FUSARIUM TROPICAL RACE 4 EPIDEMIOLOGY AND DIAGNOSTIC

Overview of the available tools for classical and molecular TR4 diagnostic, their usefulness, and minimum tools needed to perform a correct first diagnosis of TR4 in banana crops

Fernando A. García-Bastidas PhD

START PRESENTATION

@Ferchucky

CONTENT

- Fusarium Wilt (history)
- Fungal biology and genetic diversity
- Epidemiology
- Fusarium TR4: Dispersion and Current situation
- Diagnostic
- Final remarks

Fernando A. García-Bastidas

FUSARIUM WILT IN BANANAS

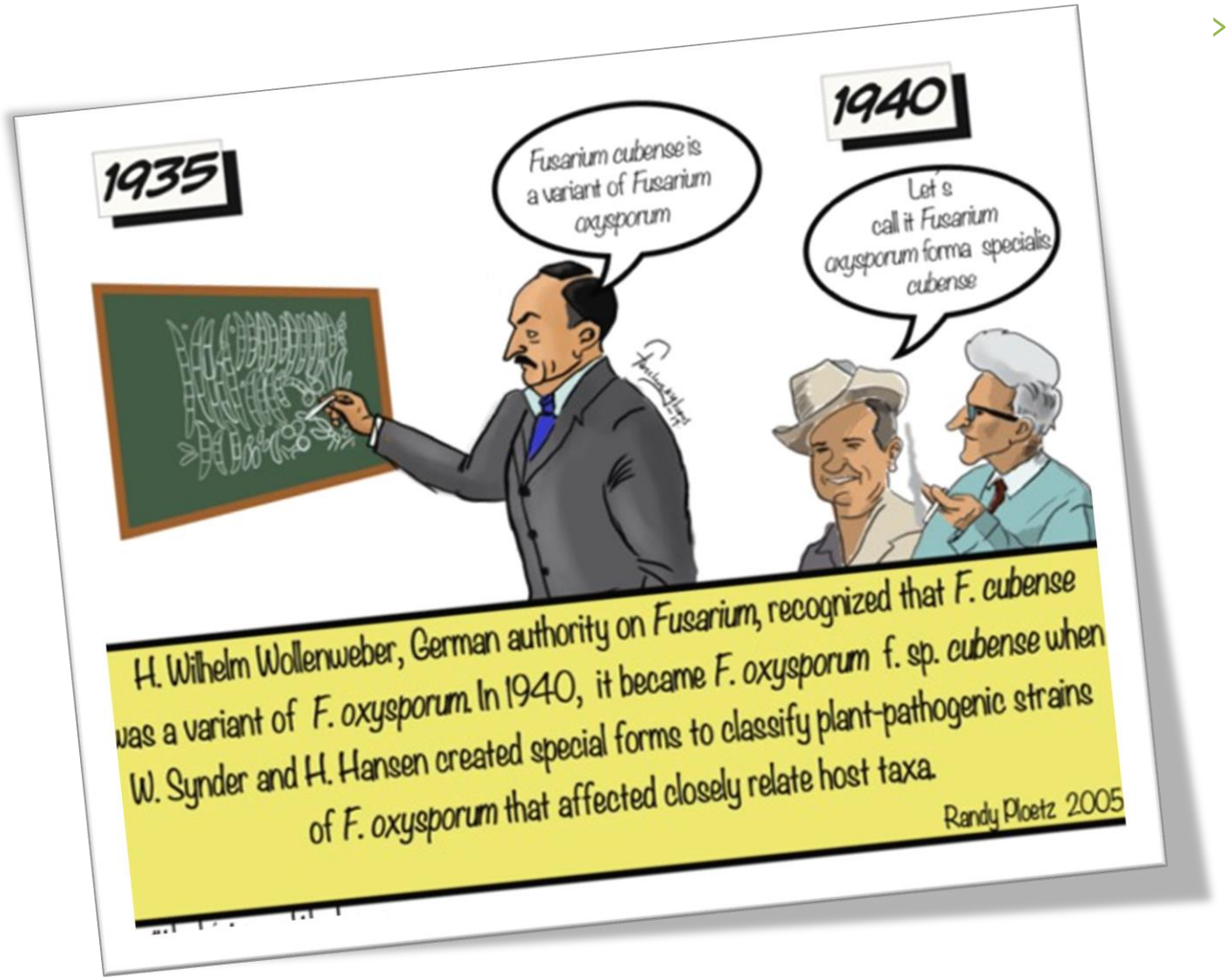
- It is one of the most destructive diseases in modern times. Even considered one of the most important epidemics in the history of agronomy.
- The disease was discovered in Australia but is believed to have originated in Southeast Asia.
-

A BIT OF HISTORY

- The disease was discovered by Dr. Joseph Bancroft in 1874 in Australia
- Shortly thereafter outbreaks of the disease were reported in Central America (Costa Rica and Panama (Col) 1890)
- Due to its discovery in Panama and its effect on plantations the disease became known as Panama Disease
- At that time the causative agent was not known.

- The causative agent of the disease was isolated in 1920 by Dr. Erwin Smith thanks to samples obtained in Cuba.

100 years later we still use the same concepts



- Several decades passed until it was recognized as a variant of *Fusarium oxysporum*. It would later be classified as *Fusarium oxysporum* f.sp. *cubense*

- For many years this has remained the name assigned to the pathogen responsible for Fusarium Wilting or Panama Disease. More recent DNA-based studies have helped to understand a little better the nature of the fungus and have led to the proposal of a new taxonomy for some of the isolates associated with this disease.

The disease spread thanks to the introduction of propagating material (contaminated rhizomes/corms/suckers) which "almost" always looks asymptomatic.

It reached its greatest dispersion in Latin America during the Gros Michel Era, cultivating implemented for the export business.

Fotos cortesía: Prof. Randy Ploetz

THE GROS MICHEL ERA

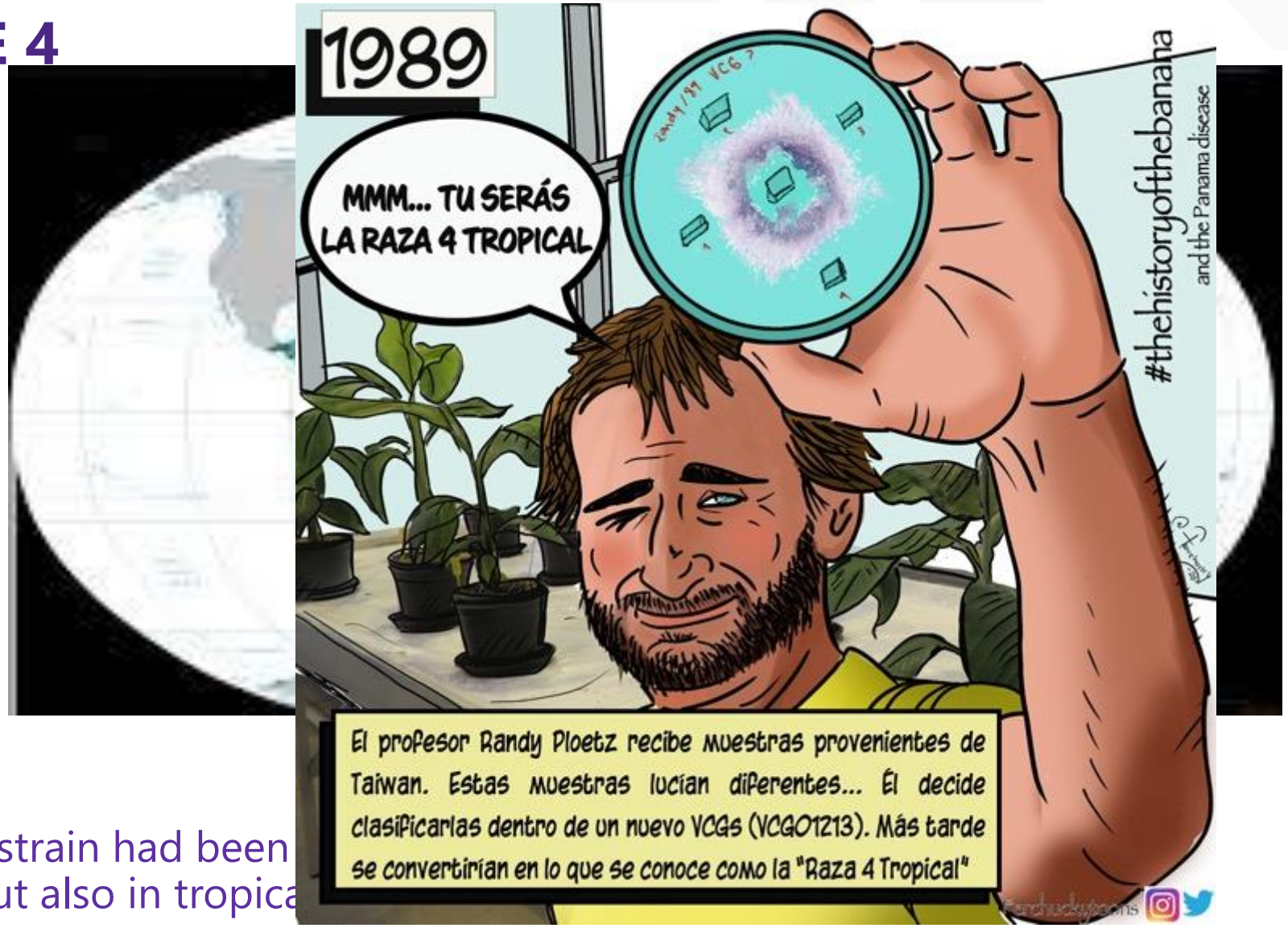
~ 1926

CAVENDISH SAVES THE BANANA INDUSTRY

~1950 ...

ALERT, CAVENDISH SICK! SUBTROPICAL RACE 4

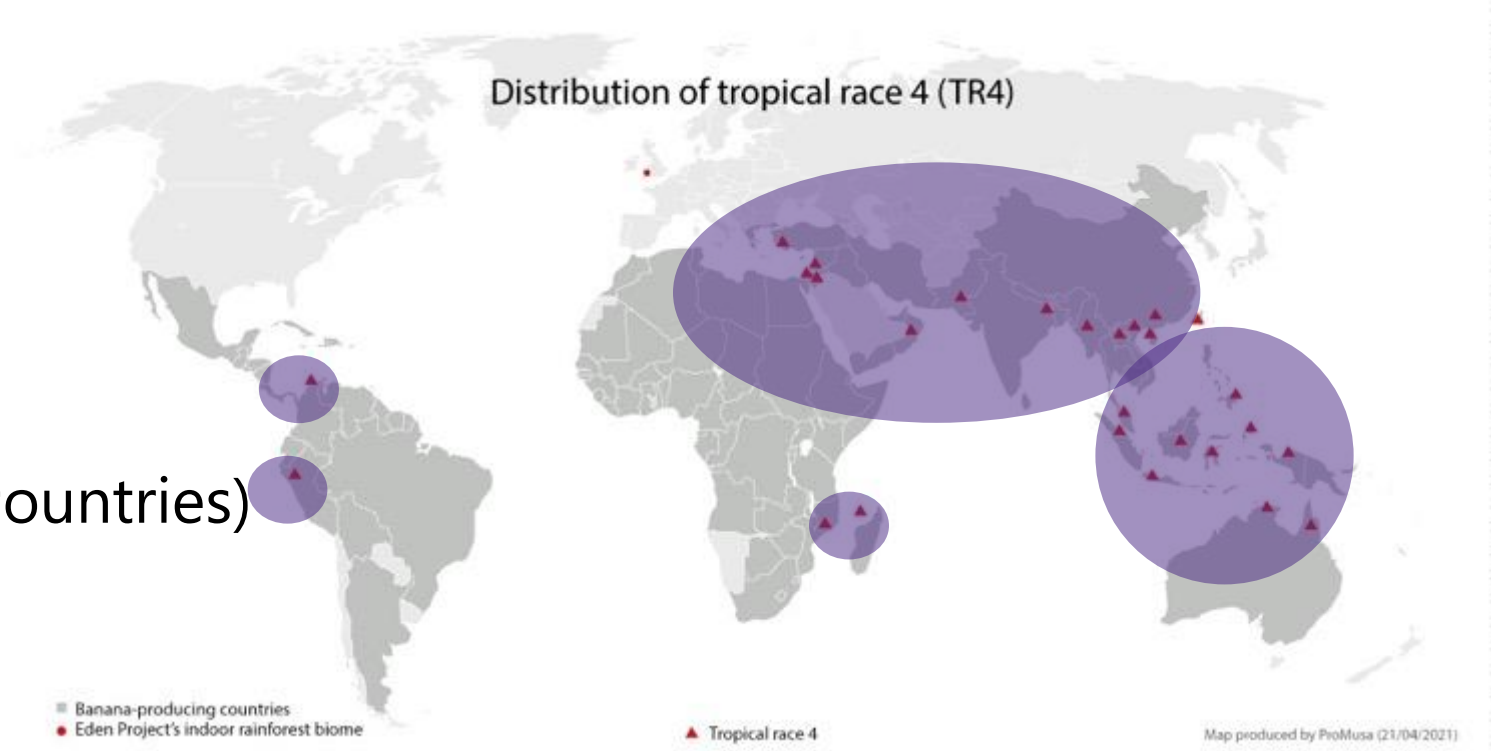
- Clones belonging to the Cavendish group displaying symptoms associated with Panama Disease in subtropical areas since the 20's in places such as the Canary Islands, South Africa, Australia and Taiwan.



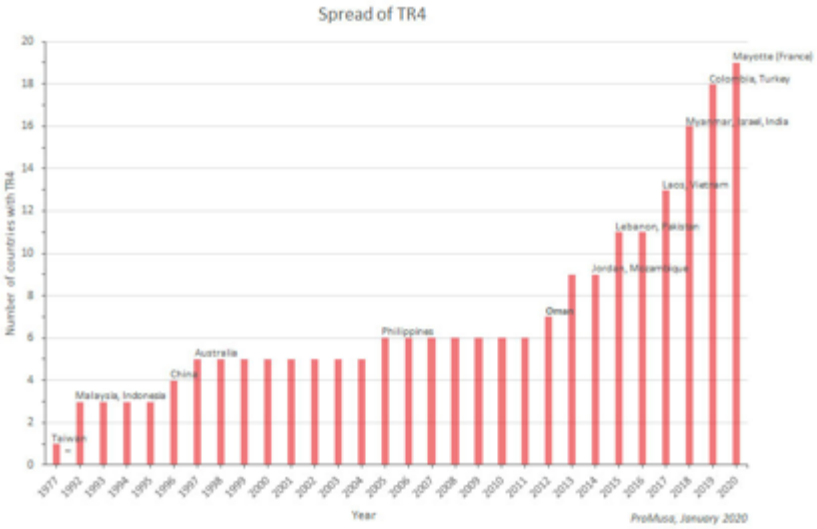
- By the late 90s a new strain had been
in subtropical areas but also in tropica

FUSARIUM TROPICAL RACE 4 FROM A LOCAL PROBLEM TO A PANDEMIC.

Panama Disease version 2.0
Tropical Race 4
Cavendish = Susceptible
2013 (5 countries) – 2022 (>20 countries)



1990 ——— 2022

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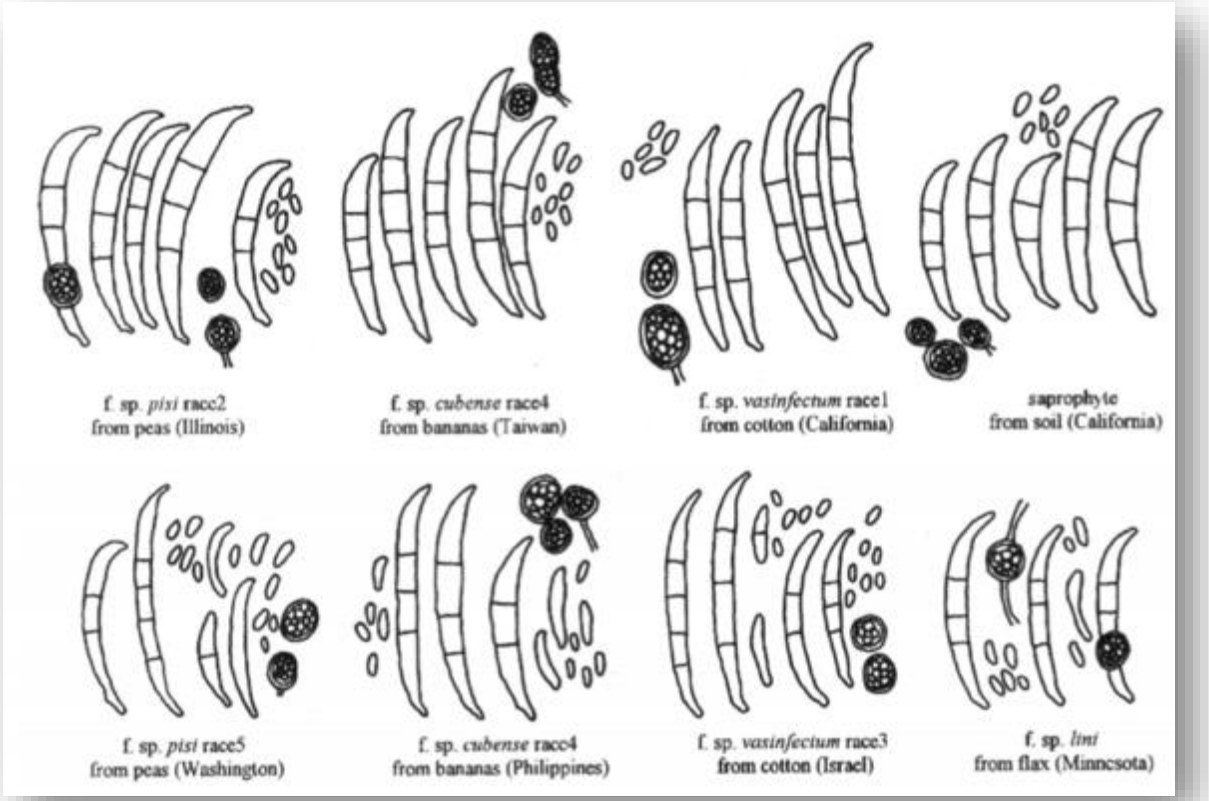
The Pathogen

FUSARIUM

- Genus *Fusarium* spp. (100 species)
- *Fusarium oxysporum* complex
- Endophytes, saprophytes and pathogens
- Causing wilting and root diseases in a wide range of hosts.
- Produce mycotoxins in cereals
- formae speciales (f.sp)
- Physiological races



THE FUSARIUM OF BANANAS

- Fusarium wilt or Panama Disease
- *Fusarium oxysporum* f.sp. *cubense* (revised taxonomy Mariany et al., 2018)
- Four races described and more than 20 VCGs
- Contaminates soils for decades
- There is no control
- Spreads easily (Contaminated soil, infected plant parts, contaminated water)

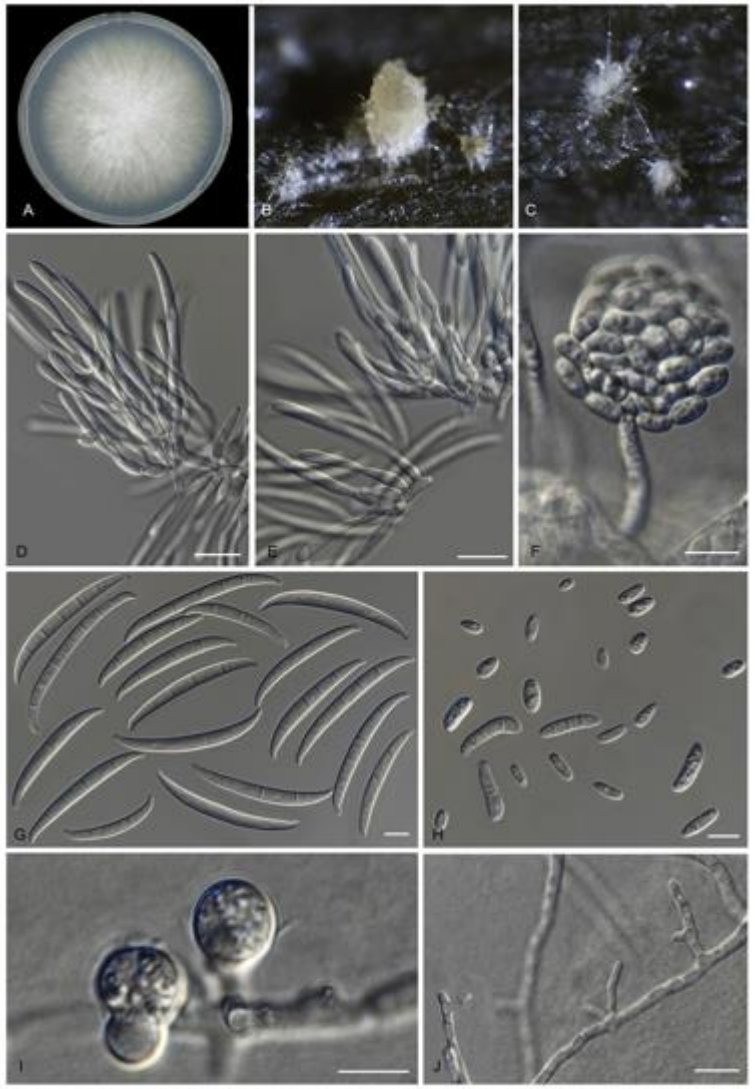
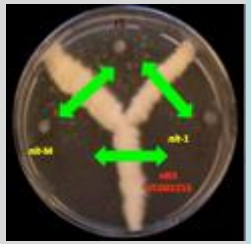


Fig. 7. *Fusarium odoratissimum* (InaCC F817). **A.** Culture grown on PDA. **B–C.** Sporodochia on carnation leaves. **D–E.** Sporodochial branched conidiophores with monophialides. **F.** False head. **G.** Falcate-shaped macroconidia. **H.** Microconidia. **I.** Chlamydospores. **J.** Polyphialides. Scale bars: D–J = 10 µm.

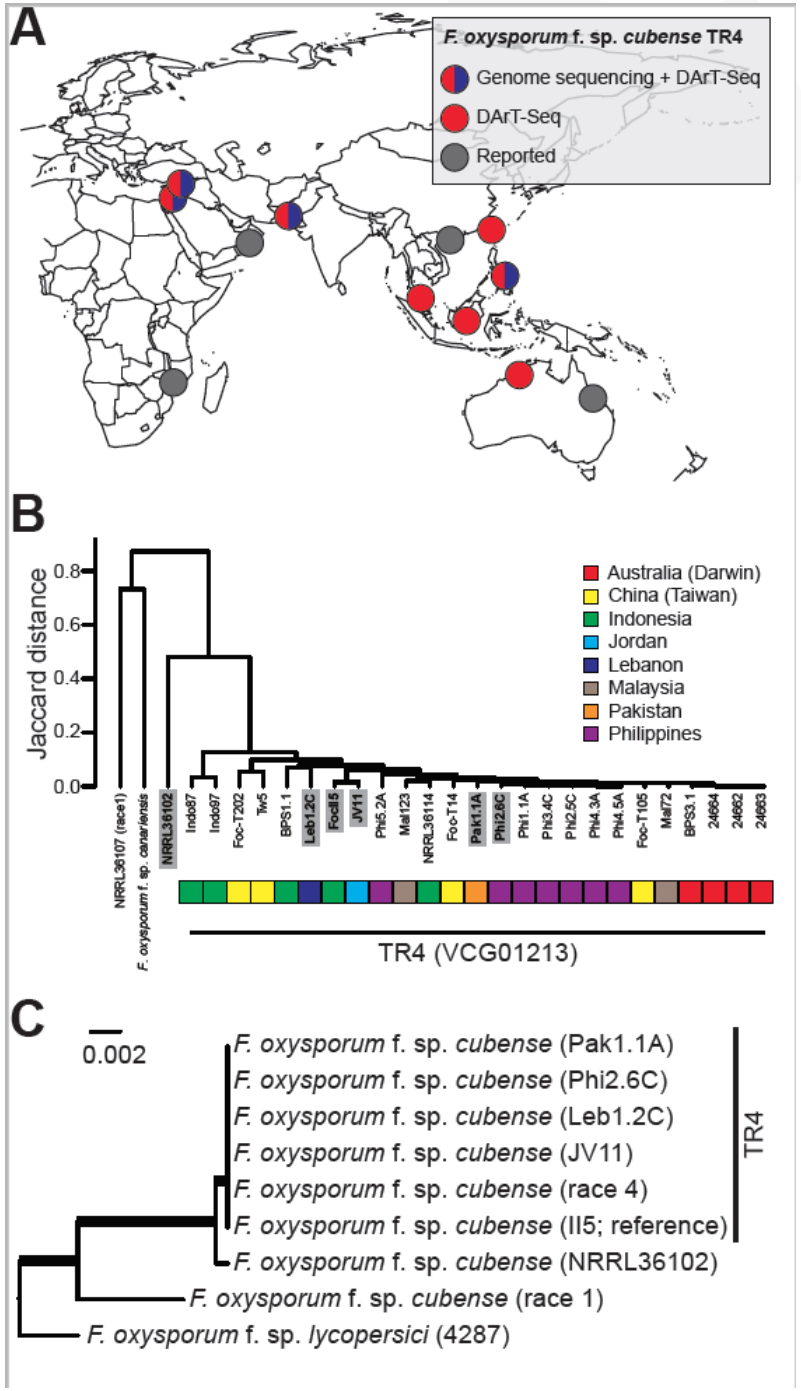
Fusarium oxysporum f.sp. *cubense* diversity

Races	Vegetative Compatibility groups	Genetic diversity
	24 reported VCGs	Chromosome number(CN) 9-14 Genome size(Gs) 32.1-58.9Mbp Molecular markers
Race4-Cavendish AAA TR4 ST4	01213/16	Clade 1 Low CN-Gs Usually isolated from pure A genome bananas
	0120, 0121, 0124, 0129	
Race1-Gros Michel AAA	0120, 0124/5, 0125, 0126, 01210	Clade 2 high CN-Gs Usually isolated from partial or pure B genome bananas
	0123, 0124	
Race2-Plantain ABB & cooking banana	0122, 0128	



Ordoñez et al., 2015

GENETIC UNIFORMITY



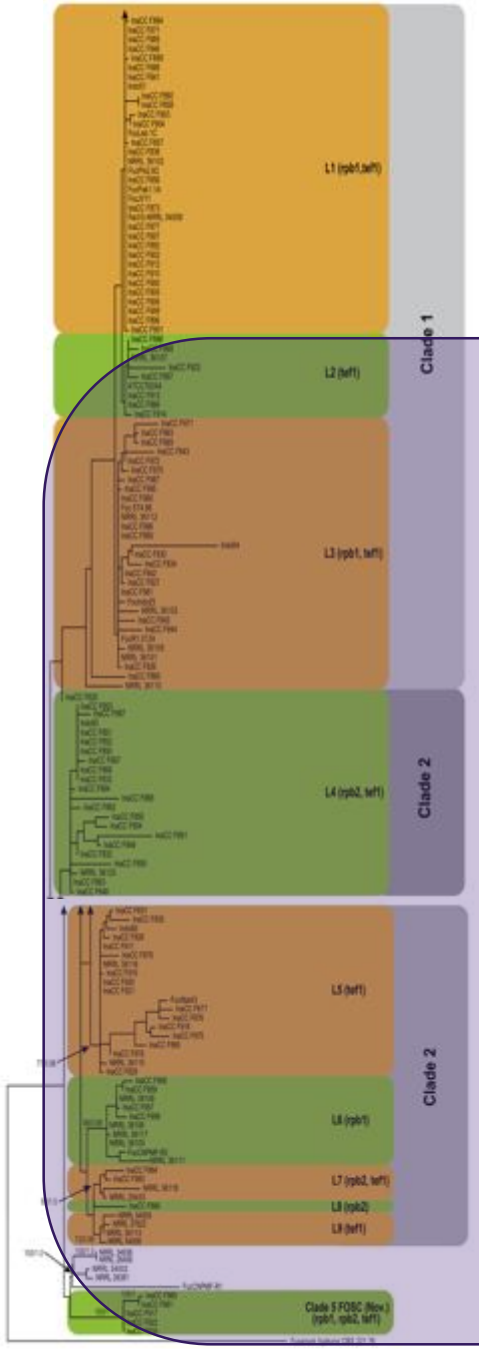
Ordoñez et al., 2015

Phylogeny and genetic diversity of the banana Fusarium wilt pathogen *Fusarium oxysporum* f. sp. *cubense* in the Indonesian centre of origin

N. Maryani[1,2,3*], L. Lombard[4], Y.S. Poerba[5], S. Subandiyah[6], P.W. Crous[2,4], and G.H.J. Kema[1,2*]

Fig. 1. Map of sampling collection in 2014–2015 in the island of Java, Sumatra, Kalimantan, Sulawesi, Papua, and Flores.

Maryani et al., 2018



Fusarium odoratissimum = Raza 4 Tropical

Fusarium purpurascens

Fusarium phialophorum

Fusarium grosmichelii

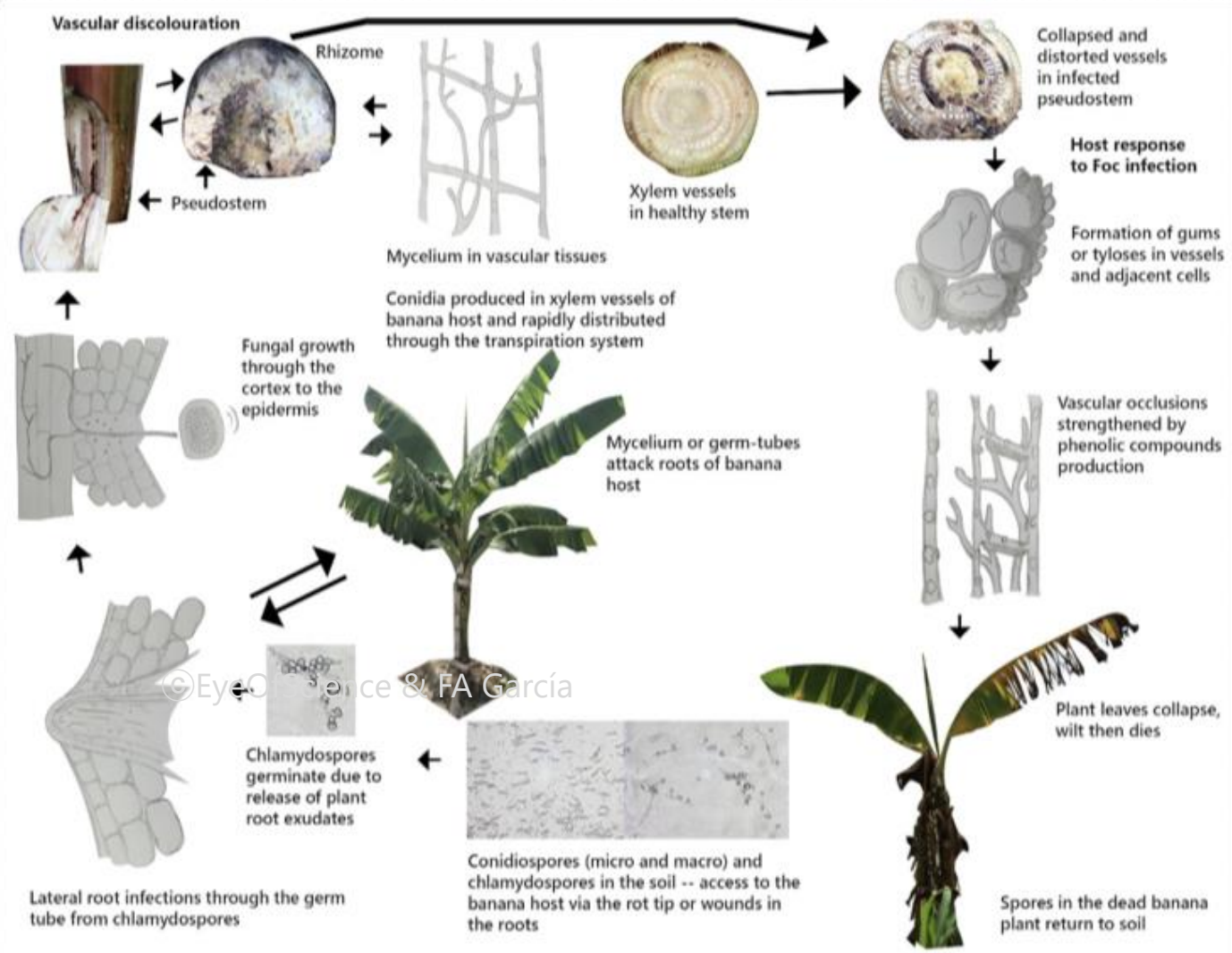
Fusarium duoseptatum

Fusarium tardichlamydosprorum

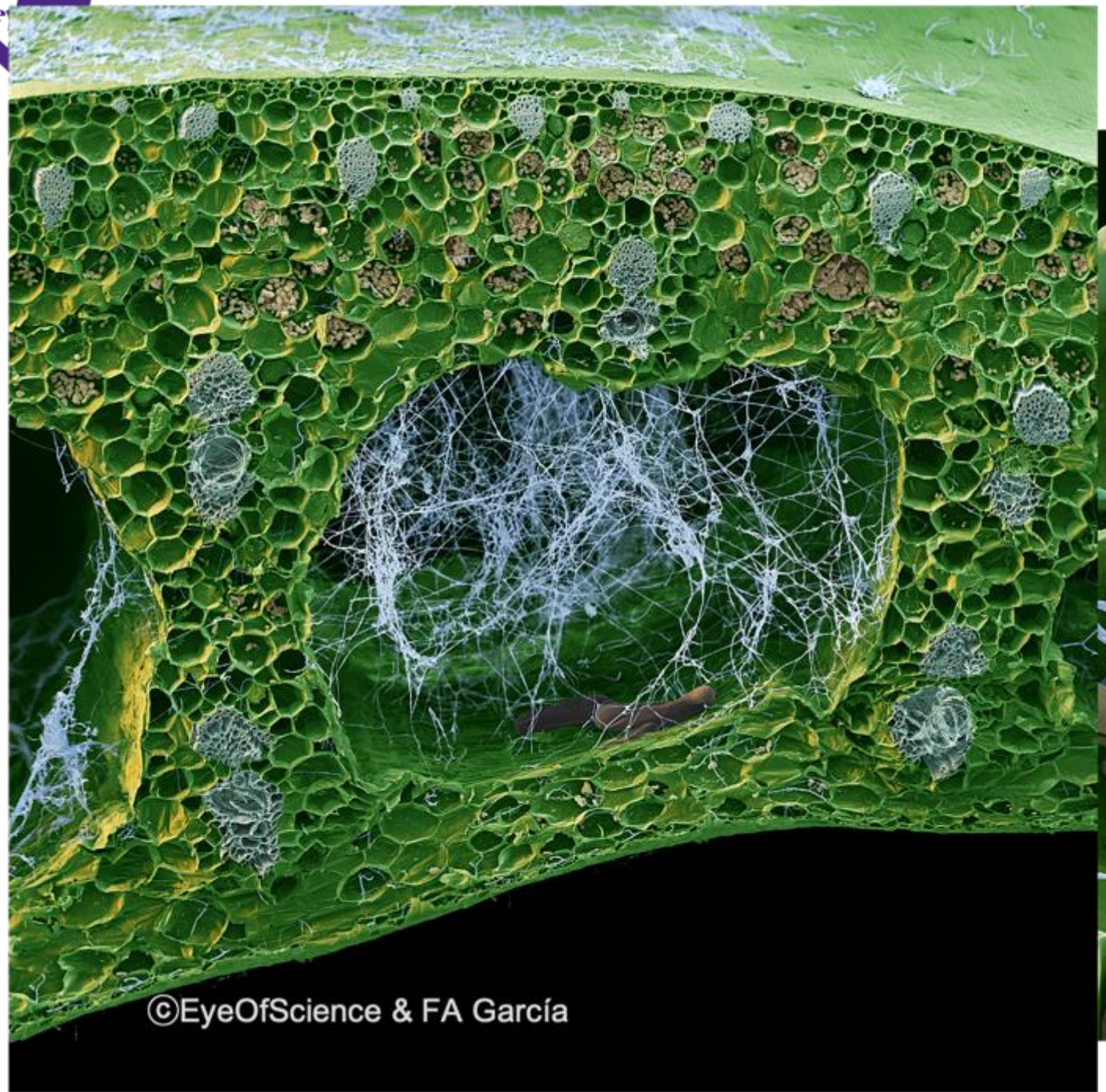
Fusarium cugenagense
Fusarium hexaseptatum
Fusarium tardicrescens

Fusarium kalimantanense

Disease cycle



Salacinas *et al.*, 2019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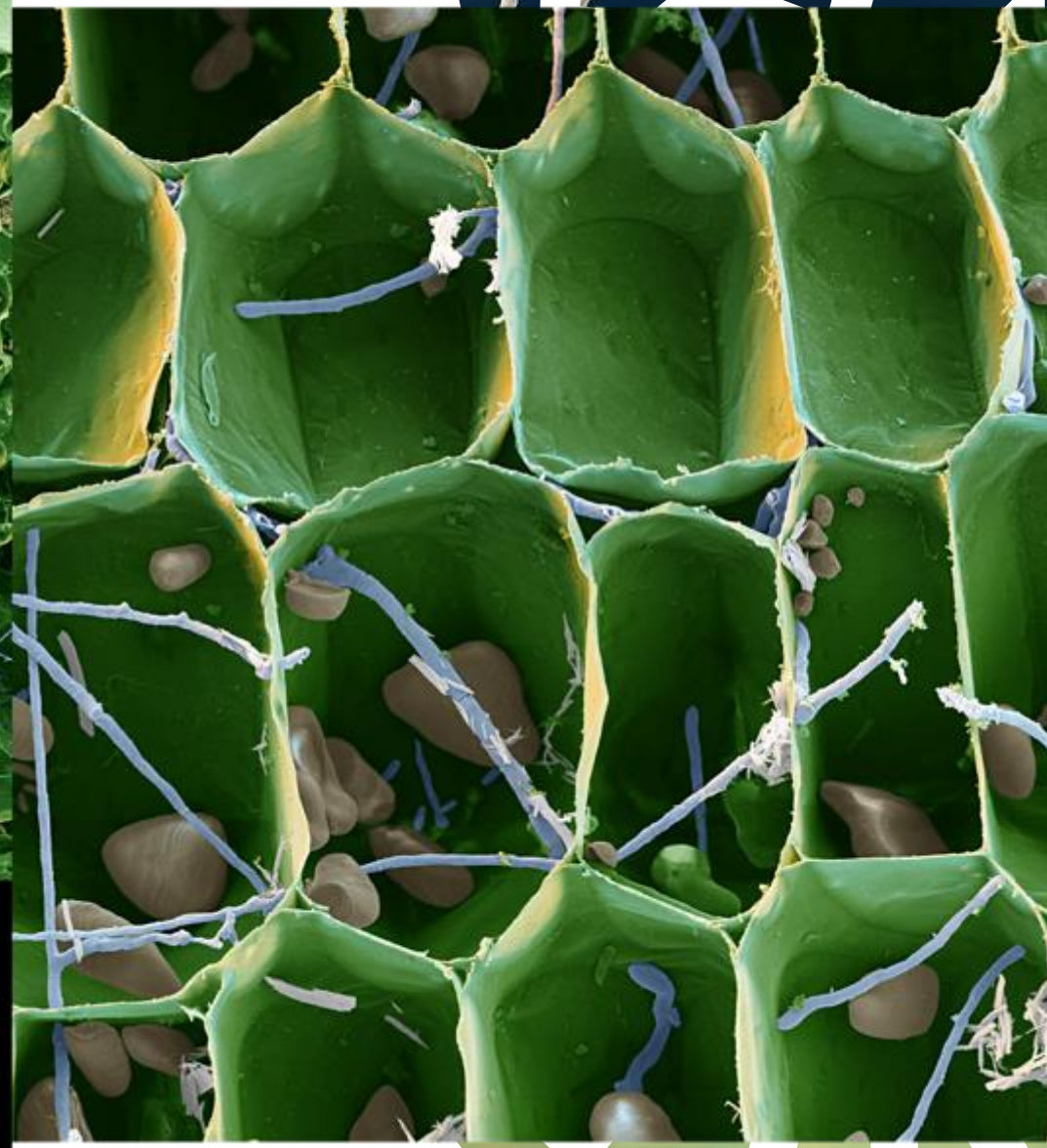

EXTERNAL SYMPTOMS

Foc R1- Banano Manzano AAB

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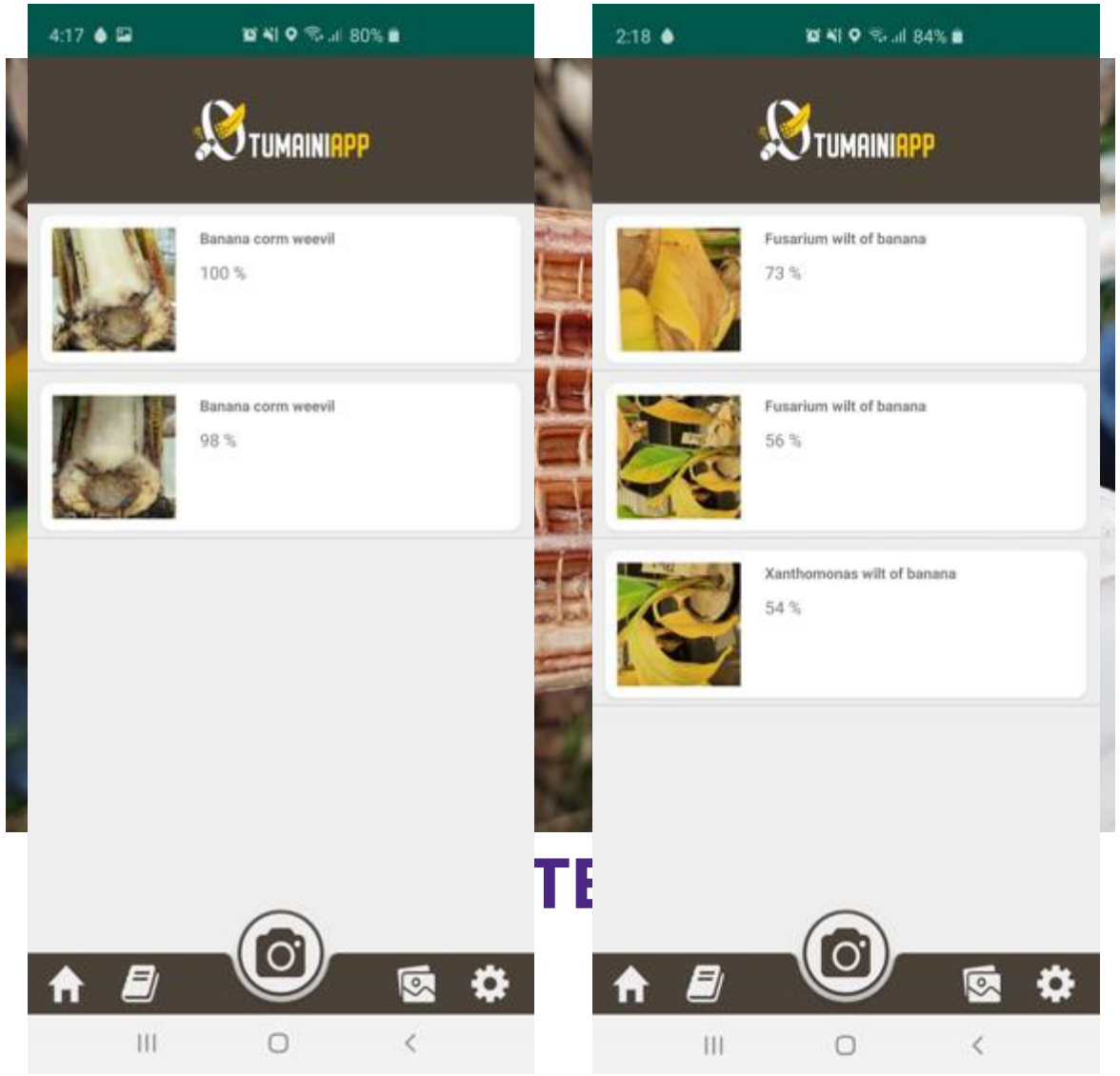
Foc R2-plátano popocho ABB

© J. Vargas, Urabá

TR4-Cavendish AAA

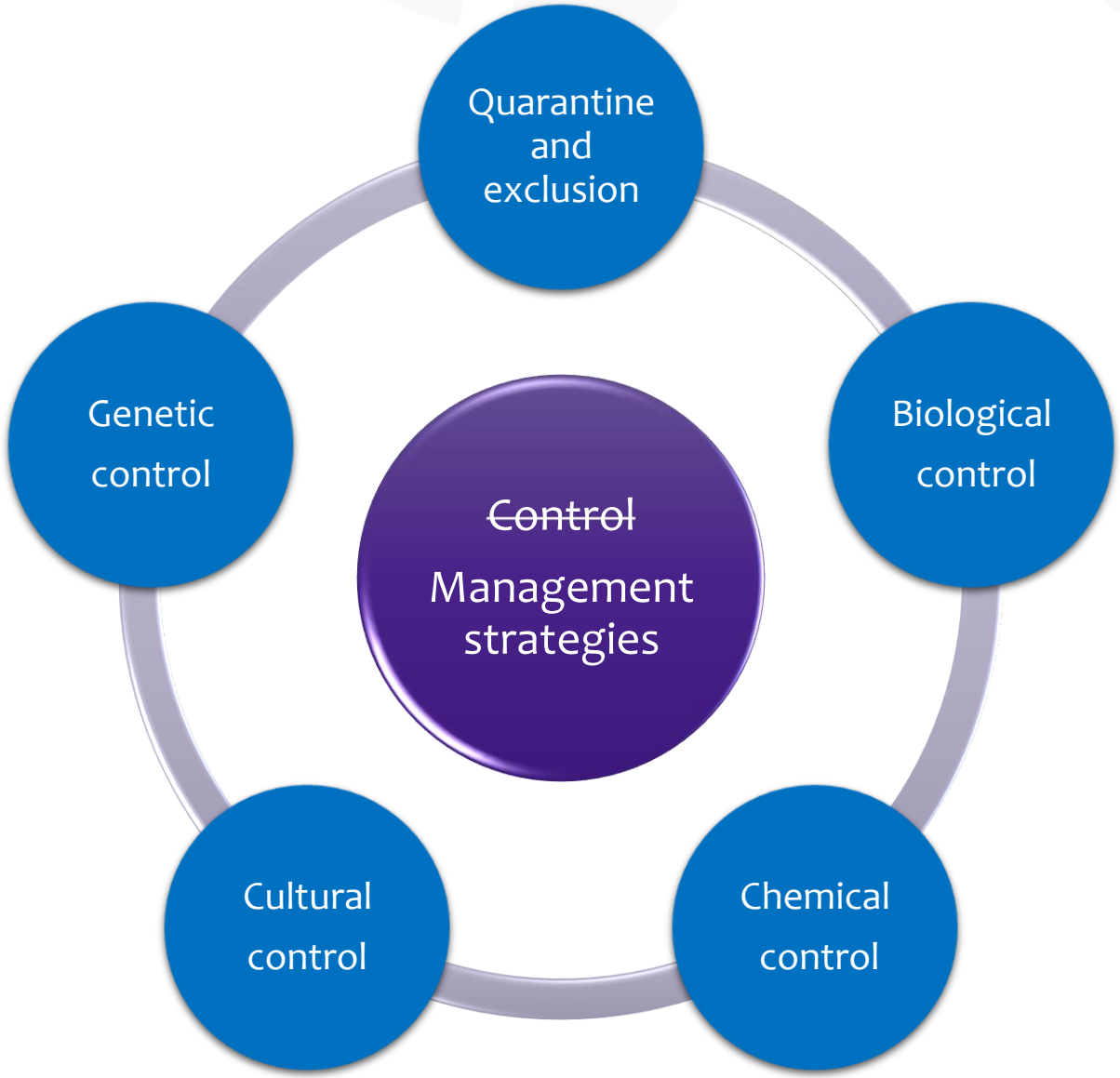
© FA Garcia, Filipinas


cenibanano
centro de investigaciones del banano
augura

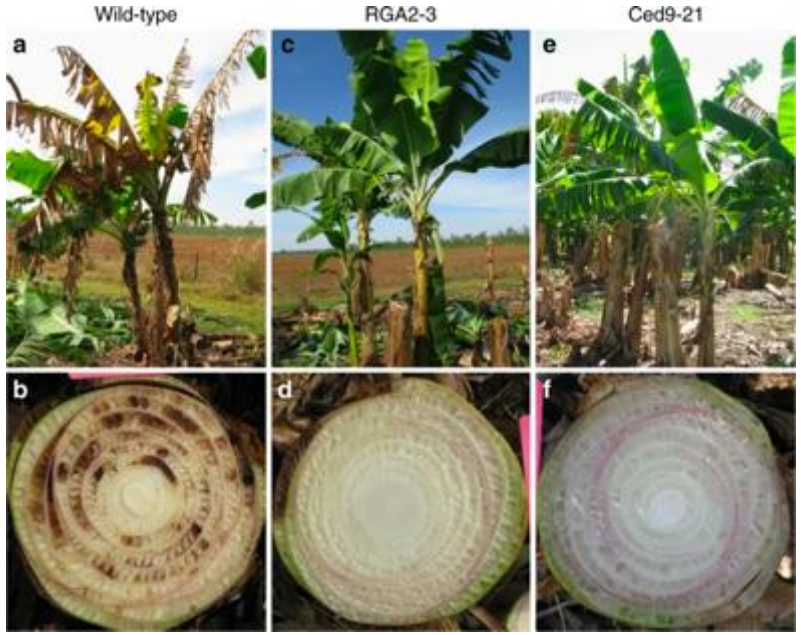


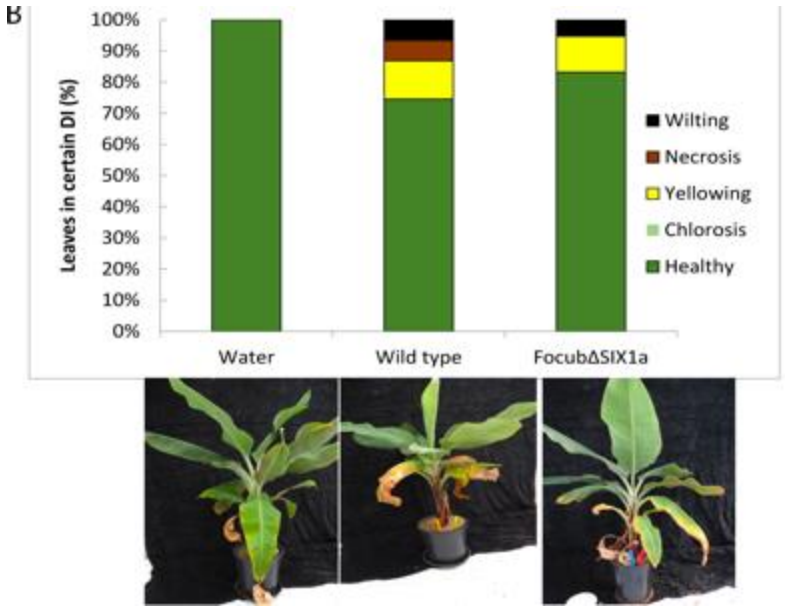
https://youtu.be/XnK03qXvJfs

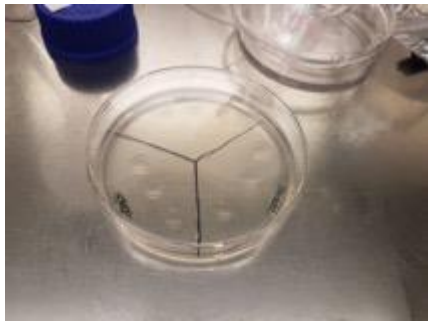
DIAGNOSTICS CRUCIAL FOR DISEASE MANAGEMENT

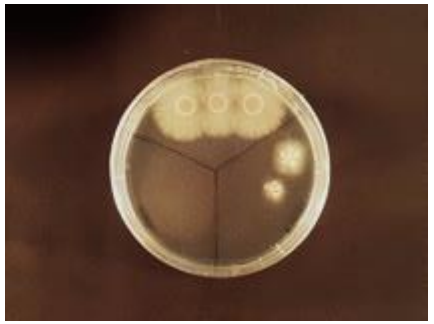


INDIRECT DIAGNOSTIC









nature COMMUNICATIONS

Article | Open Access | Published: 14 November 2017

Transgenic Cavendish bananas with resistance to Fusarium wilt tropical race 4

James Dale, Anthony James, Jean-Yves Paul, Harjeet Khanna, Mark Smith, Santy Peraza-Echeverria, Fernando Garcia-Bastidas, Gert Kema, Peter Waterhouse, Kerrie Mengersen & Robert

A *SIX1* homolog in *Fusarium oxysporum* f.sp. *cubense* tropical race 4 contributes to virulence towards Cavendish banana

S. Widinugraheni, J. Niño-Sánchez, H. C. van der Does, P. van Dam, F. A. García-Bastidas, S. Subandiyah, H. J. G. Meijer, H. C. Kistler, G. H. J. Kema, M. Rep

Published: October 22, 2018 • https://doi.org/10.1371/journal.pone.0205896

Evaluation of commercial products (active ingredients)

"Official" reports of the disease

First Repor[illegible]*sarium oxysporum* f. sp. *cubense* Tropical Ra[illegible]ssociated with Panama Disease of Banana out[illegible]outheast Asia

First Report of *Fusarium*[illegible] Tropical Ra[illegible] Cavendish[illegible]s in Pak[illegible] and Let[illegible]

Ne[illegible] Geograp[illegible]cal Insights of t[illegible] La[illegible] Expans[illegible] of *Fusarium oxy[illegible]orum* f.s[illegible]*cubense* Tropi[illegible] Ra[illegible] Into th[illegible]reater Mekon[illegible] Sub[illegible]gion

[illegible]as caused by Tropical Race 4 in [illegible]

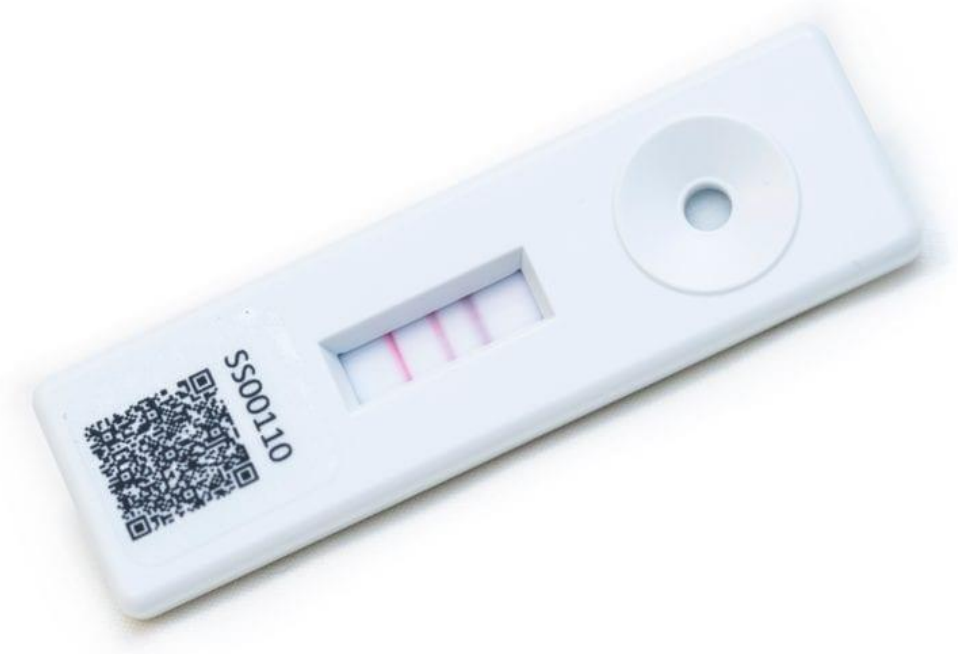
F.A. G[illegible]Bastidas[1†], J.C. Quintero-Vargas[illegible]ala-Vasqu[illegible]², T. Schermer³, M.F. Seidl³, [illegible]ntos-Paiva⁴, A.M. Noguera², C. [illegible]-Galvez[illegible] Wittenberg[5] R. Hofste[illegible]

201[illegible] 2016 2018 2019

[illegible]rdania Pakistán Vietnam Laos Myanmar Colombia

100% GRATIS!

TECHNIQUES FOR THE DIAGNOSIS OF FUSARIUM RACE 4 TROPICAL FROM MONTHS TO MINUTES.

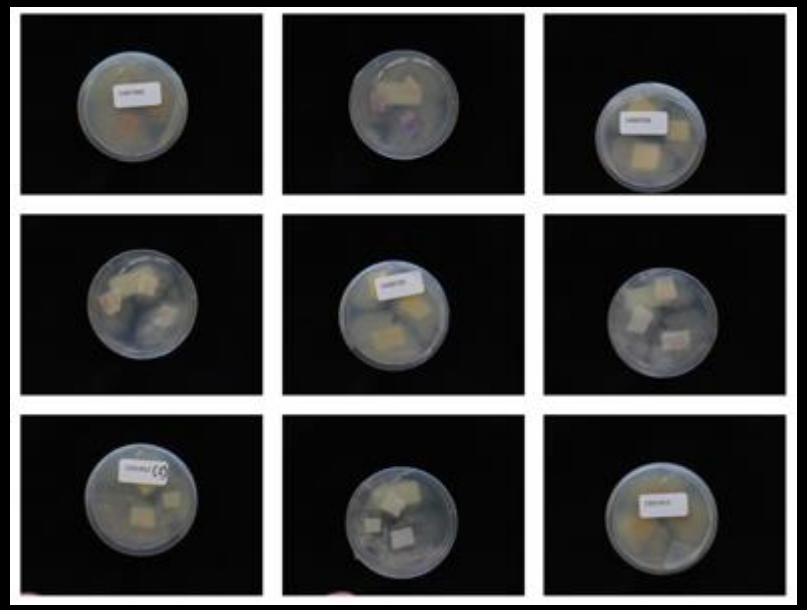


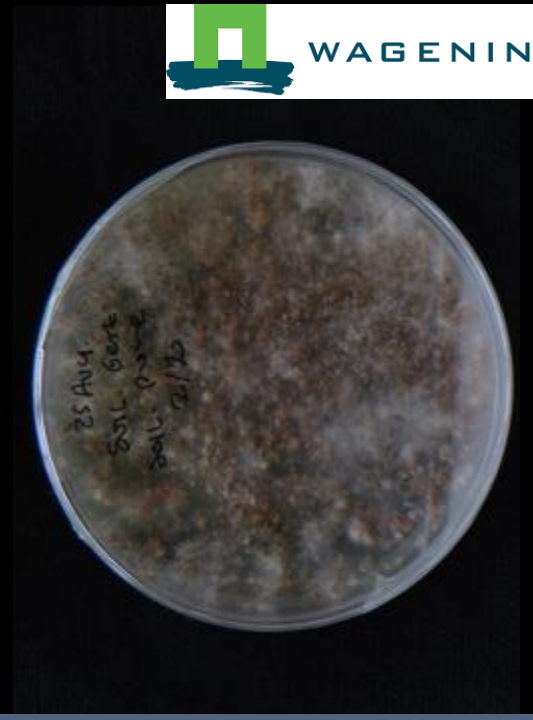
VCGs

Field
(eye)

FIRST DIAGNOSTIC (PRE-DIAGNOSTIC)

DIAGNOSTICS TROPICAL RACE 4





- Positive TR4

DIAGNOSTICS TROPICAL RACE 4

- Jordan
- Lebano
- Pakistan
- Indonesia
- Philippines
- China

- Australia
- Colombia
- México
- Surinam
- Costa Rica
- Tailandia (suelo)
- Perú
- Ecuador

Andean Guide For the Diagnosis of Fusarium Tropical Race 4

KeyGene

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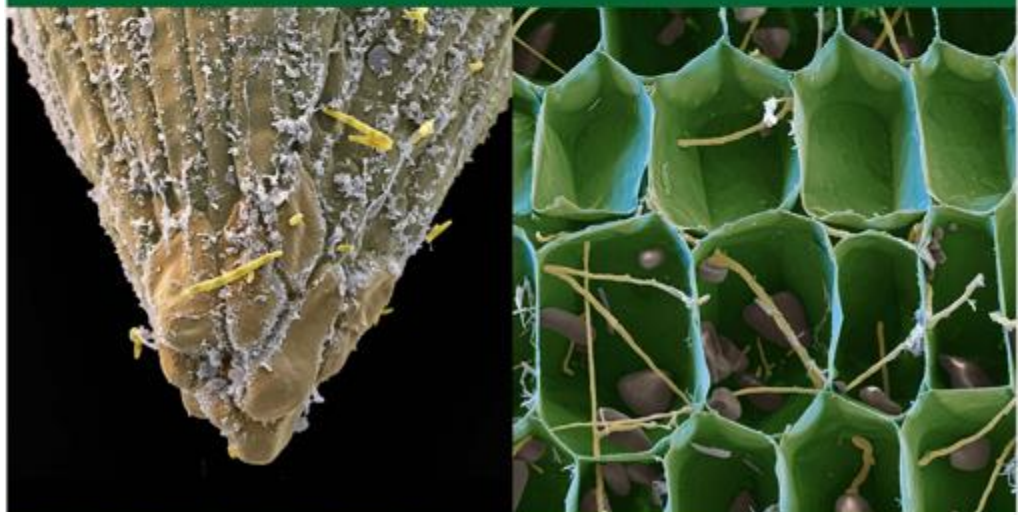
SECRETARÍA GENERAL

AÑO INTERNACIONAL DE LA SANIDAD VEGETAL 2020

GUÍA ANDINA PARA EL DIAGNÓSTICO DE

Fusarium Raza 4 Tropical (R4T)

***Fusarium oxysporum* f.sp. *cubense* (syn. *Fusarium odoratissimum*)**
agente causal de la marchitez por *Fusarium*
en musáceas (plátanos y bananos)



Con el apoyo de:

SENASA BOLIVIA · ICA Instituto Colombiano Agropecuario · AGROCALIDAD · SENASA PERU · KeGene · WAGENINGEN UNIVERSITY & RESEARCH

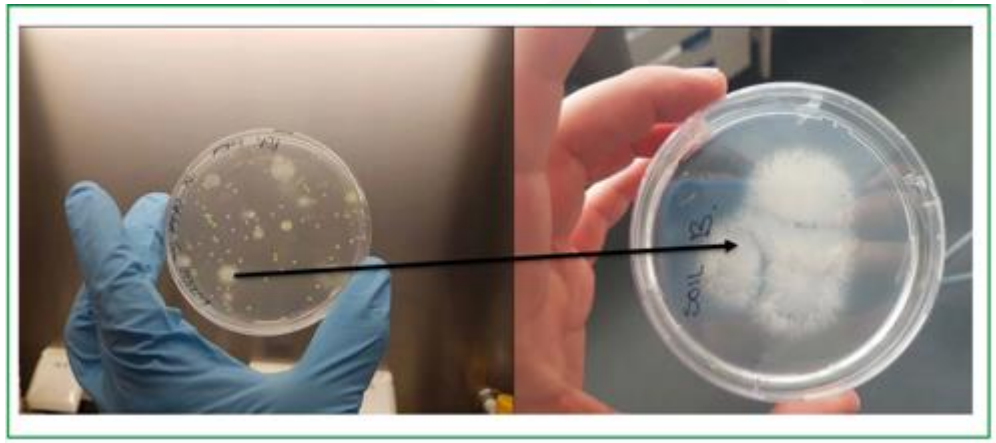


Figura 14. Manejo de las muestras de suelo en el laboratorio y dilución de la muestra.

7. Continuar con protocolo de extracción de ADN.

5.4. Protocolo de extracción de ADN

En caso de no disponer de kits comerciales para extracción de ácidos nucleicos (ADN), tales como DNAsy de Qiagen, Wizard® Magnetic DNA Purification System for Food de Promega, Clear Detections entre otros, que han sido evaluados y validados satisfactoriamente tanto para tejido vegetal como para material fúngico, se describen a continuación dos protocolos estandarizados con reactivos de fácil adquisición.

5.4.1 Extracción de ADN de Musáceas y *Fusarium spp.*

Estandarizado por F.A. García-Bastidas 2013 - Adaptado de Bernatzky y Tanksley, 1990)

Materiales:

- Mortero y pistilo, nitrógeno líquido (equipo de liofilizado, perlas de circonio)
- Tubos para microcentrífuga
- Baño María o plancha de calentamiento
- Pipetas y puntas de 1000, 200 y 10 µl
- Racks
- Marcadores
- Guantes
- Toallas

Material vegetal o micelio/esporas:

1. Introduzca por lo menos 100 mg de material en un tubo de 2 ml debidamente rotulado.
2. Aplicar nitrógeno líquido dentro del tubo y macerar con ayuda de un pistilo (mortero, liofilización o cualquier otro procedimiento de lisis son aceptados).
3. Inmediatamente adicione en 320 µl del **Buffer De Extracción Sorbitol**[6]+ 100 µl de **buffer Sarcosine**[7] + 320µl de **buffer de Lisis nuclear**

6. 350 mM de Sorbitol (63,77g/1L); 100 mM de Trizma base (12,10 g/1L); 5 mM de EDTA (1,86 g/1L); 0,2% de β-mercaptoetanol (2 mL/1L). Prepare 1 L en ddH2O y ajuste a pH 8,2. No requiere autoclave. Conserve a temperatura ambiente y pre-enfríe a 4oC antes de usar.

7. N-Lauroyl Sarcosine al 5%.

8. 55 mM de CTAB (20 g/1L), 200 mM de Trizma base (24,22 g/1L), 50 mM de EDTA (18,61 g/1L), 2 M de NaCl (116,88 g/1L). Prepare 1L con ddH2O y ajuste a pH 7.5. No requiere autoclave. Conserve a temperatura ambiente.

https://bit.ly/3hnDywY

Figura 13. Manejo de las muestras de suelo en el laboratorio y dilución de la muestra.

5.8. *Prediagnóstico mediante técnica LAMP*

Protocolo original en inglés suministrado amablemente por el equipo LAMP de la Universidad de Wageningen .

Después de la extracción de ADN utilizando el protocolo recomendado por los desarrolladores del kit LAMP o la obtención de ADN por cualquier otro protocolo. La prueba suministra resultados en un promedio de tiempo de entre 25 a 30 minutos. La interpretación de los resultados es bastante simple. Un ejemplo real de la amplificación obtenida para el caso de Colombia se puede observar en la figura 32C.

User protocol for the detection of *Fusarium odoratissimum* Tropical Race 4 (R4T) using Loop-mediated Isothermal Amplification (LAMP)

Precautions:

- Read the protocol carefully before the first-time use.
- Store all kit components at the recommended storage temperature.
- Mix the Extraction buffer and Chelex resin always before every pipetting step (Chelex quickly sediments).
- Wear gloves when following the extraction pro-

Figura 25: Imagen del equipo utilizado para ejecutar protocolo LAMP ara R4T

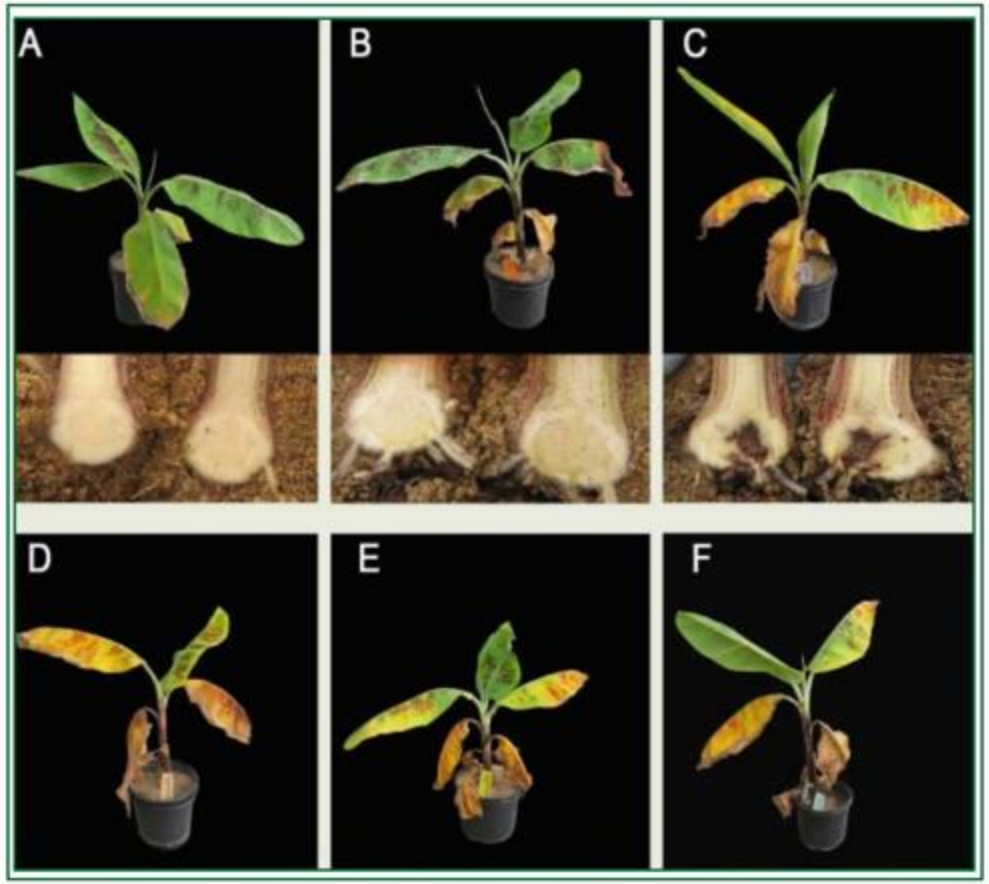
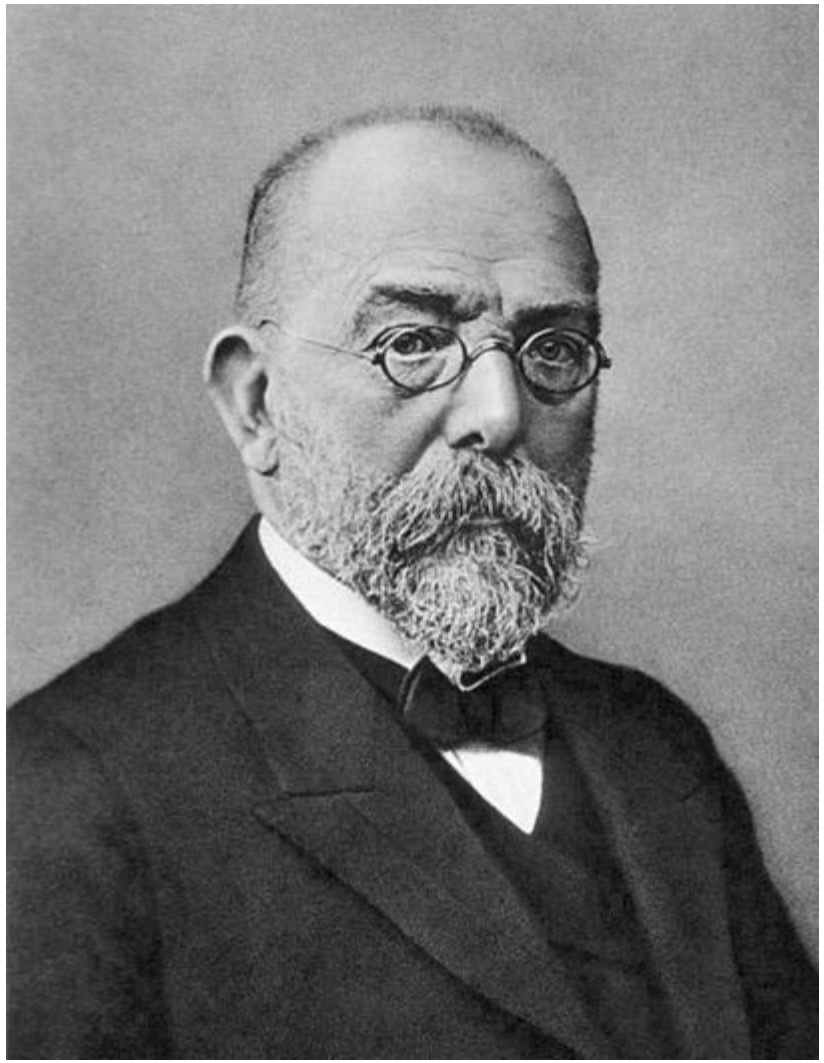


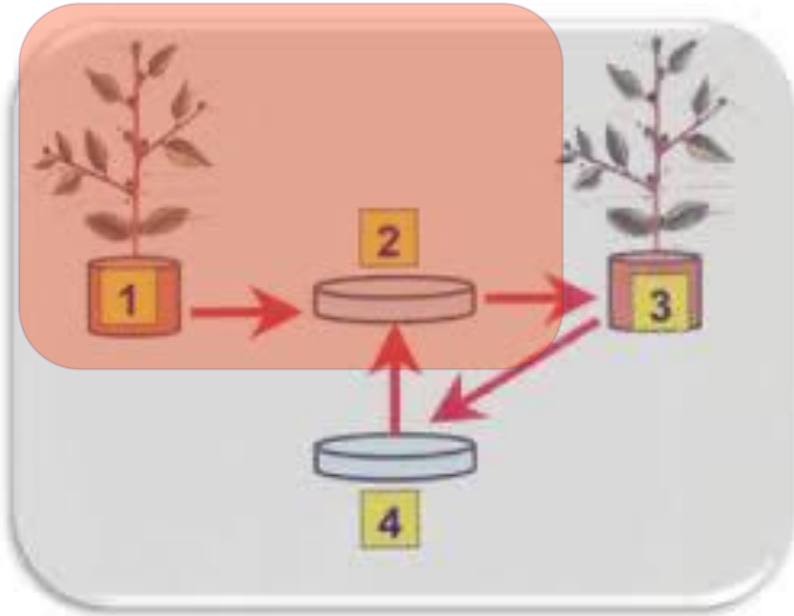
Figura 33. Resultado de la prueba de patogenicidad para un reporte inicial de la enfermedad. A y B controles negativos agua y raza 1 respectivamente. C. control positivo (R4T indonesia II-5) y D – F cepas bajo estudio. (Tomado de García-bastidas *et al*., 2019)

DIAGNOSTIC – KOCH'S POSTULATES



Robert Koch (1843 - 1910)

1- The agent must be present in each case of the disease and absent in the healthy.
2- The agent should not appear in other diseases.
3- The agent must be isolated in a pure culture from the lesions of the disease.
4- The agent must cause the disease in an organism susceptible to be inoculated.
5- The agent has to be isolated again in experimentation.



1 – 3 meses

PLANT/SOIL/WATER SAMPLING

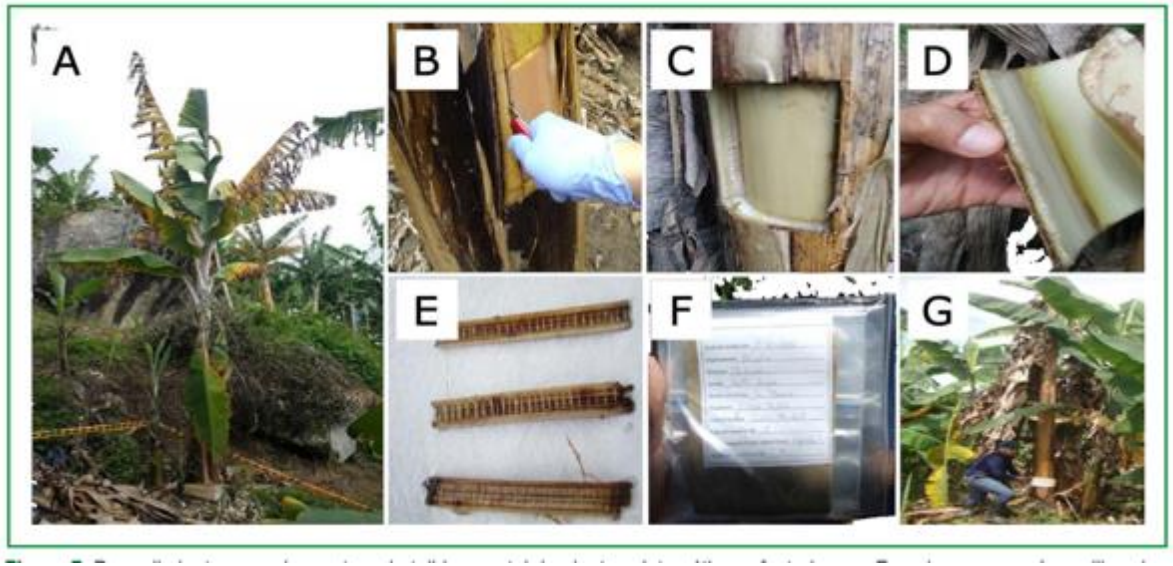
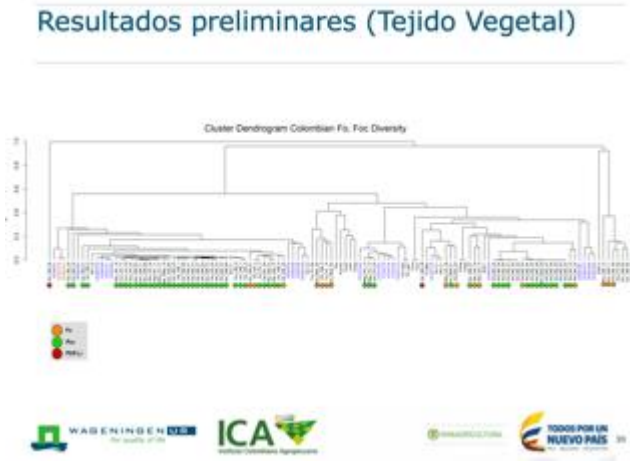


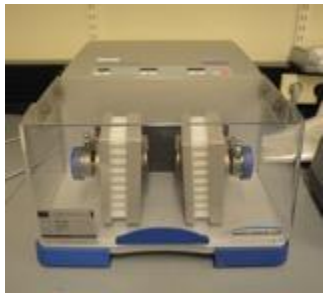
Figura 8. Representación esquemática del procedimiento de muestreo de suelo.



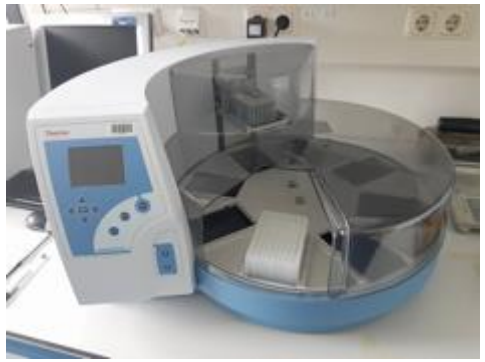
DNA ISOLATION AND TRIALS

Sample

Processing

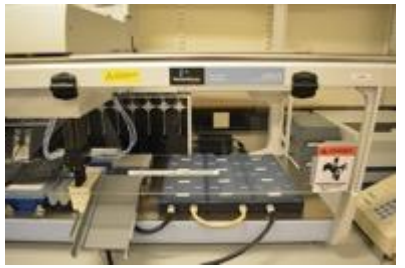


Isolation



Quantification

Technique/Equipment



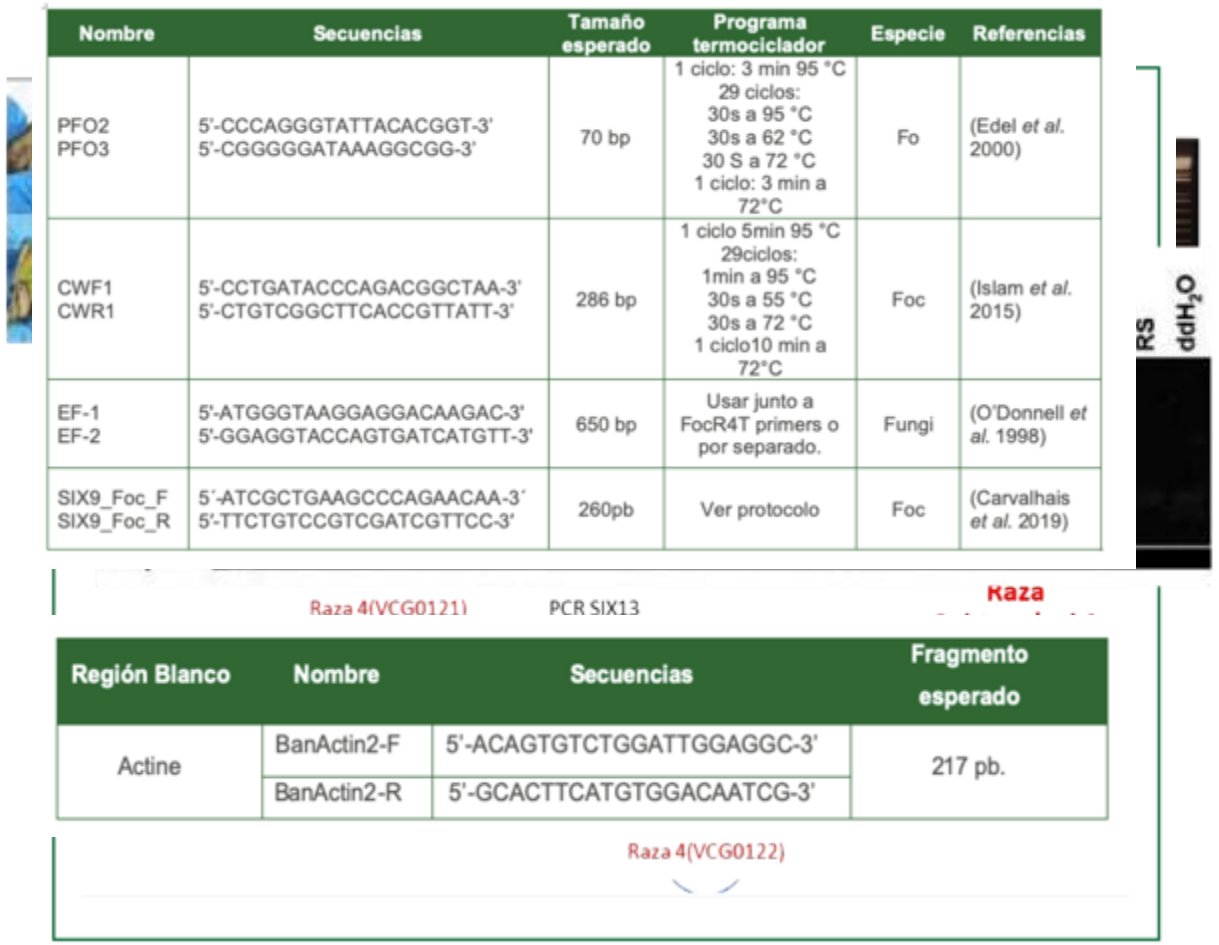
DIAGNOSTIC TECHNIQUES (FIRST DIAGNOSIS)

- PCR
  - FocR4T (Dita *et al.*, et al 2010. Wageningen)
  - SIX genes (Carvalhais., *et al* 2019, Australia)
  - W2987 (Li *et a*l., 2013. China)

- PCR Real Time
  - Kit ClearDetections
  - qPCR (Aguayo., 2017)

- LAMP
  - Wageningen University 2019

Nombre	Secuencias	Tamaño esperado	Programa termociclador	Especie	Referencias
PFO2 PFO3	5'-CCCAGGGTATTACACGGT-3' 5'-CGGGGGATAAAGGCGG-3'	70 bp	1 ciclo: 3 min 95 °C 29 ciclos: 30s a 95 °C 30s a 62 °C 30 S a 72 °C 1 ciclo: 3 min a 72°C	Fo	(Edel *et al.* 2000)
CWF1 CWR1	5'-CCTGATACCCAGACGGCTAA-3' 5'-CTGTCGGCTTCACCGTTATT-3'	286 bp	1 ciclo 5min 95 °C 29ciclos: 1min a 95 °C 30s a 55 °C 30s a 72 °C 1 ciclo10 min a 72°C	Foc	(Islam *et al.* 2015)
EF-1 EF-2	5'-ATGGGTAAGGAGGACAAGAC-3' 5'-GGAGGTACCAGTGATCATGTT-3'	650 bp	Usar junto a FocR4T primers o por separado.	Fungi	(O'Donnell *et al.* 1998)
SIX9_Foc_F SIX9_Foc_R	5´-ATCGCTGAAGCCCAGAACAA-3´ 5'-TTCTGTCCGTCGATCGTTCC-3'	260pb	Ver protocolo	Foc	(Carvalhais *et al.* 2019)



Región Blanco	Nombre	Secuencias	Fragmento esperado
Actine	BanActin2-F	5'-ACAGTGTCTGGATTGGAGGC-3'	217 pb.
	BanActin2-R	5'-GCACTTCATGTGGACAATCG-3'	

DIAGNOSTIC TECHNIQUES (FIRST INCURSIONS)

- PCR
  - FocR4T (Dita et al., et al 2010. Wageningen)
  - SIX genes (Carvalhais., et al 2019, Australia)
  - W2987 (Li et al., 2013. China)
- PCR Tiempo real
  - Kit Comercial ClearDetections
  - qPCR (Aguayo., 2017)
- LAMP
  - Wageningen University 2019

"The proper use of these methodologies depends on the skill and accuracy of the individuals making the diagnosis, which is critical to preventing false positives or worse, false negatives."

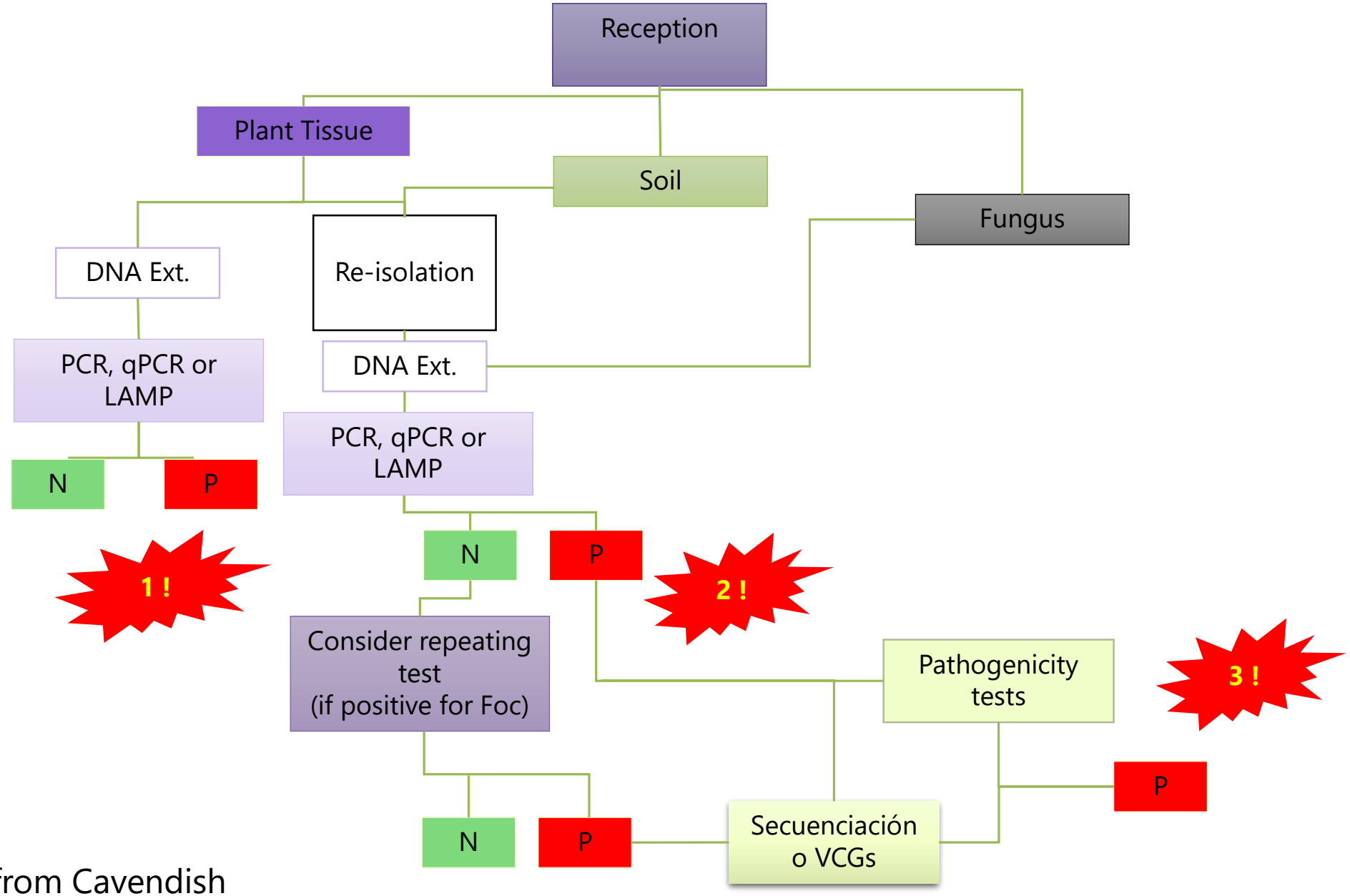
- PCR
- qPCR
- LAMP

- VCGs*
- Genome Sequencing (not fragments)

- Pathogenicity Tests

- PCR
- qPCR
- LAMP

TR4 DIAGNOSTIC SCHEME



*For samples from Cavendish

FINAL REMARKS

- The current pandemic is caused by a single clone know as Tropical Race 4 (VCG1213/16)
- Diagnostics are crucial at every stage in disease management.
- The effectiveness of control measures is still weak. Fusarium will continue its spread.
- A combination of oligos/techniques is essential for accurate diagnostics and the prevention of false negatives & positives
- Everyone with the proper equipment and training can do diagnostics.


Thank you for your attention
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