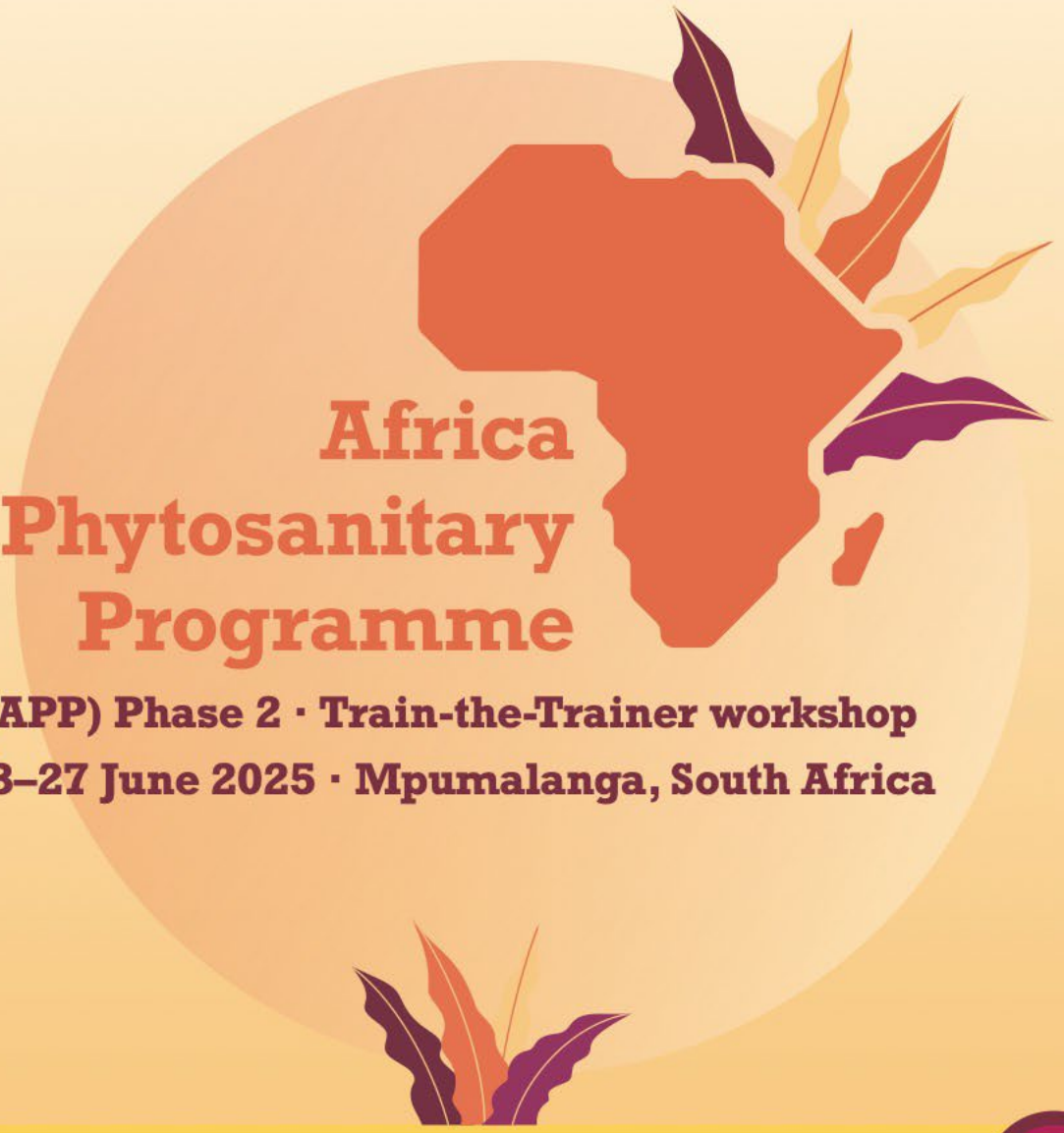


Fusarium wilt TR4 of banana

Diane Mostert

Stellenbosch University

Fusarium wilt importance and impact

- Fusarium wilt is considered one of the most damaging plant pathogens in history
- Caused by the soilborne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc)
- Accurate information on the impact of Foc TR4 is limited:
  - 'Foc TR4 risk of spread scale': Foc TR4 could affect 1.65 million ha by 2040 (value over US$ 10 billion)
  - In Mozambique weekly losses of 15 000 plants translated to USD 236 000 per week and ultimately ruined an 80 million US$ investment
  - If cooking bananas in Africa are susceptible to Foc TR4, the fungus could threaten a staple food valued at $ 4.3 billion, affecting the livelihoods of approximately 100 million people

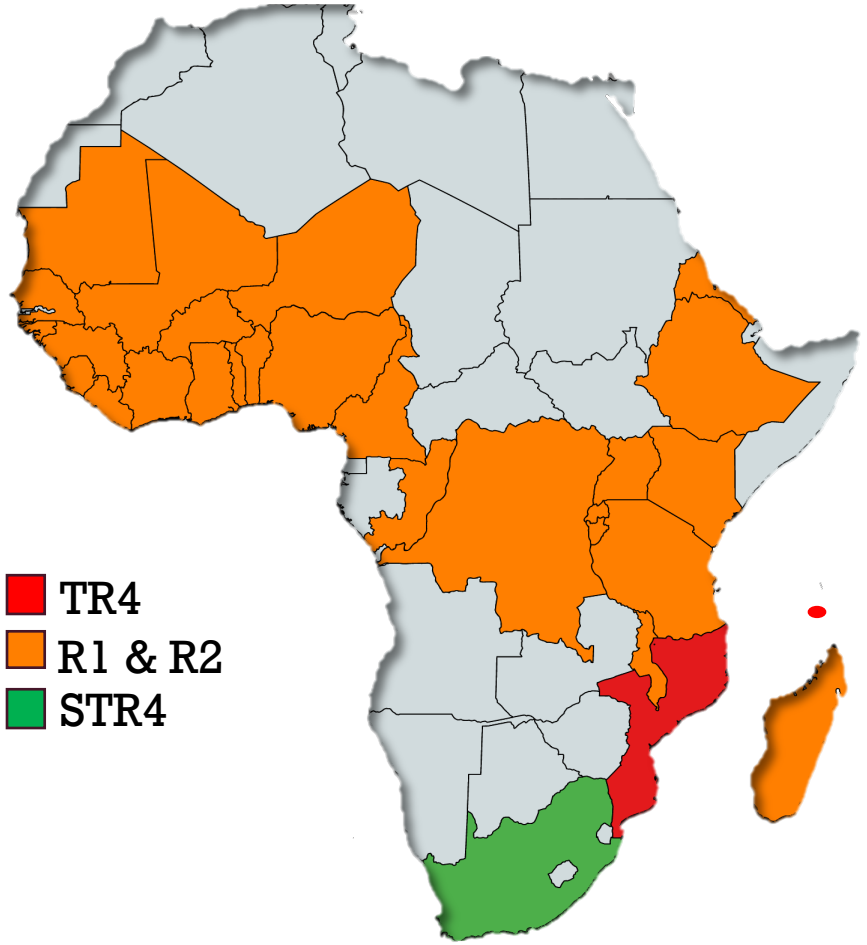
Fusarium wilt in Africa

Race 1 ⇒ Gros Michel, Silk, Pome, Pisang Awak

Race 2 ⇒ 'Bluggoe' (ABB)

Race 4 ⇒ Cavendish, most other bananas susceptible to races 1 and 2

- "sub-tropical"
- "tropical"



Causal agent of Fusarium wilt

- Foc is part of the FOSC
- Foc can only affect banana not other hosts
- It is an asexual fungus
- Importantly it displays three different spore morphologies
- Microconidia and macroconidia which are formed during the infection process (Both can be infective)
- Chlamydospores which it produces to survive in the soil for decades

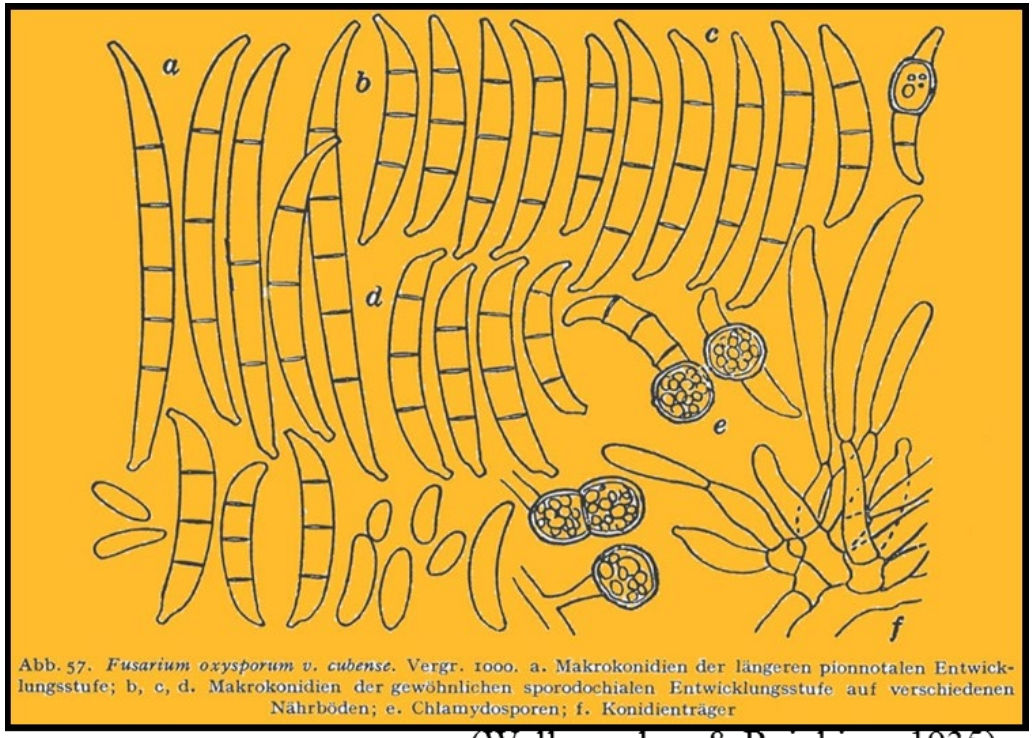
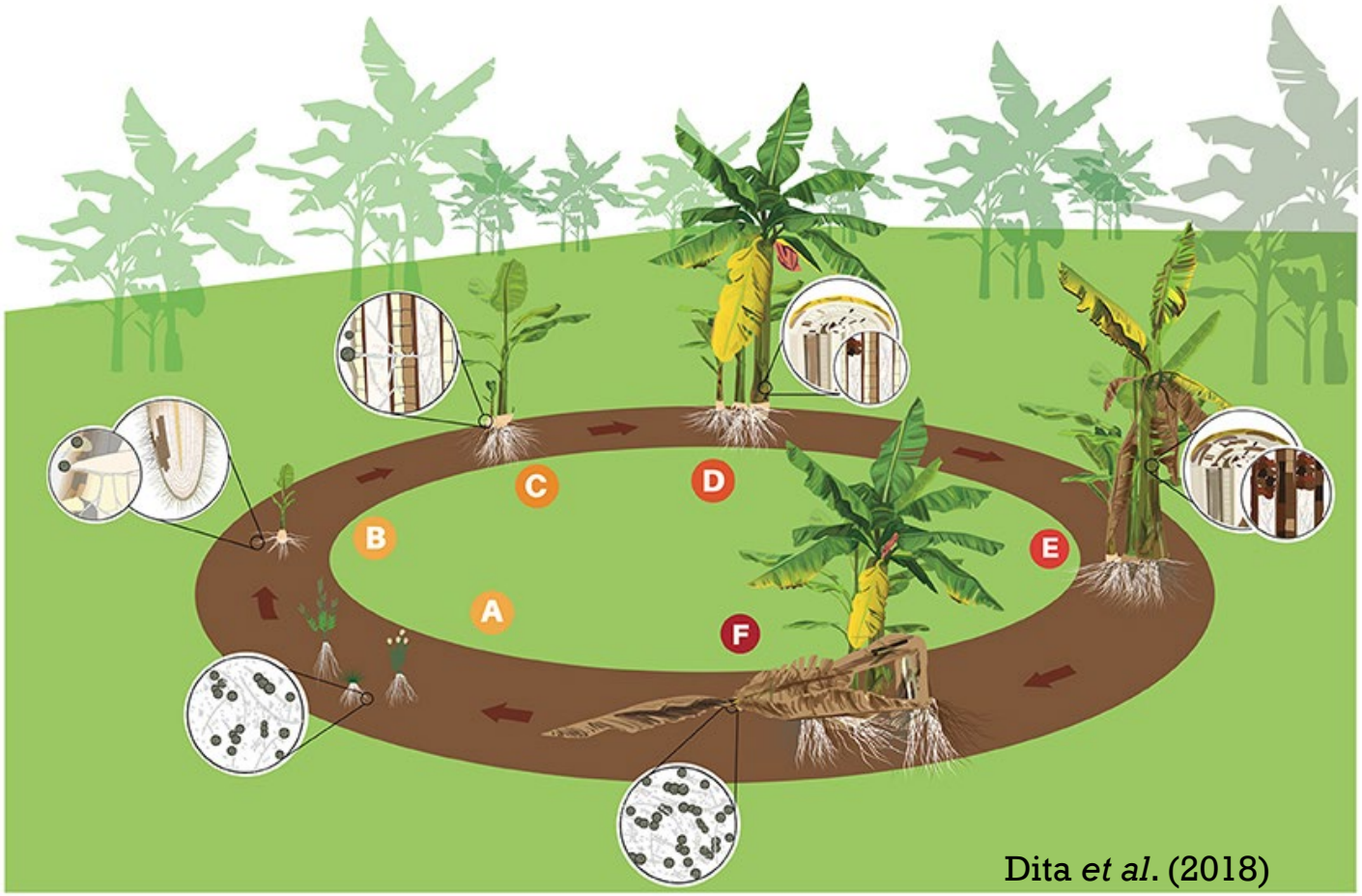


Abb. 57. *Fusarium oxysporum v. cubense*. Vergr. 1000. a. Makrokonidien der längeren pionnotalen Entwicklungsstufe; b, c, d. Makrokonidien der gewöhnlichen sporodochialen Entwicklungsstufe auf verschiedenen Nährböden; e. Chlamydosporen; f. Konidienträger

(Wollenweber & Reinking, 1935)

Fusarium wilt disease cycle

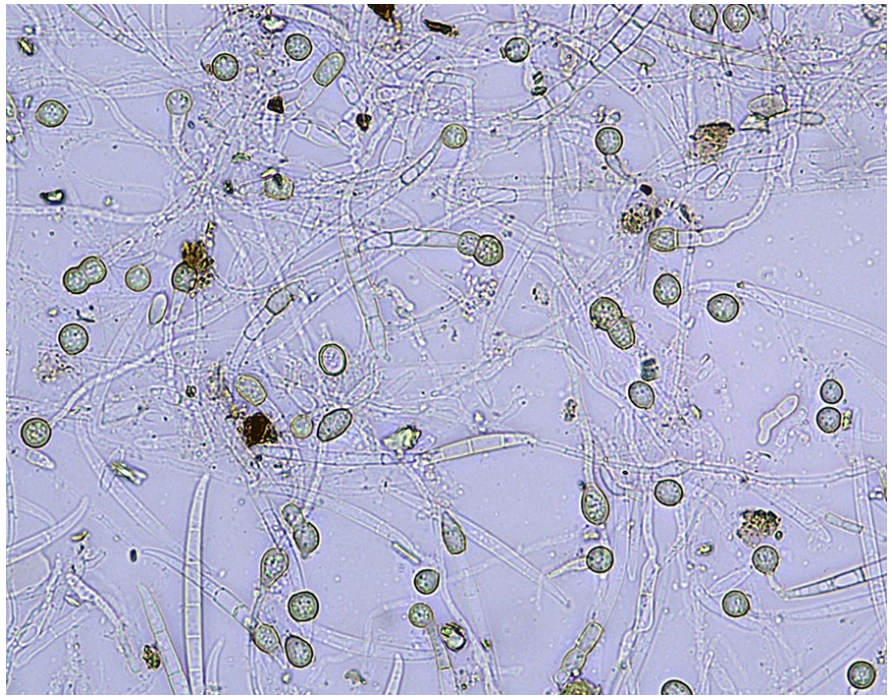
- Disease is initiated when root exudates stimulates the fungus to grow towards the roots
- It can either enter through wounds or lateral roots
- In plant that is susceptible it will enter the vascular system:
  - The host will produce gums and tyloses to try and occlude the pathogen
  - The pathogen will produce millions of spores as well as fusaric acid as a precursor molecule
- These two events will cause blockages of water and nutrients in the vascular system
- Resultant vascular discoloration and deterioration and progressing leaf yellowing and necrosis from the older to the younger leaves
- When the plant dies millions of spores go back into the soil ready to infect another plant



Dita *et al*. (2018)

Survival of Foc

- It can remain dormant in the remnants of decayed host tissue until stimulated to germinate by root exudates from banana, alternative hosts or pieces of fresh plant remains
- It is virtually impossible to eliminate from infested soil by crop rotation and even by bare fallowing
- It can survive for more than 40 years in abandoned fields
- Chlamydospores produced by Foc in dead and dying banana plants are released into the soil when the plant material decays
- Chlamydospores can persist for an extended period in plant debris in soil in the absence of a suitable host plant.



Pegg *et al.*, 2019

Survival of Foc

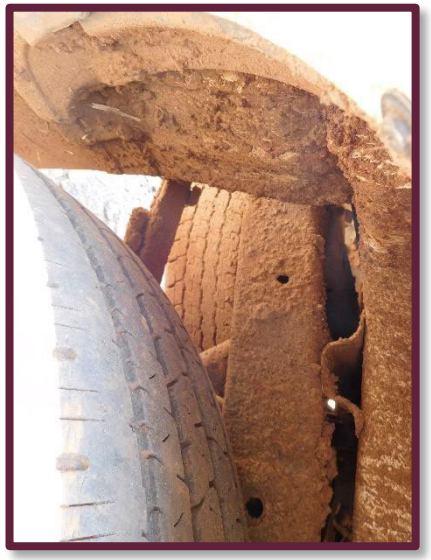
- Between countries:
  - Planting material (not tissue culture)
  - Shoes and clothing
  - Plantation tools
  - Containers?

Within countries:

Planting materials

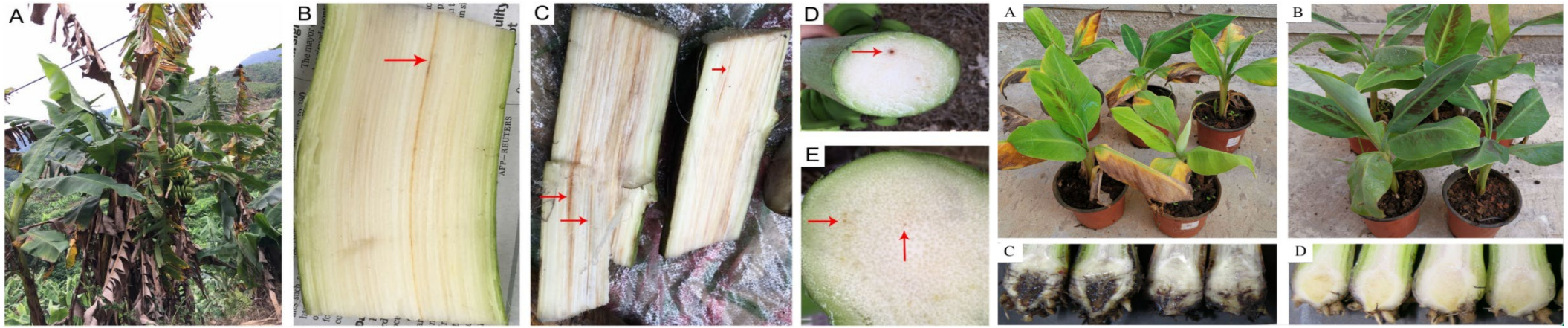
Shoes and vehicles

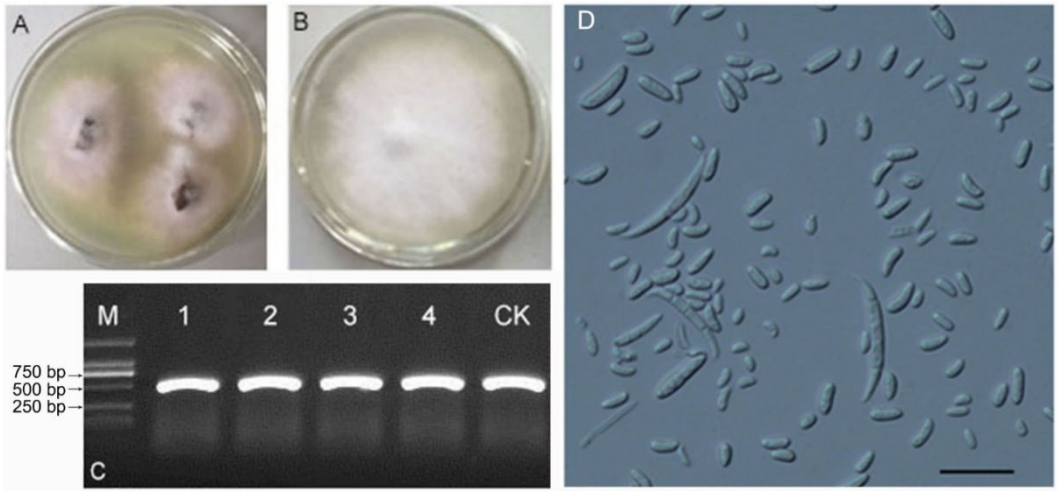
Machinery and tools Irrigation and flooding Feral animals



Methods of spread: Planting material

Foc TR4 can infect the peduncle





Plant Health Progress ◆ 2020 ◆ 00:1–5 https://doi.org/10.1094/PHP-08-20-0067-RS

Research

An Additional Threat to 'Cavendish' Banana Growers and Traders: The Infection of Banana Peduncles by *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4 (*Foc* TR4)

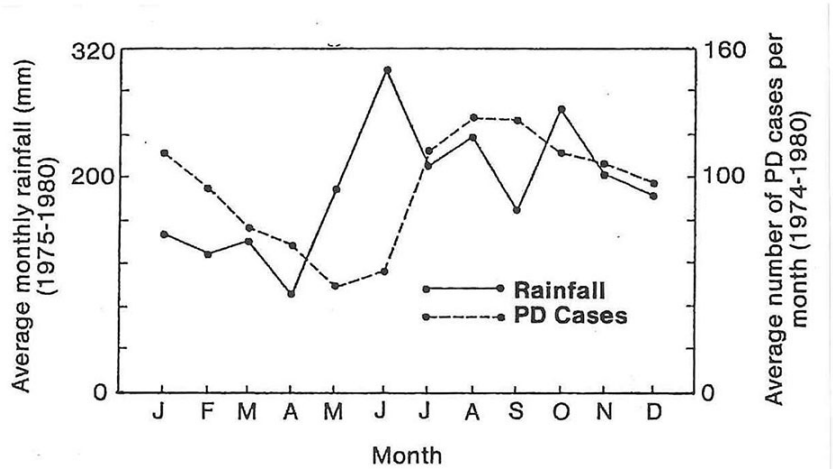
Tingting Bai,[1] Meng Qin,[2] Xundong Li,[1] Huacai Fan,[1] Shengtao Xu,[1] Li Zeng,[1] and Si-Jun Zheng[1,3,†]

[1] Agricultural Environment and Resources Institute, Yunnan Academy of Agricultural Sciences, Kunming 650205, Yunnan, China
[2] National Agro-Technical Extension and Service Center, Chaoyang District, Beijing 100125, China
[3] Bioversity International, Kunming 650205, Yunnan, China

Accepted for publication 24 August 2020.

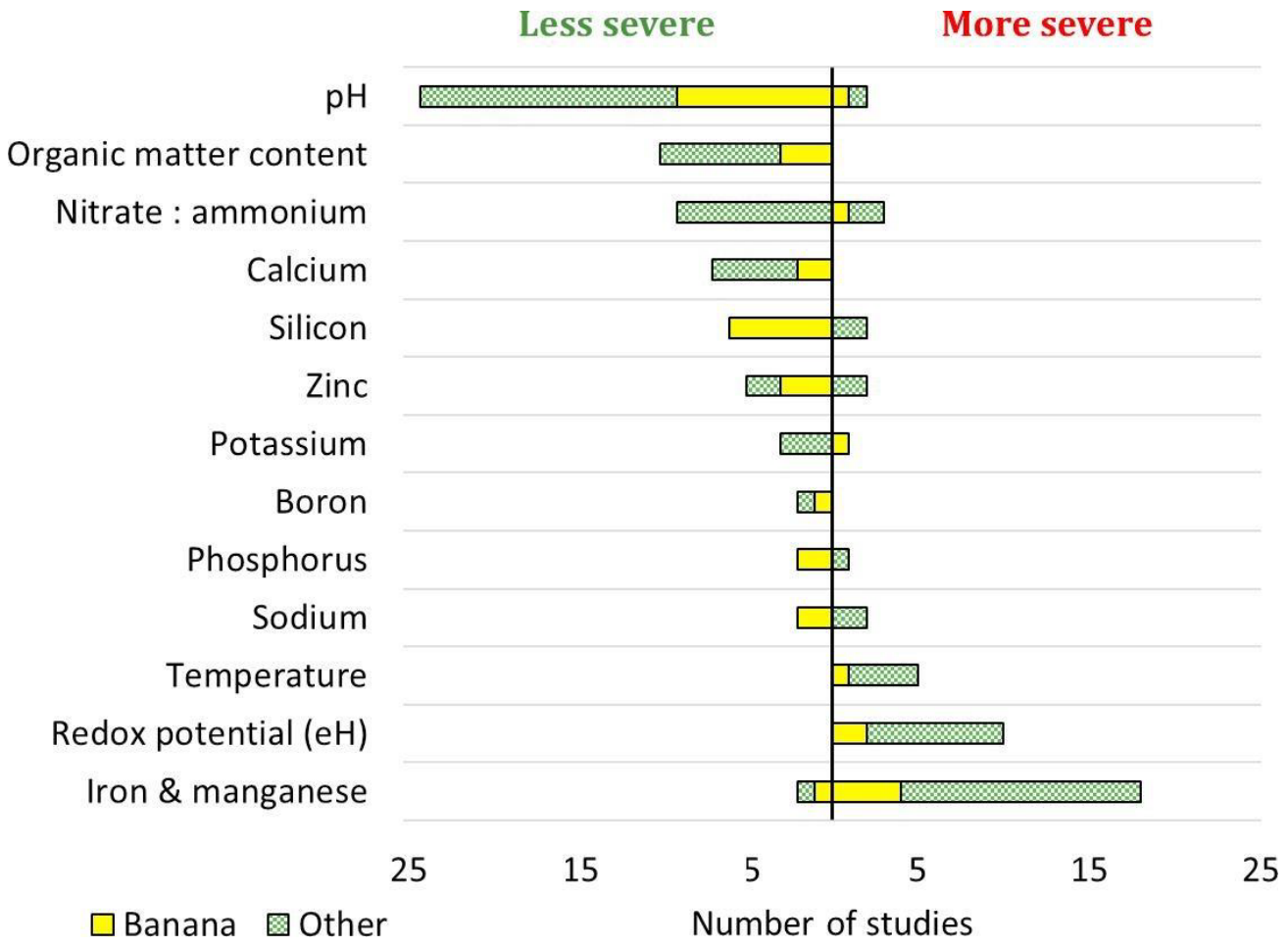
Methods of spread: Water

- Can survive in water containing soil and organic material for longer than 3 months
- Foc spore viability was reduced in stagnant water, probably due to anaerobic conditions when spores settled at the bottom
- Irrigation sources including rivers and dams
- Flooding events
- Lack of proper drainage



Methods of spread: Humans

Conditions promoting Foc TR4 severity



- Temperatures between 21-28 is optimal for Foc growth
- Fertile soils with good physical structure, and balanced fertilization will prolong the life of an infested plantation including high levels of organic matter, zinc, calcium, potassium and magnesium
- Nitrate (NO_3^-) reduces, whereas ammonium (NH_4^+) increases severity
- Silicon applications slightly reduced Foc under greenhouse conditions

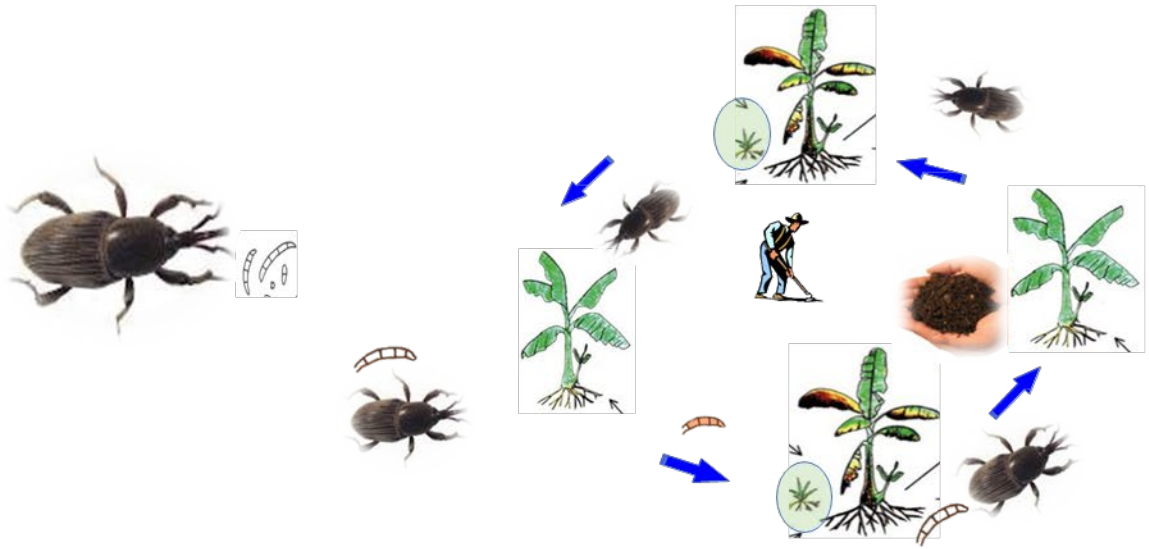
Conditions promoting Foc TR4 severity

The presence of nematodes and weevils that can cause wounds for infection can **increase severity** of disease but its **not** vectors of the disease

Australasian Plant Pathol. (2013) 42:543–549
DOI 10.1007/s13313-013-0214-2

Are banana weevil borers a vector in spreading *Fusarium oxysporum* f. sp. *cubense* tropical race 4 in banana plantations?

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



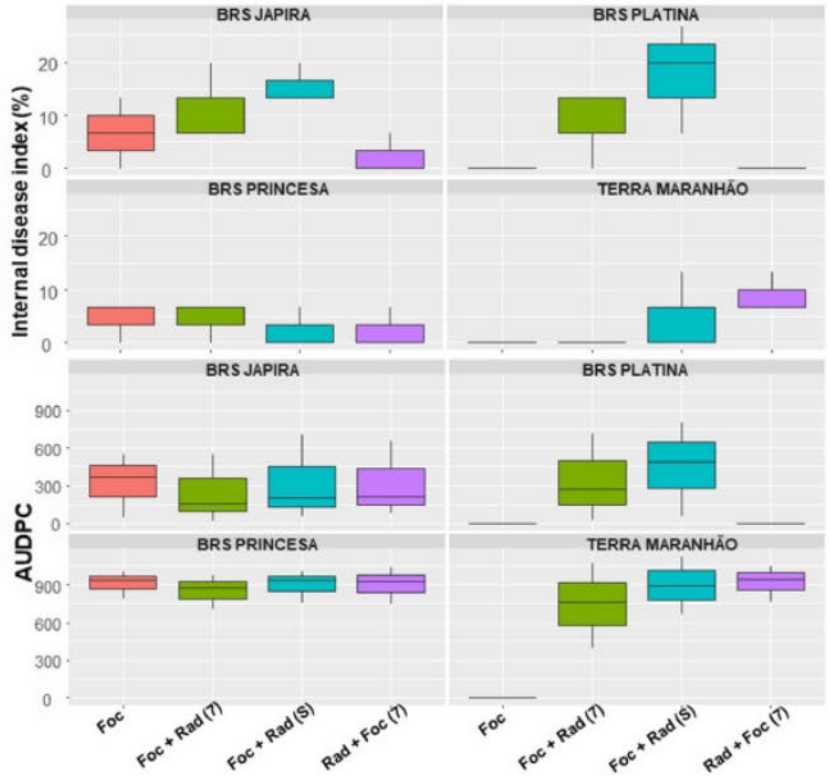


Fig. 2 Effects of the interaction between *Fusarium oxysporum* f. sp. *cubense* (isolate 0801, race 1) and *Radopholus similis* in banana cultivars. Foc: Foc inoculated isolated; Foc + Rad (7): Foc inoculated in the planting and *R. similis* inoculated after 7 days; Foc + Rad (S): Foc and *R. similis* inoculated simultaneously; Rad + Foc (7): *R. similis* inoculated in the planting planting and Foc inoculated after 7 days. AUDPC: area under the disease progress curve

Before starting surveillance

- Obtain permission from the Ministry of Agriculture and owners to survey their properties
- Provide the background and process of the surveillance to property owners
- Obtain information on the history and production practices of bananas
- It is an offence to obstruct an inspector or any other authorised person to conduct surveillance on properties planted to bananas

Surveillance equipment

- Boots and protective clothing to prevent the spread of the fungus
- Gloves
- Spray bottles
- Water, disinfectants and 70% alcohol/spiritus
- Machetes and cutting knives
- Scalpels and tweezers
- Envelopes and paper bags for the collection of samples
- Sterile paper towels
- Notebooks and pens
- Spray paint, marker pens, flagging tape and masking tape
- Electronics to take GPS coordinates and a camera
- Digital or paper based surveillance questionnaire

Finding a suspect plant

- Look for plants with yellow or wilted leaves and/or stem splitting
- Sampling must be conducted on all suspect plants
- **Suspect Cavendish** banana plants should be marked with flagging tape or spray paint, as well as the end of the row or block
  - Plants should only be cut down if superficial internal symptoms in the pseudostem cannot be observed
  - Felled plants should be burned if symptomatic to destroy inoculum
  - Felling plants could prevent further investigations if laboratory tests are inconclusive

Fusarium wilt symptoms: External symptoms

Leaves wilt and progressively buckle at the petiole resulting in a skirt of dead leaves draped around the plant. This is followed by a wilt and buckling of the leaves at the petiole base resulting in a skirt of dead leaves draped around the plant.

Courtesy of Altus Viljoen

Fusarium wilt symptoms: Advanced external symptoms

Courtesy of Altus Viljoen

Fusarium wilt symptoms: Pseudostem splitting

Courtesy of Altus Viljoen

Fusarium wilt symptoms: Rhizome discoloration

Courtesy of Altus Viljoen

Fusarium wilt symptoms: Internal symptoms

The most characteristic symptom may be seen by cutting through the pseudostem of an infected plant. Dark-brown to black discoloration of the water conducting tissues is evident.

Courtesy of Altus Viljoen

Fusarium wilt symptoms: Expression on different varieties

Cavendish

Pisang Awak

Gros Michel

Courtesy of Altus Viljoen

Fusarium wilt “like” symptoms

Banana bacterial wilt

Banana weevil

Calcium deficiency

Courtesy of Altus Viljoen

Bacterial wilt symptoms

Courtesy of Altus Viljoen

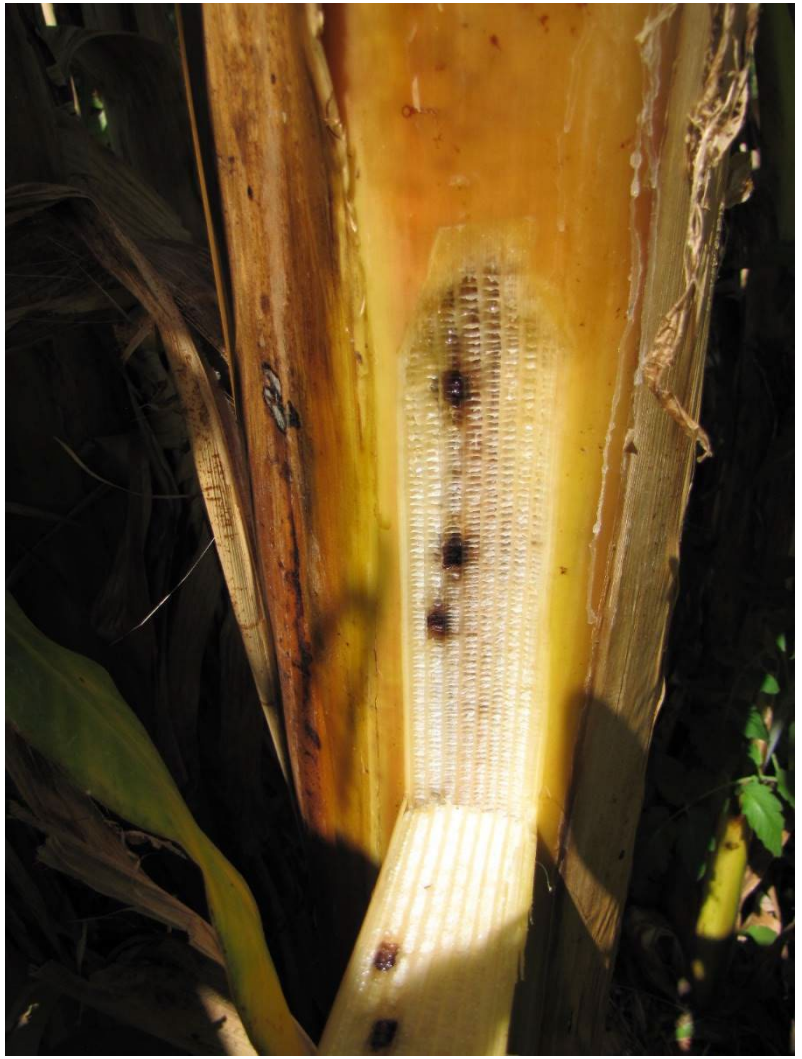
Armillaria corm rot

Courtesy of Altus Viljoen

Challenge of surveillance

Courtesy of Altus Viljoen

Challenge of surveillance



Courtesy of Altus Viljoen

Fusarium wilt sample collection

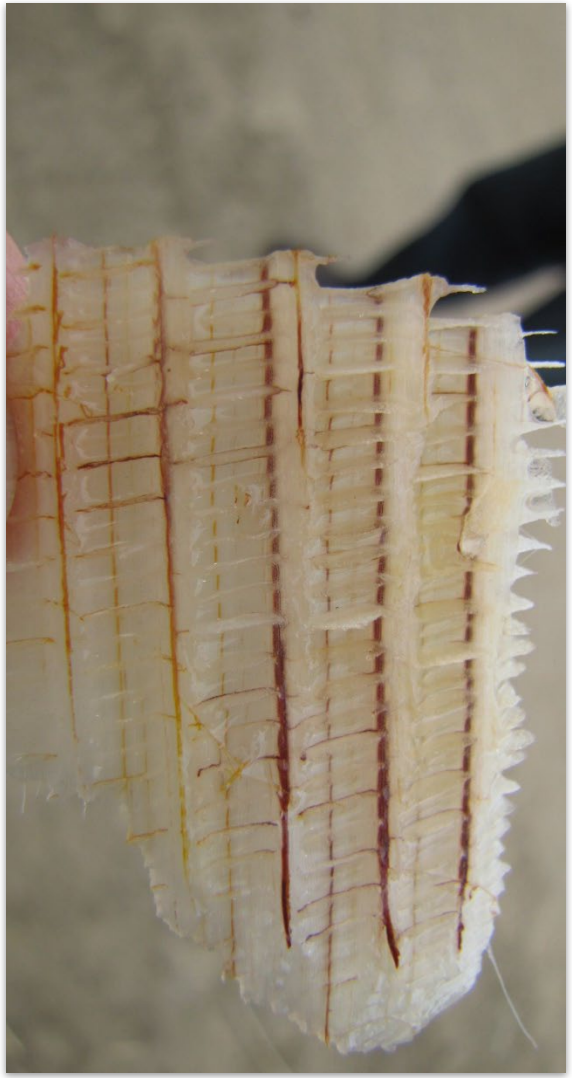
Collect vascular strands from banana pseudostems with typical Fusarium wilt symptoms (A). This can be achieved in a non-destructive way by slicing open the pseudostem on the side of yellow leaves (B), and by dissecting out discoloured xylem tissue (C).

Courtesy of Altus Viljoen

Fusarium wilt sample collection

Courtesy of Altus Viljoen

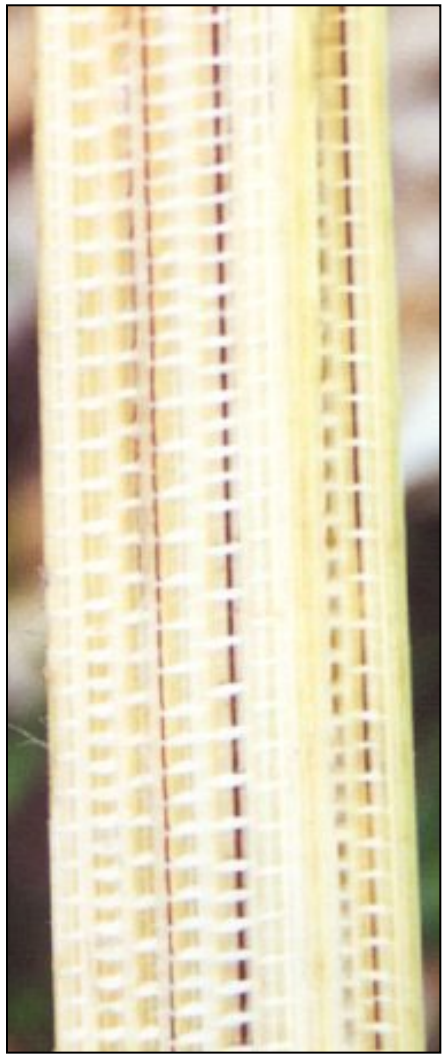
Fusarium wilt sample collection



Courtesy of Altus Viljoen

Fusarium wilt sample collection

- Collect specimen from pseudostem tissue with continuous vascular discoloration
- Take sample from as low in the pseudostem as possible but not from areas where decay is advanced
- The sample should be taken from as close to the center of the pseudostem as is possible as opposed to the outermost leaf bases
- If no symptoms in the pseudostem, check the rhizome
- Cut discoloured vascular strands out of pseudostem section as soon as possible after collection
- Take more than you need



Courtesy of Altus Viljoen

Fusarium wilt sample collection

Courtesy of Altus Viljoen

Fusarium wilt sample collection

- Banana tissue is very wet
- The risk of bacterial contamination of samples is high particularly in warm weather when samples can deteriorate rapidly
- The chance of recovering healthy cultures of *Foc* decreases as the sample deteriorates
- Samples should be kept in heavy **paper** bags or wrapped in paper (e.g. newspaper) until the strands can be excised
- **Avoid using plastic bags** as this causes the samples to sweat and promotes growth of bacteria

Courtesy of Altus Viljoen

Fusarium wilt sample collection

- Sample number (one sample number per plant)
- Date
- The variety of the host plant, including local names (and uses if known)
- Genomic constitution of host if known (e.g. AA, AAB, ABB etc.)
- Whether the plants sampled are growing in a garden, commercial plantation, village or wild situation
- Location
  - name of province or state, how far in what direction from nearest town, name of road, name of property if sample is from a commercial plantation etc.
-
- A map with sample numbers marked on it or GPS coordinates can be very useful
- Name of surveyor
- Other useful observations might include
  - the source of the planting material, whether the plant is growing in water-logged soil, how many plants are affected, what other varieties are growing around the diseased plant and are these diseased or healthy?

Courtesy of Altus Viljoen

Fusarium wilt sample collection

agriculture, forestry & fisheries

Department: Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Page 1

Directorate Plant Health

Permit No. P0089665

PERMIT FOR THE IMPORTATION OF CONTROLLED GOODS

In terms of the provisions of section 3(1) of the Agricultural Pests Act, 1983 (Act 36 of 1983) and subject to the conditions stated here under, authorisation is hereby granted to-

PROF ALTUS VILJOEN

Tel No: 021 808 4797

DEPT OF PLANT PATHOLOGY
STELLENBOSCH UNIVERSITY
PRIVATE BAG X1, MATIELAND
7602

to import into the Republic the following controlled goods REGULATED ARTICLES FOR LABORATORY ANALYSIS

AS PER ATTACHED CONDITIONS

300 SAMPLE (S)

Name and address of foreign supplier VARIOUS SUPPLIERS/COUNTRIES

Conditions 1. AS ATTACHED

Port of Entry : O R TAMBO INTERNATIONAL AIRPORT

Import authorized from 2018/07/06 TO 2019/07/06

IMPORTANT : This permit does not exempt the holder from the provisions of any other Act, ordinance or agreement

DEPARTMENT: AGRICULTURE FORESTRY AND FISHERIES
DIRECTORATE PLANT HEALTH
2023 -07- 06
DIRECTORATE PLANT HEALTH
P.O. BOX 40024 ARCADIA 0007

Date

Reference Number 9/18/87

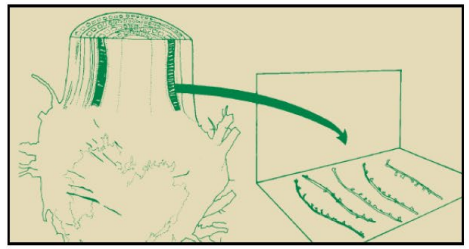
Executive Officer

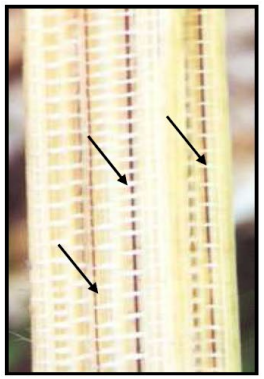
INQUIRIES : TEL.: (012)319 6102 (Christina Makgoba) FAX: (012)319 6370

Preparation of Fusarium wilt sample for *Foc* analysis

How to collect samples:

- Samples should be taken from 3-4 randomly selected plants in an infected field site
- Cut out sections of tissue from low in pseudostem where many discolored strands are present
- Dissect out 5-8 individual discoloured vascular strands and place between sterile blotting paper to air dry
- When dry, place the blotting paper and strands in a paper envelope (never a plastic bag!), seal and label with the sample number, cultivar name, locality, collector's name and date
- Send the sample by fast mail to Prof Altus Viljoen, Dept. Plant Pathology, Lombardi building 2024, Corners Neethling and Victoria streets, University of Stellenbosch, Stellenbosch, South Africa





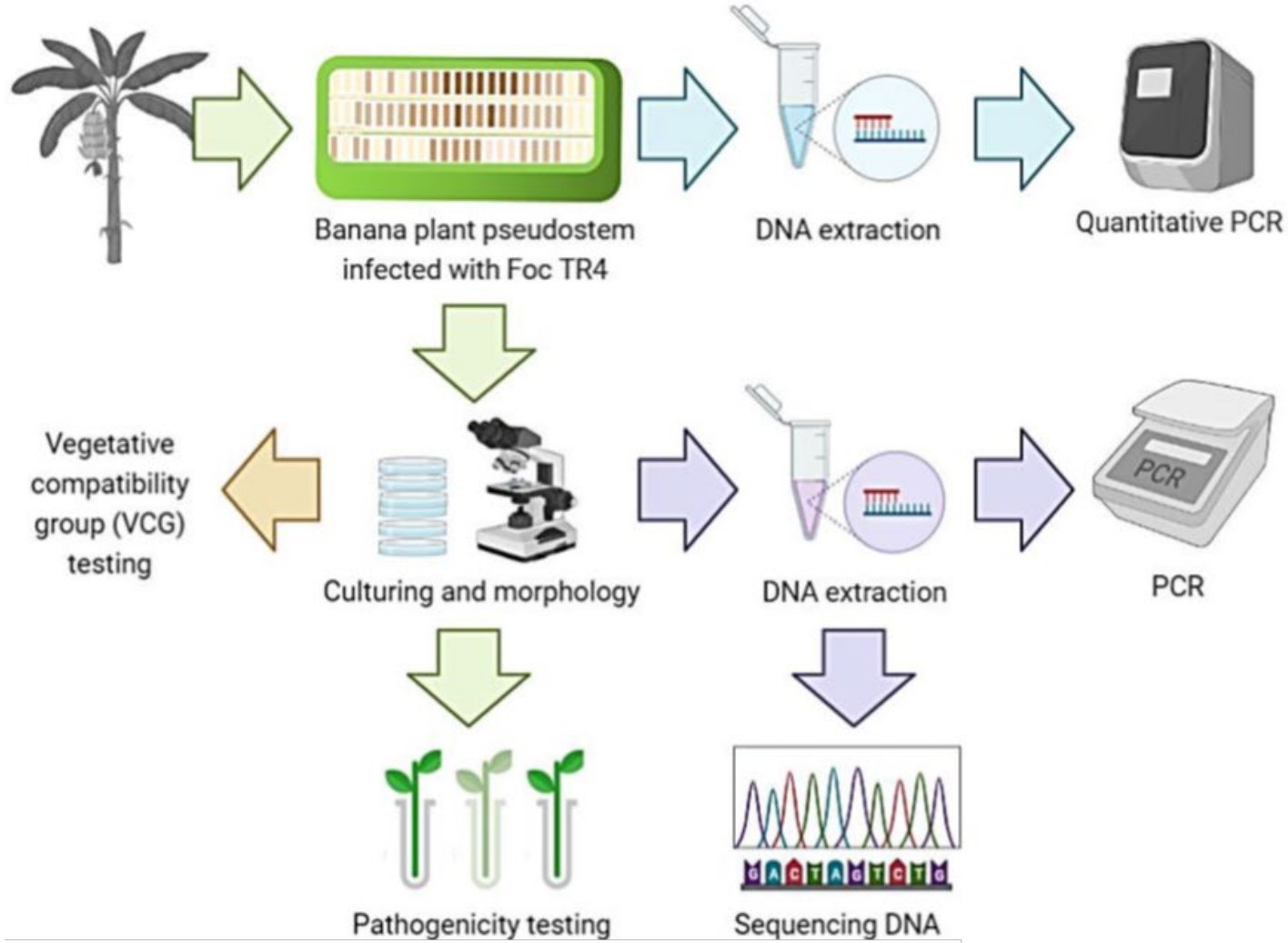
Courtesy of Altus Viljoen

Decontamination of surveillance team

- Please be aware that **you** can also spread the disease during the survey
- Take disinfecting solutions with to the surveillance sites and install a disinfection station at each new site
- Make sure the disinfection solution is working properly
- Usually quaternary ammonium compounds is very effective
- Remove soil before disinfection
  - Brush or wear disposable covers
  - Soil lowers the efficacy of disinfectants
- Disinfect sampling tools after each sample is collected
- Disinfect your hands and shoes
- Disinfect vehicle tyres when visiting an infected team (Or don't drive onto known infected properties)
- Always visit known or suspected infected properties last

Courtesy of Altus Viljoen

Fusarium wilt diagnostic process



Courtesy of M. Matthews

Importance of robust diagnosis

Cultivation-dependent and -independent approaches for determining the distribution of *Fusarium oxysporum* f. sp. *cubense* tropical race 4 in soil of banana plantations in Côte d'Ivoire

doi.org/10.33500/ijambr.2021.09.008

Toualy Serge Ouina[1,3]*, Voko Bi Rosin Don Rodrigue[1], Stéphanie Gente[2], David Garon[2], Jean-Philippe Rioult[2], Jean-Michel Panoff[3] and Marina Koussémon-Camara[4]

[1]Laboratory of Agrovalorisation, Department of Biochemistry-Microbiology, University Jean Lorougnon Guédé, BP 150 Daloa, Côte d'Ivoire.
[2]University of Caen Normandy, Food, Bioprocess, Toxicology, Environments (Team 4651) - Environmental Toxicology, Aerial Environments and Cancer, 14000 Caen, France.
[3]Food, Bioprocess, Toxicology, Environments (Team 4651) - Food Matrix and Microbiota - Campus 1, Institute for Fundamental and Applied Biology, University of Caen Normandy, Esplanade de la Paix, 14032 Caen cedex 5, France.
[4]Laboratory of Biotechnology and Food Microbiology, Faculty of Sciences and Food Technology, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Cote d'Ivoire.

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Article Type:
Full Length Research Article

ABSTRACT

Banana *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *cubense* tropical race 4 (Foc TR4), is a worrying destructive disease affecting banana plantations with devastating effects on food safety and economy of many tropical countries. There is few information about this disease in Côte d'Ivoire. This study was aimed at assessing the distribution of Foc TR4 in banana plantations of the Lôh-Djiboua region (Côte d'Ivoire) and then finding the most sensitive research methods of Foc TR4 in the soil of these plantations. Foc TR4 was searched for in soils of 8 banana plantations through culture-dependent (CDMA) and culture-independent molecular approaches (CIMA). CDMA consisted of the isolation of *F. oxysporum* from culture media and subjecting it to molecular confirmation tests at subspecies level. CIMA consisted of searching for Foc TR4 directly from soil samples with molecular probes. Molecular detection limit of Foc TR4 was also searched for in the mix: i) Foc TR4 DNA and ultra-pure water and ii) Foc TR4 DNA and pool of DNA from Foc TR4 free soil. Foc TR4 was found in 5 plantations. For 16 of the soil samples analyzed, CDMA showed that 8 samples belonging to 5 plantations harboured Foc TR4 whereas with CIMA, Foc TR4 were isolated from 3 samples belonging to 2 plantations. FocTR4 DNA was detected in the soil by polymerase chain reaction (PCR) from 73 ± 6.02 ng. The combination of CDMA and CIMA led to a better estimate of Foc TR4 distribution. CDMA was more sensitive for detecting Foc TR4 in the soil of the plantations investigated.

INTRODUCTION

ABC RURAL

Independent review blames unreliable test, not Biosecurity Queensland, for false panama diagnosis

ABC Rural / By Charlie McKillop

Posted Mon 15 Feb 2016 at 5:38am, updated Mon 15 Feb 2016 at 11:39pm

"We're just trying to digest it and it was good to be able to tell the crew at smoko that we have won this long battle."

Mr Reppel said the flaws in the diagnosis process had broader implications for the banana industry, in Australia and globally.

"The PCR test has been held up as the Holy Grail of testing and it's been proved to be less than reliable," he said.

"From the start, I was always concerned and that's mainly because when we took the initial samples of the (suspect) tree, it had no evidence of any vascular issues.

"So that's where I've been questioning it and really waited with bated breath for our second and third lot of testings to come back.

"And, as they kept came back negative, I suppose my - I wouldn't call it scepticism - but my questioning got deeper and deeper."

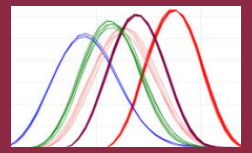
This Tully banana farm is still locked down with Panama Tropical Race 4, despite another farm being given the all-clear by Biosecurity Queensland. *(Charlie McKillop)*

The PCR test has been held up as the Holy Grail of testing and it's been proved to be less than reliable.

Workflow for diagnosis of Foc

Sample

DNA extraction from plant material and qPCR



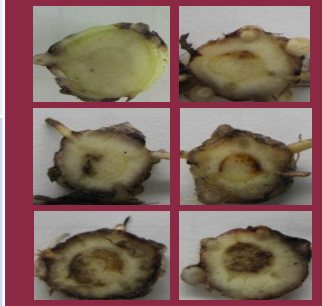
Isolation and purification

DNA extraction and PCR

Gene sequencing

VCG testing

Pathogenicity testing



	Direct qPCR	Purification and PCR	Sequencing	VCG testing	Pathogenicity
Time:	1-2 days	10-14 days	2-3 weeks	3-6 weeks	2-3 months
Pro's:	• Fast and sensitive • Direct detection from environmental material	• Affordable and accessible • DNA does not pose quarantine risk	• Comparison to public databases • DNA does not pose quarantine risk	• Very accurate • Can distinguish different Foc VCGs • Relatively cheap	• Only method that can reliably confirm virulence
Con's:	• Expensive • Specialized skill • Risk of detection of false positives • Based on a single gene region • Sampling bias that can be introduced	• Some PCR protocols have picked up false positives • Based on single gene region	• Expensive • Specialized skill • Cannot distinguish between closely related Foc VCGs	• Time consuming • Specialized skills • Quarantine risk that standard testers strains pose	• Time consuming • Access to plant material • Specialiszd skill • Quarantine risk • Cannot distinguish between closely related Foc VCGs • Isolates of the same VCGs might show differences in virulence

Africa Phytosanitary Programme

Train-the-Trainer workshop

Thank you

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