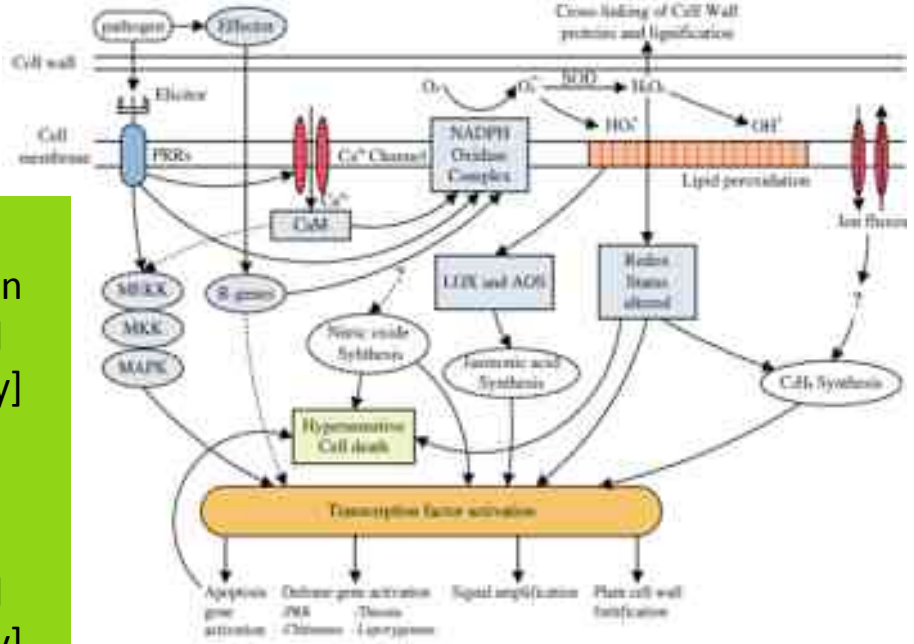
BMC Genomics

RESEARCH ARTICLE Open Access

Transcriptome profiling of resistant and susceptible Cavendish banana roots following inoculation with *Fusarium oxysporum* f. sp. *cubense* tropical race 4

Chun-yu Li[1,2†], Gui-ming Deng[3†], Jing Yang[3†], Altus Viljoen[4], Yan Jin[1,2], Rui-bin Kuang[1,2], Cun-wu Zuo[3], Zhi-cheng Lv[3], Qiao-song Yang[1,2], Ou Sheng[1,2], Yue-rong Wei[1,2], Chun-hua Hu[1,2], Tao Dong[1,2] and Gan-jun Yi[1,2*]





PTI [pathogen triggered immunity]

ETI [Effector triggered immunity]

Comparative proteomic analyses: Musa- FOC interactions

Li et al. Proteome Science 2013, 11:41
http://www.proteomesci.com/content/11/1/41

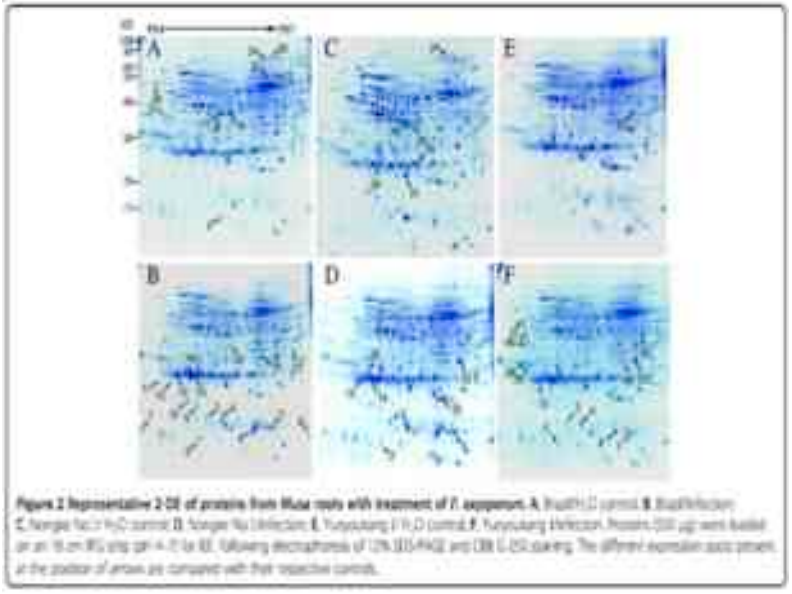
PROTEOME SCIENCE

RESEARCH Open Access

Proteomic analysis of *Fusarium oxysporum* f. sp. *cubense* tropical race 4-inoculated response to Fusarium wilts in the banana root cells

Xingshen Li[1,2], Tingting Bai[1,2], Yunfeng Li[2], Xiaolei Ruan[1,2] and Huaping Li[1,2*]

Figure 1 Disease symptoms from Musa roots with treatment of *F. oxysporum*. A Banana xoco/H_2O control, and B Banana xoco/Infection (susceptible Brazil, moderately resistant Nongke No.1 and highly resistant Yueyoukang I were treated at 30 after *F. oxysporum* inoculation, compared with water-treated control.



Comparative proteomic analyses: Musa- FOC interactions

Li et al. Proteome Science 2013, 11:41
http://www.proteomesci.com/content/11/1/41

RESEARCH Open Access

Proteomic analysis of *Fusarium oxysporum* f. sp. *cubense* tropical race 4-inoculated response to Fusarium wilts in the banana root cells

Xingshen Li[1,2], Tingting Bai[1,2], Yunfeng Li[2], Xiaolei Ruan[1,2] and Huaping Li[1,2*]

Conclusions:

This report is the first to use proteomic profiling to study the molecular mechanism of banana roots infected with Foc4.

The differentially regulated proteins involved in different defense pathways are likely associated with different resistant levels of the three banana cultivars.

Planta (2014) 239:735–751
DOI 10.1007/s00425-013-2024-8

REVIEW

Plant defense response against *Fusarium oxysporum* and strategies to develop tolerant genotypes in banana

V. Swarupa · K. V. Ravishankar · A. Rekha

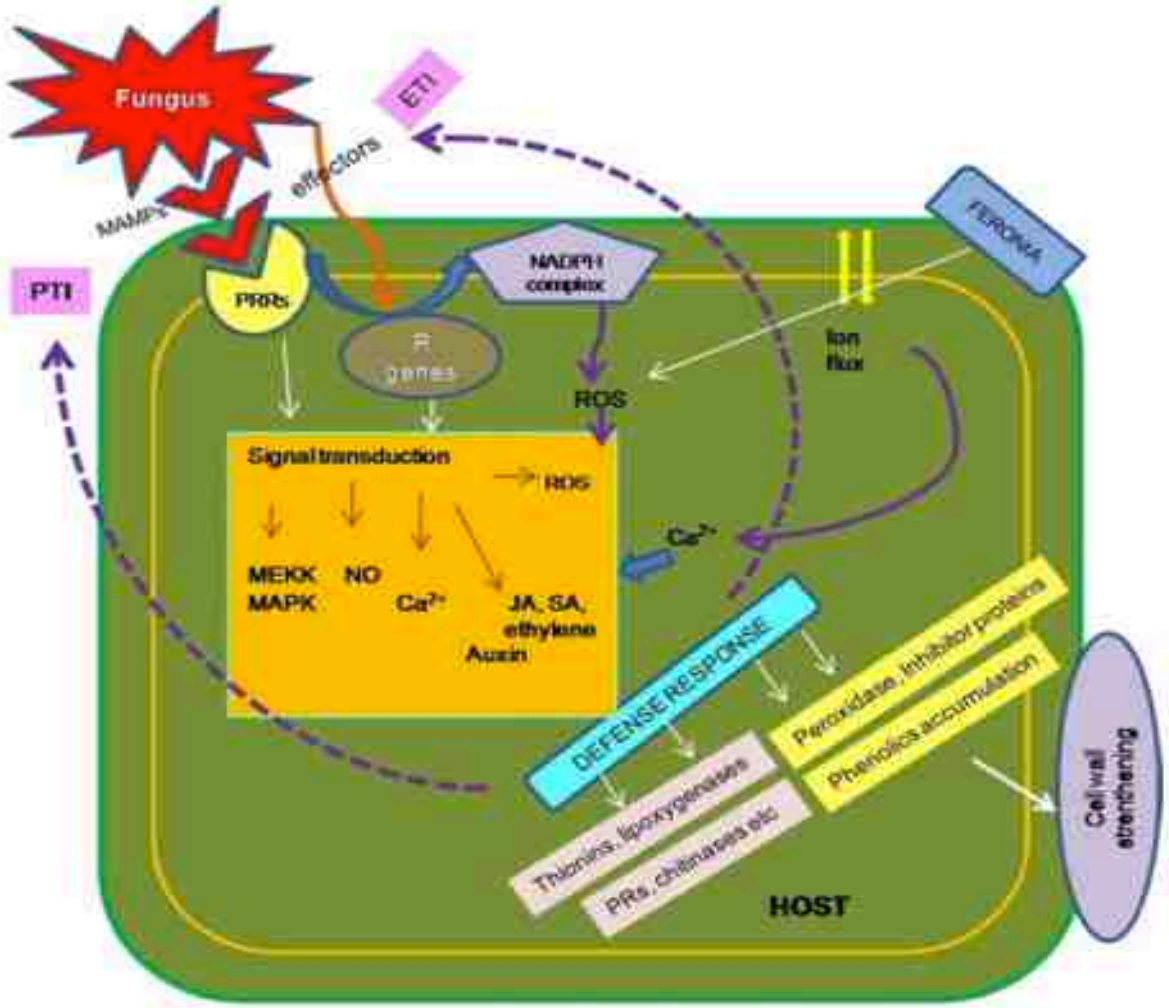


Fig. 1 Recognition of the pathogen and elicitation of defense response by the host. When the pathogen attacks the host through their typical molecular signatures called microbe-associated molecular patterns (MAMPs) and/or effectors, they are recognized by the host through specific pathogen recognition receptors (PRRs) and R-genes leading to host defense response via the activation of multiple signal transduction pathways such as salicylic acid (SA), jasmonic acid (JA) and ethylene. Pathogen recognition results in change in membrane potential leading to activation of NADPH complex that results in production of reactive oxygen species (ROS), which acts as major signaling molecules and in turn, may induce other signaling molecules. Change in ion flux also induces Ca^{2+} signaling. Defense responses against MAMPs are called pathogen-triggered immunity (PTI) and against effectors are called effector-triggered immunity (ETI)

Is Transgenic an alternative ? What do we know so far?

Journal of Integrative Plant Biology
Formerly *Acta Botanica Sinica* 2005, 47 (8): 971–977
http://www.blackwell-synergy.com
http://www.chineseplantscience.com

Creation of Transgenic Bananas Expressing Human Lysozyme Gene for Panama Wilt Resistance

Xin-Wu PEI[1], Shi-Kai CHEN[2], Rui-Ming WEN[2], Shang YE[2], Jia-Qin HUANG[2], Yong-Qiang ZHANG[1], Bing-Shan WANG[1], Zhi-Xing WANG[1] and Shi-Rong JIA[1*]

(1. *Biotechnology Institute, Chinese Academy of Agricultural Sciences*, Beijing 100081, China;
2. *Xinyi Institute of Agricultural Science and Technology*, Xinyi, Guangdong 523530, China)

Plant Biotechnology Journal

Plant Biotechnology Journal (2011) 9, pp. 1141–1148 doi: 10.1111/j.1467-7652.2011.00639.x

Apoptosis-related genes confer resistance to Fusarium wilt in transgenic 'Lady Finger' bananas

Jean-Yves Paul[1], Douglas K. Becker[1], Martin B. Dickman[2], Robert M. Harding[1], Harjeet K. Khanna[1] and James L. Dale[1,*]

[1]*Centre for Tropical Crops and Biocommodities, Faculty of Science and Technology, Queensland University of Technology, Brisbane, Qld, Australia*
[2]*Institute for Plant Genomics and Biotechnology, Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, USA*

Is Transgenic an alternative ? What do we know so far?

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Transgenic banana cv. Rasthali (AAB, Silk gp) harboring *Ace-AMP1* gene imparts enhanced resistance to *Fusarium oxysporum* f.sp. *cubense* race 1

Sukhada Mohandas [a,*], H.D. Sowmya [a], A.K. Saxena [a], S. Meenakshi [a], R. Thilaka Rani [a], Riaz Mahmood [b]

[a] Indian Institute of Horticultural Research, Hessaraghatta, Bangalore 560 089, India
[b] Department of Studies and Research in Biotechnology & Bioinformatics, Kuvempu University, Shankaraghatta 577 451, India

Conclusion: Significantly higher AMP-protein content in transgenic lines was negatively correlated and higher root colonization was positively correlated with disease symptoms. **Six transgenic lines were selected for field evaluation.**

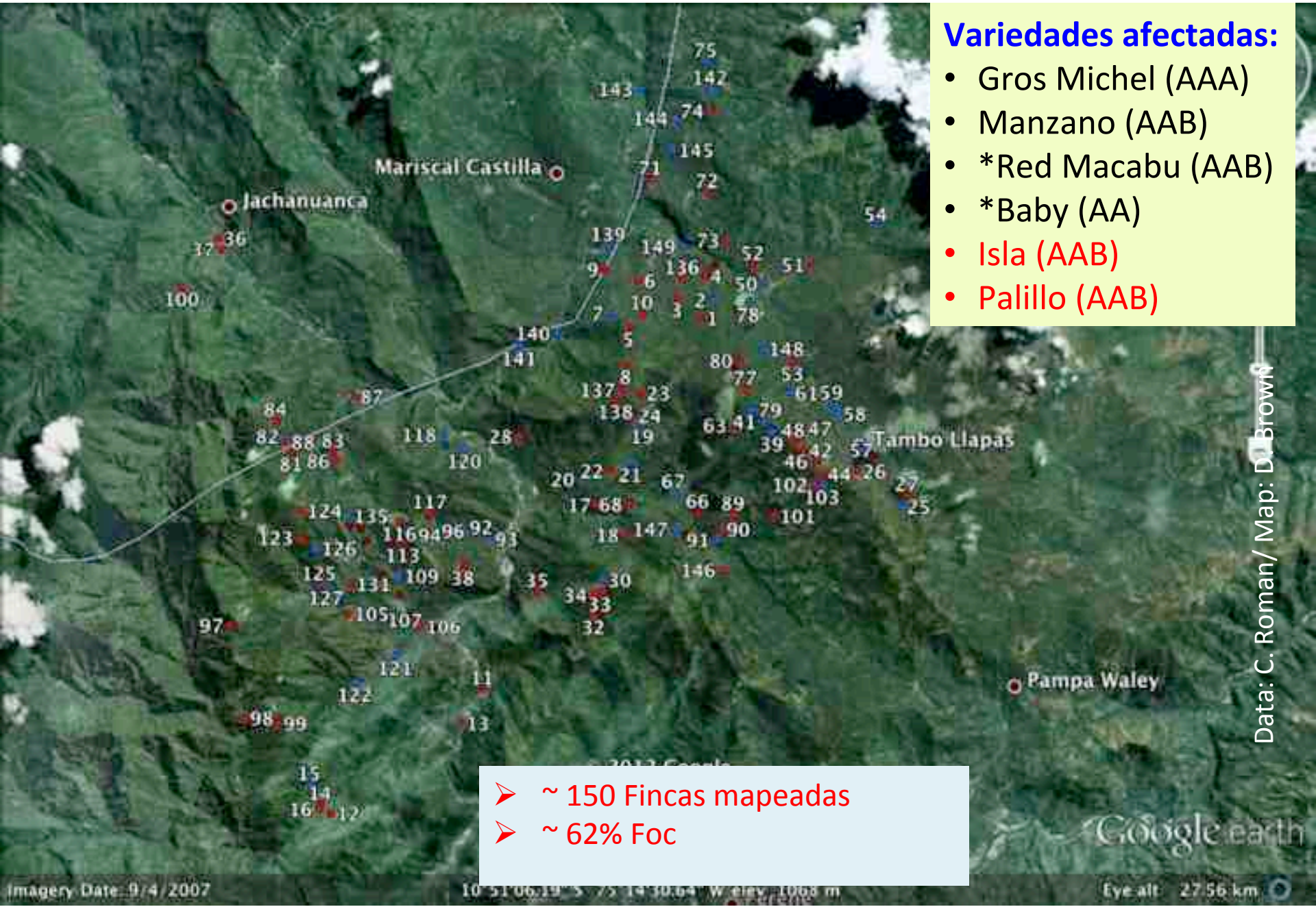
Fig. 6. Progress of disease symptoms caused by *Fusarium oxysporum* f.sp. *cubense* race 1 after root challenging of transformed banana plants with untransformed control. (a) External symptoms of transformed banana plants (plants – 6, 13, 14, 18 and 19) and control plants fully devastated on the left. (b) Vascular discoloration of transgenic plants ((c–e) Plants 19, 14 and 6) along with control treated with Foc.


© Miguel Dita
http://www.scientificamerican.com/article.cfm?id=find-the-dna-in-a-banana-bring-science-home

There are no resistant varieties, so what ?

Mapping and epidemiological studies of Foc in Peru

Mapping and epidemiological studies of Foc in Peru

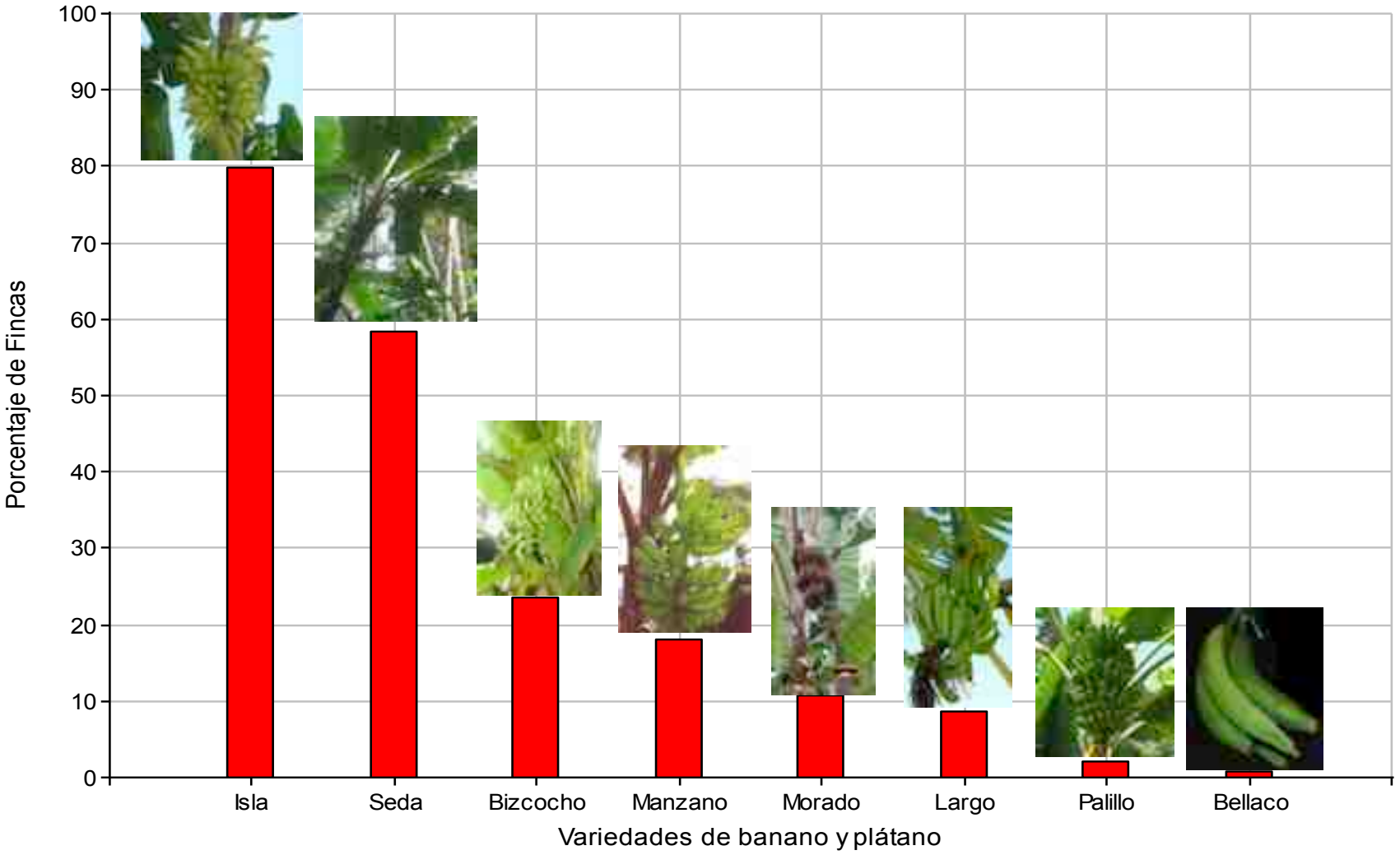


Variedades afectadas:

- Gros Michel (AAA)
- Manzano (AAB)
- *Red Macabu (AAB)
- *Baby (AA)
- Isla (AAB)
- Palillo (AAB)

- ~ 150 Fincas mapeadas
- ~ 62% Foc

Banana varieties in San Luis de Shuaro District, Peru



Use of bioinputs

Fences

Destronque

Prunning

Long spacement between plants

Elements of managemnt present on farm with absence or lower levels of Fusarium wilt incidence

CaOH

Coffe debris

Capacitation

Eliminate disese plants
Apply wood ash

Cut & apply CaOH

Association of some practices with Fusarium wilt intensity in Peru

(Roman, 2012)

Activity	Association with FOC
Introduction of planting material and soil from other farms	
Leave infected plants in the farm without isolation	
Presence of transit areas across the farm	
Remove roots and soil attached to suckers in the farm	
Discard infected plants in rivers or irrigation channels	
Presence of water flow towards the farm and poor drainage	
Presence of FOC in neighboring farms	
Tools are frequently sterilized	
Apply measures to avoid contaminated soils in the farm	
Selection of clean planting material	
Diversification of varieties	
Ca identify the disease correclty	
Use of natural barriers	
Use of warning systems on the entry points	
presence of fences	
Apply crop management strategies	
Organization and capacitation of workers	
Monitoring and surveillance for early detection	
Eliminate/isolate infected plants immediately	

High
Medium
Low

Cultural control

Journal of Phytopathology

J Phytopathol
© 2012 Blackwell Verlag GmbH
doi: 10.1111/jph.12005

Department of Plant Pathology, Viçosa Federal University, Brazil

Silicon Suppresses Fusarium Wilt Development in Banana Plants

Alessandro Antonio Fortunato[1], Fabrício Ávila Rodrigues[1], Júlio Cezar Parpaiola Baroni[1], Gustavo César Barbosa Soares[1], Miguel Angel Dita Rodriguez[2] and Olinto Liparini Pereira[1]

Authors' addresses: [1]Department of Plant Pathology, Viçosa| Federal University, Zip Code 36570-000, Viçosa, Minas Gerais, Brazil; [2]Embrapa Mandioca e Fruticultura, Zip Code 44380-000, Cruz das Almas, Bahia, Brazil (correspondence to F. A. Rodrigues. E-mail: fabricio@ufv.br)

Received April 25, 2012; accepted July 27, 2012

Table 5
Area under darkening of rhizome-pseudostem progress curve (AUDRPPC) and the area under relative lesion length progress curve (AURLLPC) for banana plants from the Grand Nain and Maçã cultivars grown in soil amended with silicon (+Si) or without silicon (−Si) and inoculated with *Fusarium oxysporum* f. sp. *cubense*.

	AUDRPPC		AURLLPC	
Treatments	Grand Nain	Maçã	Grand Nain	Maçã
−Si	114.29 Aa	110.86 Aa	367.96 Ba	606.92 Aa
+Si	117.29 Aa	93.14 Bb	322.05 Aa	354.79 Ab
C.V. (%)	8.87		15.13	

C.V. = coefficient of variation.
Means within each column followed by the same lowercase letter or means within each row followed by the same uppercase letter are not significantly different (P = 0.05) as determined by Tukey's test.

Cultural control

Chinese Leek (*Allium tuberosum*) suppress Foc

Dr. Yi Ganjun in the 7th BAPNET SC Meeting, Hanoi, Vietnam

Chinese leeks have properties that destroy the cell structure of Fusarium wilt rendering the virus inert and incapable of infecting banana plants.

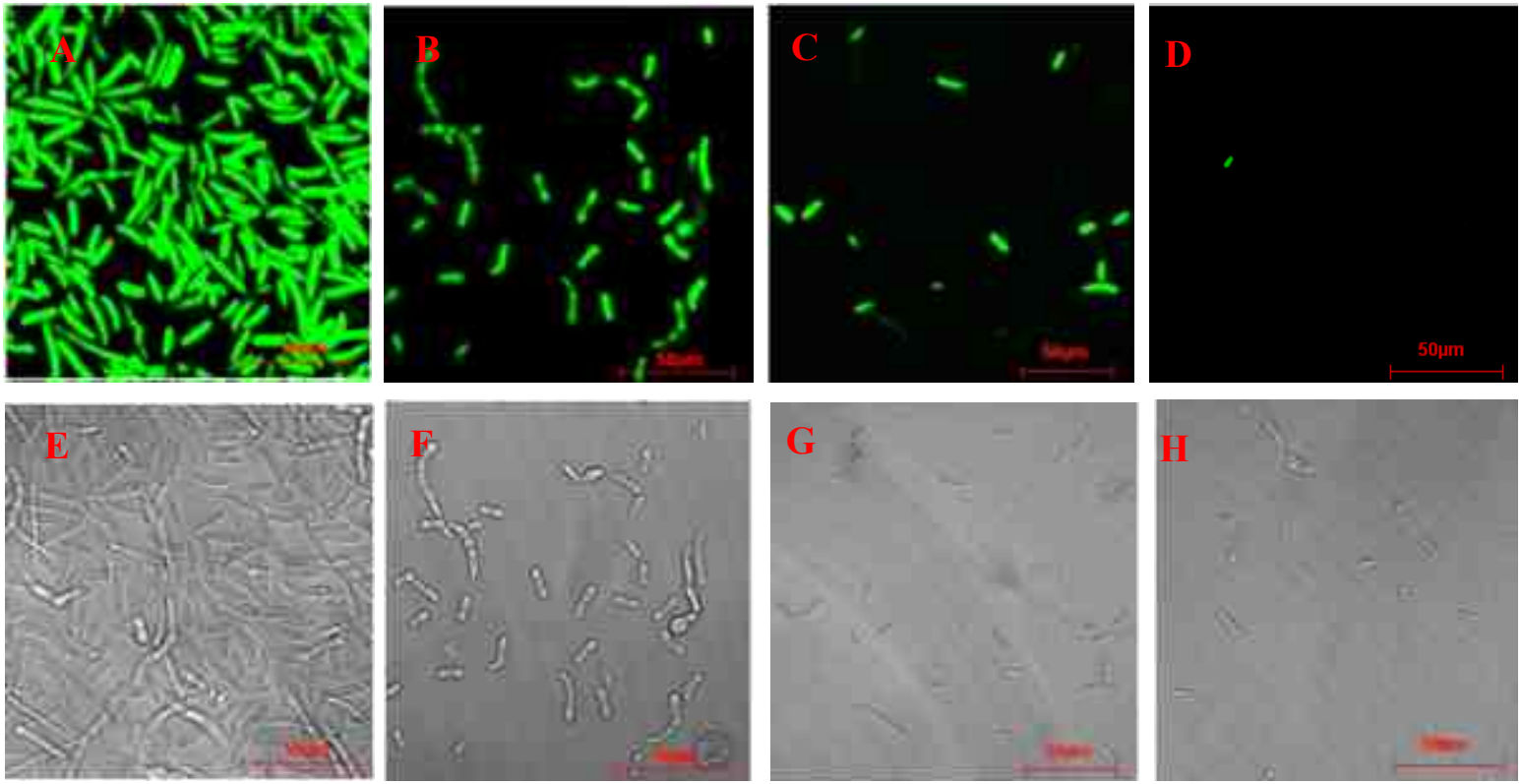
Plots planted to Chinese leeks (Photo by Yi Ganjun).

Eur J Plant Pathol
DOI 10.1007/s10658-012-0024-3

Cultural control

Control of Fusarium wilt in banana with Chinese leek

Y. H. Huang · R. C. Wang · C. H. Li · C. W. Zuo ·
Y. R. Wei · L. Zhang · G. J. Yi



Dr. Yi, Gan-Jun – Bapnet meeting 2012

Cultural control

Allium tuberosum vs. Foc

Some considerations for LAC

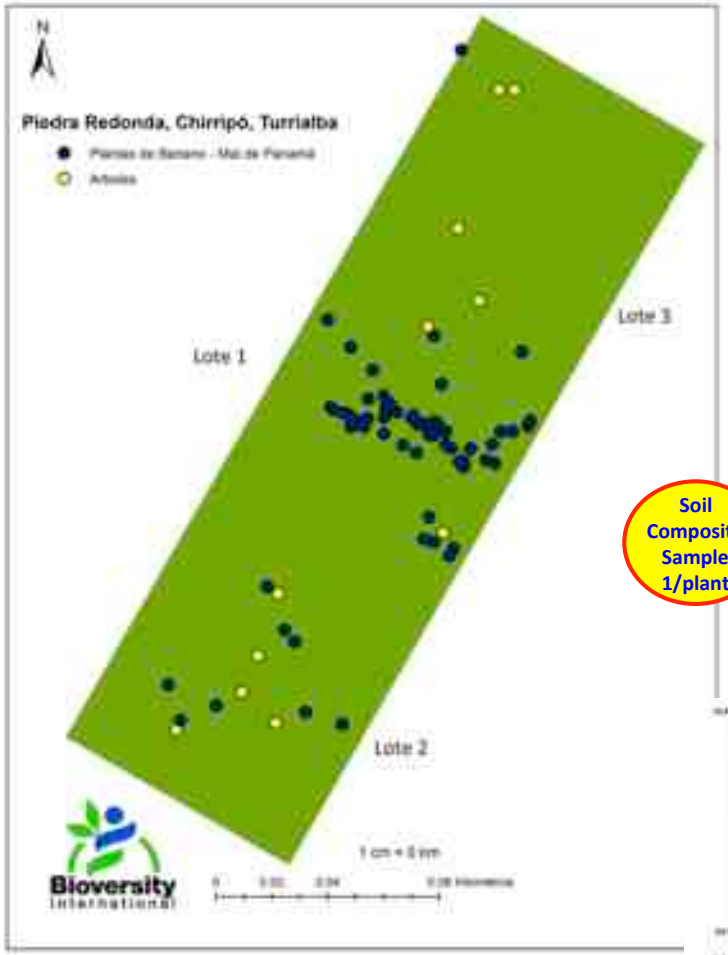
Photo by Yi Ganjun

Vs

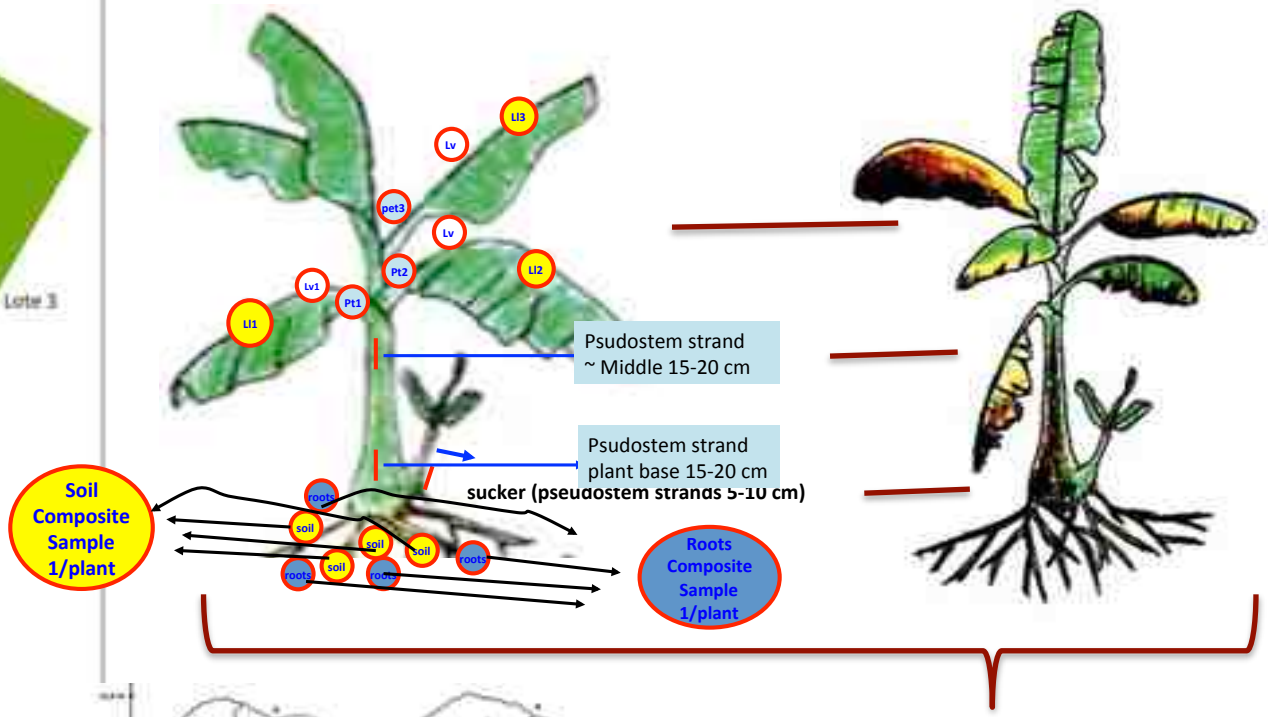
Photo by R. Ploetz

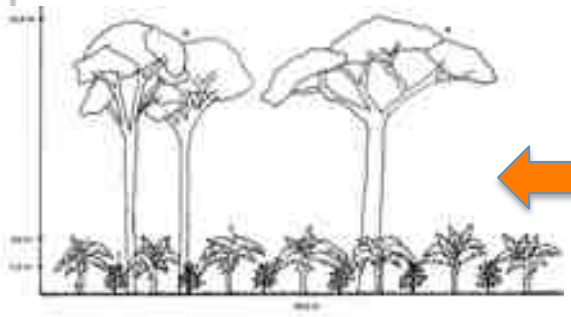
- Is this specie adapted to LAC?
- What is the practicity for bid banana plantations of monocrop – and for agroforestry systems?

Biocontrol in Foc needs to be associated to soil heath and suppressive soils need a better understanding



Farm 1: Alto Varas, Turrialba, Costa Rica
Watershade, Turrialba river





Landscapes characterization

454 sequencing

Metagenomics analysis
Fuctional diversity

Integrated approach for disease management and enhancing productivity in Foc-susceptible bananas

TC plants
+
target endophytes

Use of Beneficial Microbial Diversity Multifunctional isolates

"Biotized"/bio-fortified healthy plants acclimatized – Plant fitness increased!

Use of tailored fertilization based on organic amendments (Biochar, manures, compost) + beneficial soil microbes (Trichoderma & CIA)

Healthy plants are established on a healthy soil

Soil -health oriented practices and crop Integrated management

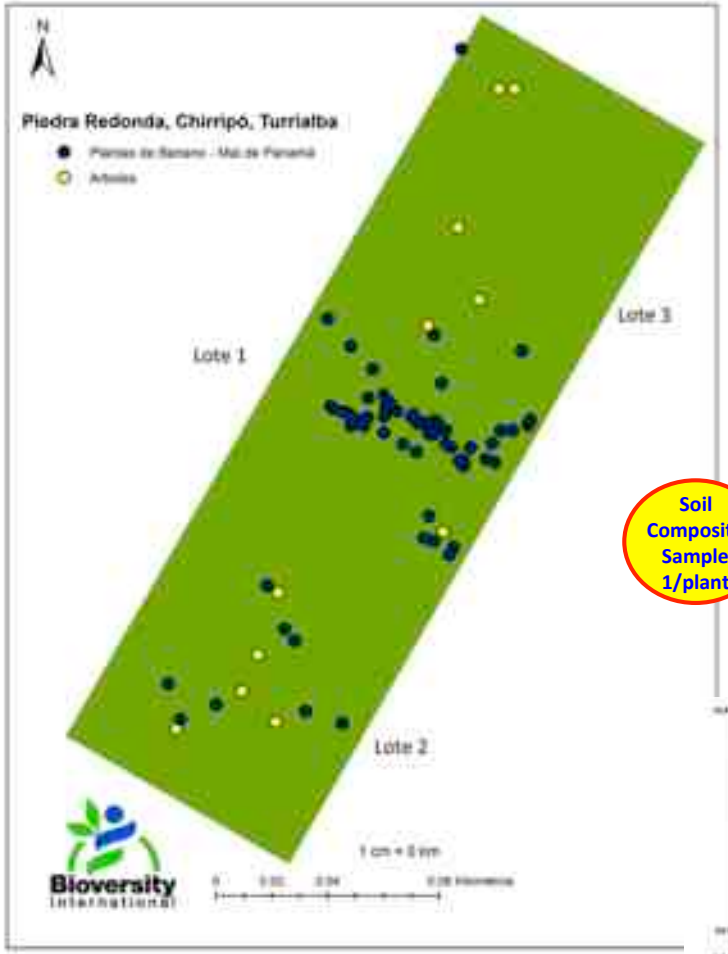
Sustainable production of banana from High value, but Foc- susceptible varieties in Market

Plant health through soil health management: boosting soils for increasing plant defenses and suppressiveness to reduce losses caused by Fusarium wilt on bananas

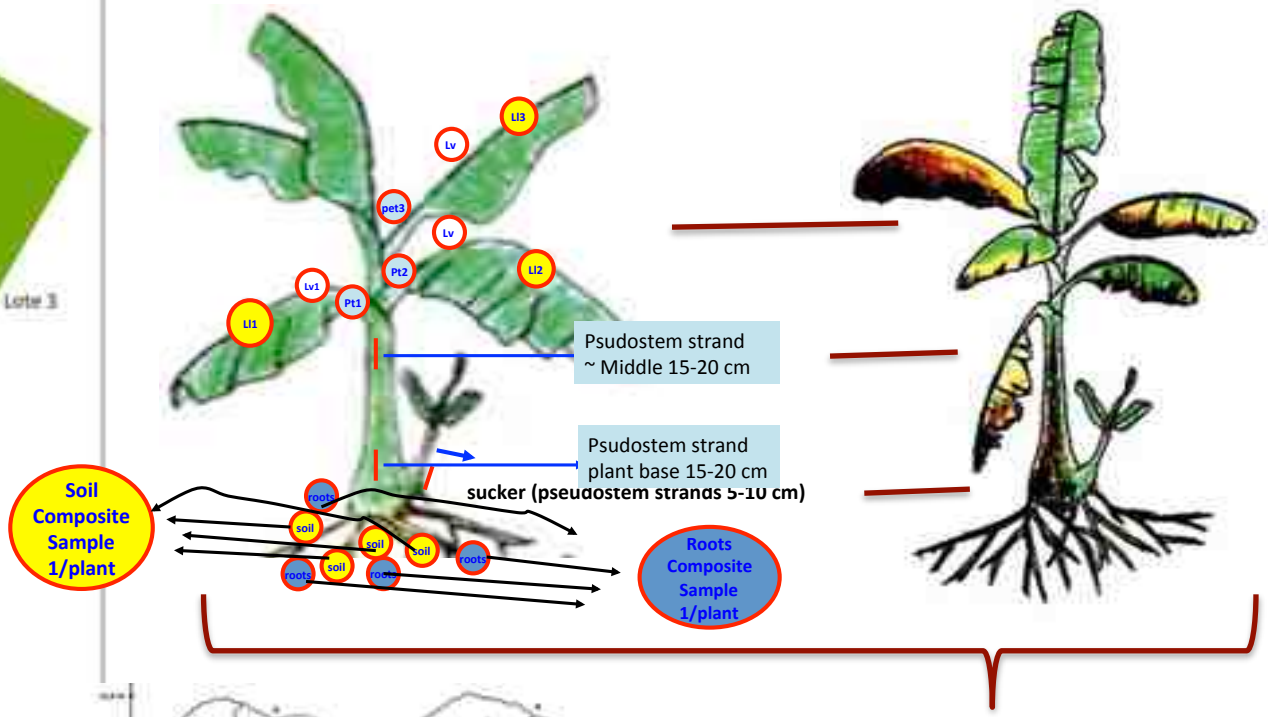
Generating a toolbox of practices based on agrobiodiveristy for sustainable management of Foc in banana

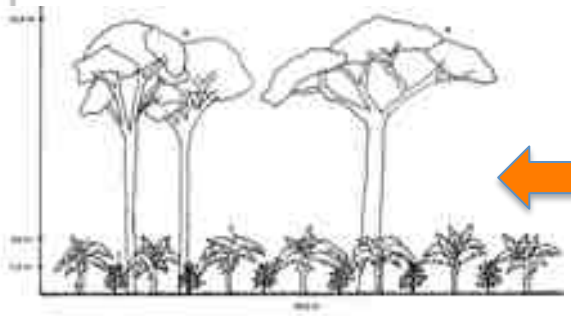
Understanding soil suppressiveness in Foc-banana

- 2 countries [Nicaragua & Costa Rica]
- 3 farms/ country, 3 areas/farm/ 5 plants/ area/



Farm 1: Alto Varas, Turrialba, Costa Rica
Watershade, Turrialba river



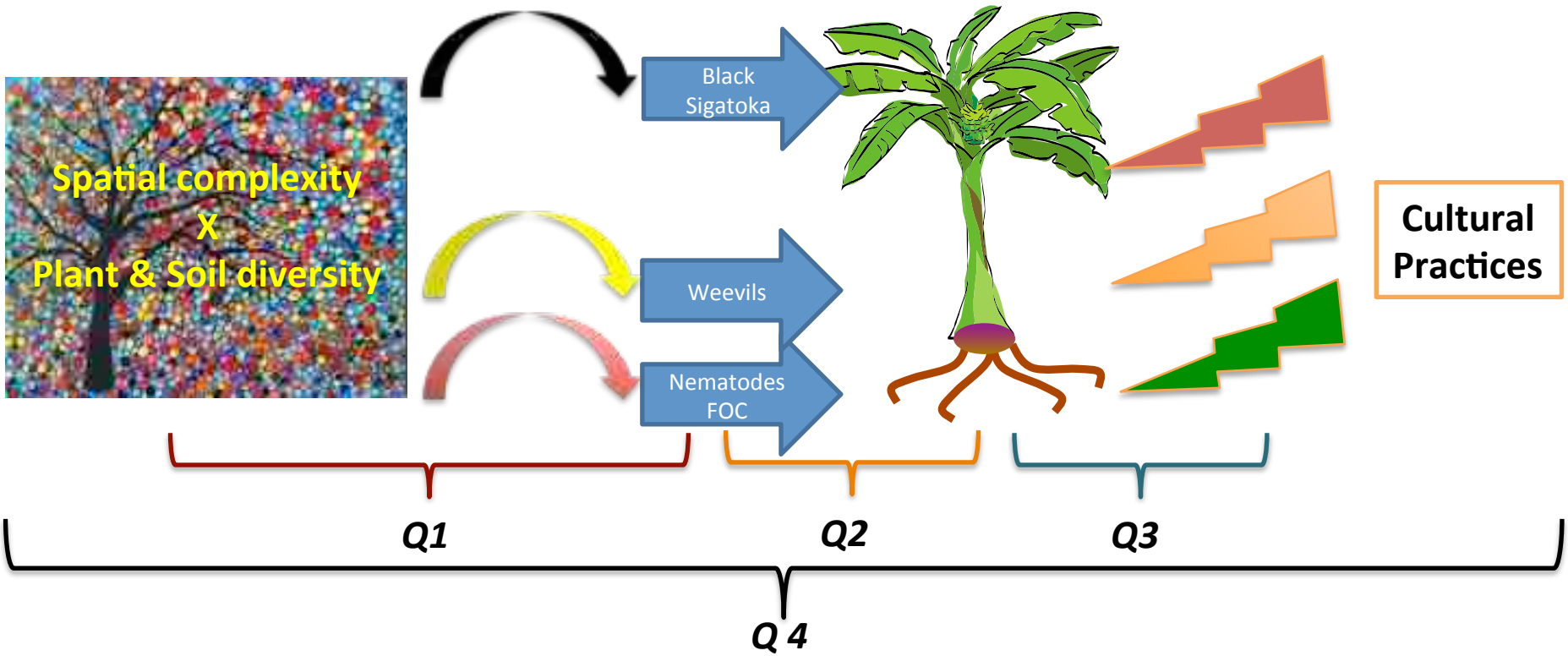


Landscapes characterization

454 sequencing

Metagenomics analysis
Fuctional diversity

Agrobiodiversity and management factors vs. Foc & overall pests

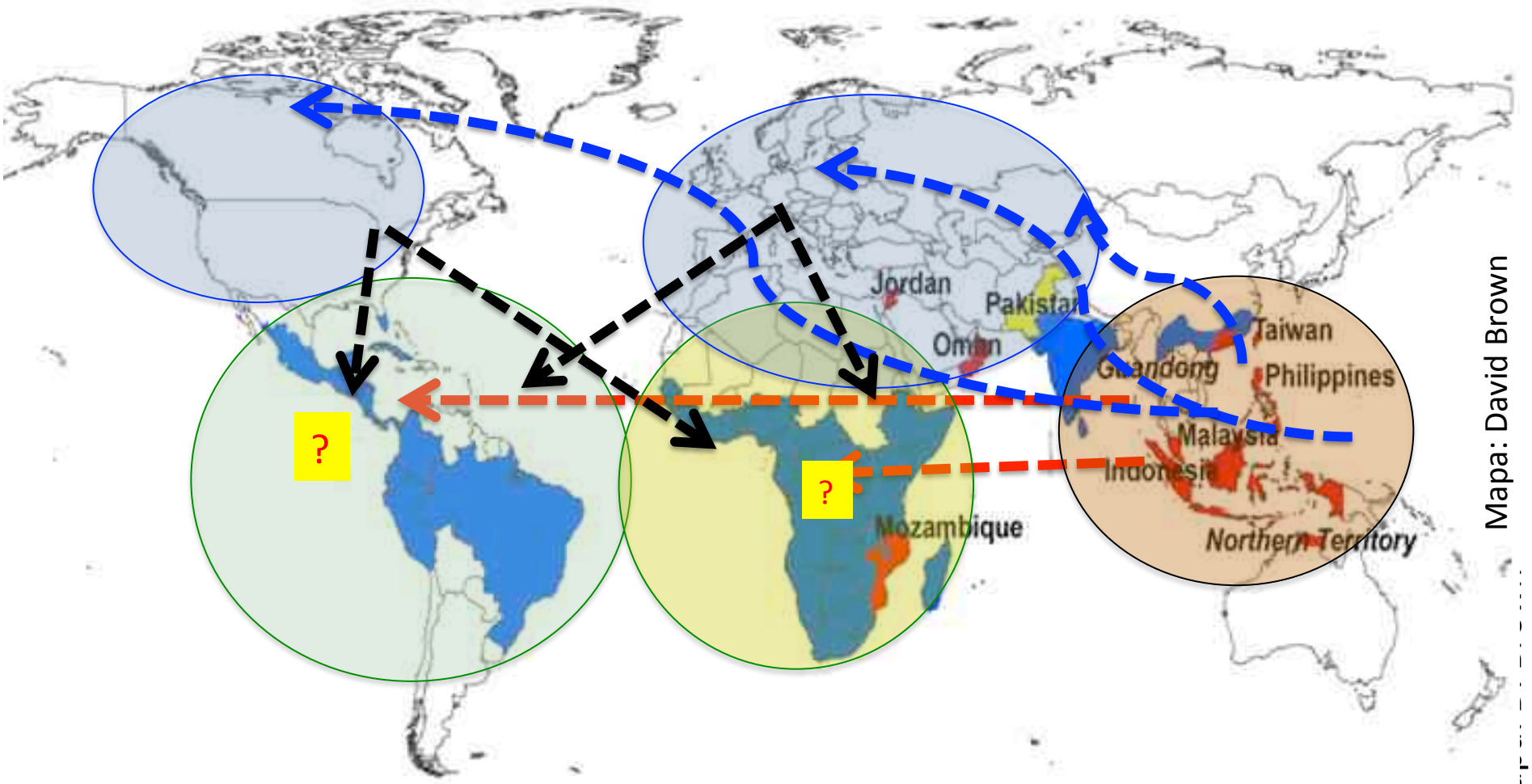


Q 1. How plant and soil diversity + spatial organization alter pests abundances?
Q 2. How a profile of pests alter plant growth?
Q 3. Which plant management practices should minimize pests effects on yield?
Q 4. How to manage spatio-temporal factors and functional biodiversity to guarantee ecosystem services and resilience without relevant yield penalties?

Adapted from P. Tixier, 2013

Fusarium wilt of banana: Global distribution

Possible scenarios for global spreading



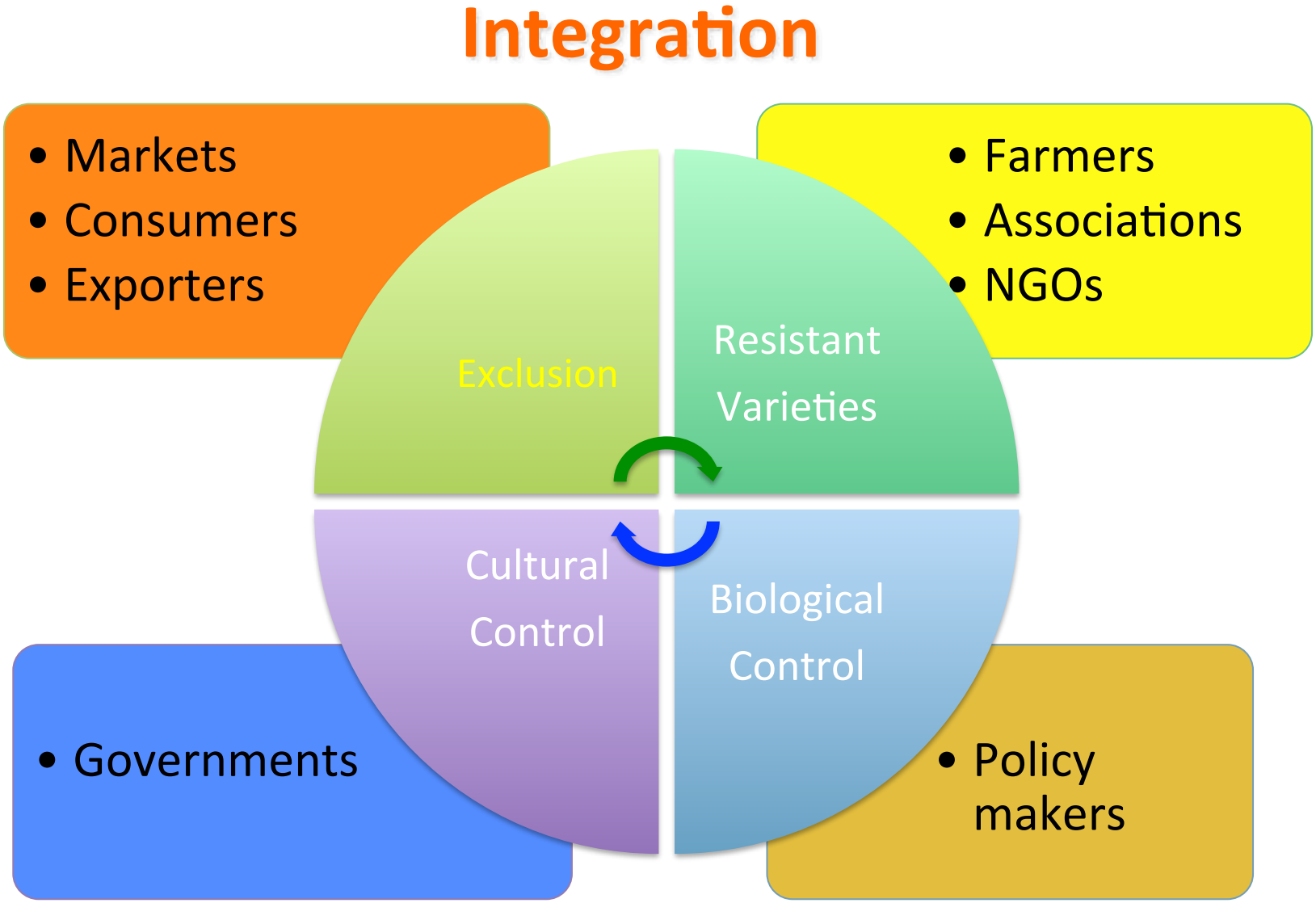
Races 1, 2

Tropical race 4, 1, 2

Under evaluation for Tropical race 4

This MAP is only a tentative approximation of the distribution of Foc to be used for academic purposes. It is not based on any scientific studies and should not be used by authorities for decision making purposes.

What would be the best scenario to fight agaisnt TR4 or any other high impact pest?



Take home message

Adapted from Staver 2011

Photo –ENDURE Guadalupe

Landscape and watershed

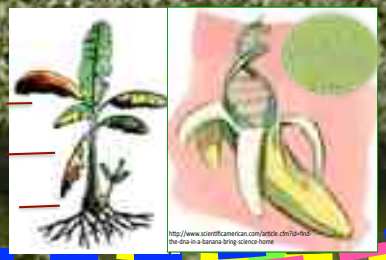
Local landscape & Multi-Farm

Farm ecosystem

Plantation ecosystem

Banana Plantation

Banana plant + Fo(DNA)



Collaborators

BIOVERSITY INTERNATIONAL
- Gus Molina

Cuba - INISAV
- Luis Pérez Vicente, Einar Martinez

Holland - PLANT RESEARCH INTERNATIONAL
- G. Kema, C. Waalwijk, M. Rep

AUSTRALIA
Andrew Daly, Wayne O'Neill

Taiwan - National Chung Hsing University
- P.F.L. Chang

Philippines - TADECO
- B.M. Corcolon

Costa Rica – UCR; Earth; Corbana
- L. Pocasangre; A. Tapia, M. Gusmám;
- J. Sandoval

USA – University of Florida
- Randy Ploetz
- Li-Jun Ma, C.Kistler

China
- Xue-Jun Xe,
- Yi Ganyu

EMBRAPA CASSAVA & FRUITS
- Edson Amorim,
- Zilton Cordeiro
- Fernando Haddad
- Sebastião Silva

EMBRAPA INFORMÁTICA
- Paula Ksuser Falcão
- Michel E B Yamagishi
- Poliana F Giachetto

EMBRAPA AGROENERGIA
- Manoel Souza

EMBRAPA AMAZONIA OCIDENTAL
- Gilvan Ferreira

UNICAMP
- Roberto H Herai

UFLA
- Luciano Paiva

GRACIAS

miguel.dita@embrapa.br