



Food and Agriculture  
Organization of the  
United Nations



International  
Plant Protection  
Convention

MAR  
2023

ENG

# Prevention, preparedness and response guidelines for Fusarium Tropical Race 4 (TR4) of banana



Funded by the  
European Union



MAR  
2023

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**Required citation:**

IPPC Secretariat. 2023. *Prevention, preparedness and response guidelines for Fusarium Tropical Race 4 (TR4) of banana*. Rome. FAO on behalf of the Secretariat of the International Plant Protection Convention. <https://doi.org/10.4060/cc4865en>

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**Publication history**

2023-03 Version 1.0 The Implementation and Capacity Development Committee (IC) established an IC Team on Fusarium TR4 that prioritized the development of prevention, preparedness, and response guidelines.

## Abstract

The guidelines focus on prevention, preparedness and response for the safe international trade movement of the plants, plant products and other regulated articles that can spread *Fusarium* TR4. The guidelines provide relevant biological and scientific information to inform the measures that need to be considered and implemented to develop a plan when the pest is still absent, comprising elements for pest risk analysis, phytosanitary regulations, official diagnostics and surveillance for detection purposes. The guidelines also provide information on first response, including surveillance for delimiting purposes and phytosanitary measures to contain an outbreak. The guidelines gather information, tools and materials, and comprise actions that national plant protection organizations (NPPOs) and relevant stakeholders may undertake before the introduction of *Fusarium oxysporum* f. sp. *cubense* (*Foc*) TR4 into new territories.



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## Acknowledgements

This document provides guidance for the prevention, preparedness and response for Fusarium TR4 outbreaks. It was created under the auspices of the Secretariat of the International Plant Protection Convention ( IPPC) as a component of the Strategic Framework for the IPPC 2020-2030: *Protecting global plant resources and facilitating safe trade*. This work has been developed and peer-reviewed by selected experts worldwide under the coordination of the IPPC Secretariat with the oversight of the IPPC Implementation and Capacity Development Committee (IC).

## Abbreviations and acronyms

AA	Diploid group cultivars of <i>Musa acuminata</i>
AAA	Triploid group cultivars of <i>Musa acuminata</i>
AAB	Triploid cultivars of plantain
ABB	Triploid cultivars of cooking banana
APHIS	Animal and Plant Health Inspection Service
BI	Bayesian inference
CABI	Centre for Agriculture and Bioscience International
CELAC	Community of Latin American and Caribbean States
CGIAR	Consultative Group for International Agricultural Research
CLA	carnation leaf agar
CPM	Commission on Phytosanitary Measures
CTAB	cetyl trimethyl ammonium bromide
DDAC	didecyldimethylammonium chloride
DNA	deoxyribonucleic acid
EFSA	European Food Safety Authority
EPPO	European and Mediterranean Plant Protection Organization
EuFMD	European Commission for the Control of Foot-and-Mouth Disease
f. sp.	<i>Formae speciales</i>
Foc	<i>Fusarium oxysporum</i> f. sp. <i>cubense</i>
FOSC	<i>Fusarium oxysporum</i> species complex
FWB	Fusarium wilt of banana
GICSV	Inter-American Plant Health Coordination Group
GIS	geographic information system
ML	maximum likelihood

<b>MP</b>	maximum parsimony
<b>NPPO</b>	national plant protection organization
<b>NRO</b>	National Reporting Obligation
<b>RPPO</b>	regional plant protection organization
<b>IC</b>	Implementation and Capacity Development Committee
<b>ISPM</b>	International Standards for Phytosanitary Measures
<b>IPP</b>	International Phytosanitary Portal
<b>IPPC</b>	International Plant Protection Convention
<b>LAMP</b>	loop-mediated isothermal amplification
<b>OIRSA</b>	Organismo Internacional Regional de Sanidad Agropecuaria
<b>PAHO</b>	Pan American Health Organization
<b>PCR</b>	polymerase chain reaction
<b>PDA</b>	potato dextrose agar
<b>PLANTPLAN</b>	Australian Emergency Plant Pest Response Plan
<b>PRA</b>	pest risk analysis
<b>ProMusa</b>	Platform for sharing news, knowledge, and information on bananas
<b>QA</b>	quaternary ammonium
<b>RealAmp assay</b>	Real-time fluorescence loop-mediated isothermal amplification assay
<b>SNA</b>	Spezieller Nährstoffärmer agar
<b>STR4</b>	Subtropical Race 4
<b>TR4</b>	Tropical Race 4
<b>UCP</b>	Unified Command Post
<b>USDA</b>	United States Department of Agriculture
<b>UNISDR</b>	United Nations International Strategy for Disaster Reduction
<b>VCGs</b>	vegetative compatibility groups
<b>WHO</b>	World Health Organization
<b>WOAH</b>	World Organisation for Animal Health

# Introduction

Fusarium wilt of banana (FWB) is a banana (*Musa* spp.) disease that was hitherto considered to be caused by the fungal pathogen *Fusarium oxysporum* f. sp. *cubense* (*Foc*). Recent data, however, show that FWB is caused by multiple Fusarium species (Maryani *et al.* 2019; Westerhoven *et al.*, 2022a). In the mid-twentieth century (1950s and 1960s), FWB affected Gros Michel (AAA) banana production in the Americas. It caused the collapse of the banana industry based on this cultivar. A feasible solution was to replace the Gros Michel cultivar with a resistant banana varietal group discovered in Southeast Asia, Cavendish (AAA), which includes banana cultivars such as Grand Naine and Williams. However, a new strain emerged that was later called Tropical Race 4 (Fusarium TR4) affecting Cavendish bananas in Taiwan Province of China in the late 1960s and, by the 1990s, severely impacting banana production under tropical conditions in Southeast Asia. At present, the Cavendish varieties, as well as other banana cultivars, including cooking bananas, succumb to Fusarium TR4. This fungus is and has been the biggest threat to banana production. This is evidenced by an increased number of Fusarium TR4 incursions in banana-producing countries where this fungus is a quarantine pest. Banana production provides social and economic benefits for producing countries. It is a source of food and income for many people worldwide, especially in countries where the production of cooking bananas and plantains has an important role in subsistence or smallholder systems. Consequently, the introduction and spread of Fusarium TR4 must be prevented.

Fusarium TR4 is a primary concern for the IPPC community. In recognition of this, the Implementation and Capacity Development Committee (IC) established an IC Team on Fusarium TR4 that prioritized the development of prevention, preparedness and response guidelines as a critical output to help countries avoid the international spread of Fusarium TR4. These guidelines gather information, tools and materials, and set out actions that national plant protection organizations (NPPOs) and relevant stakeholders may undertake before a Fusarium TR4 introduction into new territory. Such actions aim to prevent an introduction and to prepare countries for the response and containment of a first outbreak. Information and actions for Fusarium TR4 management, once introduced and established, are available in other documents or information prepared by FAO, international organizations and research centres.

These guidelines focus on prevention, preparedness and response for the safe international trade movement of plants, plant products and other regulated articles that can spread Fusarium TR4. The guidelines provide relevant biological and scientific information to inform the measures that need to be considered and implemented to develop a plan when the pest is still absent, comprising elements for pest risk analysis, phytosanitary regulations, official diagnostics and surveillance for detection purposes. The guidelines also provide information on first response, including surveillance for delimiting purposes and phytosanitary measures to contain an outbreak.

# 1. Distribution and biology of Fusarium TR4

## 1.1 DISTRIBUTION OF FUSARIUM TR4

To date, Fusarium TR4 has been reported in 20 countries worldwide (Westerhoven *et al.*, 2022a). Taiwan Province of China was the first place where FWB was found in the Cavendish cultivar in the late 1960s. That does not mean that Taiwan Province of China is the biological centre of origin of Fusarium TR4 (Maryani *et al.*, 2019, based on phylogenetic inferences, suggest Indonesia as the biological centre of origin). In Southeast Asia, Fusarium TR4 was reported in the 1990s (Buddenhagen, 2009). Then, Fusarium TR4 spread to Africa in 2013 and to Latin America in 2019 and 2021 (Westerhoven *et al.*, 2022b; García-Bastidas *et al.*, 2013; Ordoñez *et al.*, 2015; García-Bastidas *et al.*, 2020; SENASA, 2021). Under the scope of the IPPC, contracting parties agree to report the occurrence, outbreak or spread of pests that may be of potential danger. As a result, when occurrence of Fusarium TR4 is identified in new territories, NPPOs must update their national profile in the International Phytosanitary Portal (IPP).

The European and Mediterranean Plant Protection Organization (EPPO), the Centre for Agriculture and Bioscience International (CABI), and the Platform for sharing news, knowledge, and information on bananas (ProMusa) provide documents on the worldwide distribution of Fusarium TR4 based on scientific publications and other sources. These sources may be consulted for informative purposes. For regulatory purposes however, the official phytosanitary condition needs to be confirmed by the NPPO of the respective country.

## 1.2 BIOLOGY OF FUSARIUM TR4

- ◆ ***Fusarium oxysporum* species complex (FOSC):** *Fusarium oxysporum* Schlechtend.: Fr. 1824 (Ascomycota, Hypocreales, Nectriaceae) is a cosmopolitan soil-borne asexual fungus known to harbour both pathogenic (to plants, animals and humans) and non-pathogenic strains (Lombard *et al.*, 2019; Baayen *et al.*, 2000). Members of FOSC are ubiquitous soil-borne pathogens responsible for vascular wilts, rots and damping-off diseases of a broad range of important crops (O'Donnell *et al.*, 2009).
- ◆ **Formae speciales:** There are over 150 different "formae speciales" (f. sp.) of *Fusarium oxysporum*. Each one is restricted to a narrow range of host plant species. For example, *F. oxysporum* f. sp. *cubense* (E.F. Smith) Snyder and Hansen, causes Fusarium wilt of banana (FWB). However, it has now been demonstrated that *formae speciales* have evolved independently. That means *F. oxysporum* f. sp. *cubense* is polyphyletic (derived from more than one common evolutionary ancestor) with strains in different clades or lineages (Summerell 2019; Laurence *et al.*, 2015; Ploetz 2006; O'Donnell *et al.*, 1998). Additional subspecific classification systems for *formae speciales* of *F. oxysporum*, such as physiological races and vegetative compatibility groups (VCGs), have also been introduced (Lombard *et al.*, 2019).
- ◆ **The race concept:** The race concept has been used to classify strains causing FWB since the mid-1900s (Ploetz, 2006). There are three races identified based on the pathogenicity of different banana cultivars (Stover, 1990). Race 1 is known for causing FWB

EPPO	CABI	PROMUSA
		
<a href="https://bit.ly/3x5Nplo">https://bit.ly/3x5Nplo</a>	<a href="https://bit.ly/3cTXfji">https://bit.ly/3cTXfji</a>	<a href="https://bit.ly/3Qodfrz">https://bit.ly/3Qodfrz</a>

in Gros Michel (AAA genome group), Silk (AAB), Pisangawak (ABB), Abaca (AA), "Maqueño" (AAB) and Pome (AAB) cultivars. Race 2 affects cooking bananas, especially those in the Bluggoe subgroup (ABB). Race 4 is further divided into subtropical (affecting Cavendish cultivar under biotic/abiotic stresses) and TR4 responsible for causing disease on Cavendish bananas regardless of their biotic or abiotic stresses. Additionally, it has been reported to cause disease to cultivars which are race 1 and race 2 susceptible. In contrast, races 1 and 2 cannot affect Cavendish bananas since their clones are resistant to these races. Reported detection of *Foc* on *Heliconia* species suggests the existence of another race (race 3). However, it is not confirmed because the original materials are unavailable (Ploetz, 2015, 2006; Waite and Stover, 1960). Fusarium TR4 is a quarantine pest in most banana-producing countries, while *Foc* races 1 and 2 have a wide distribution.

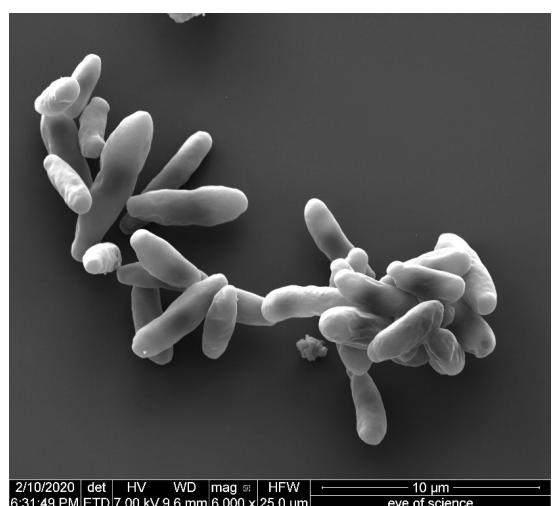
- ◆ **Vegetative compatibility groups (VCGs):** Other methods to describe diversity, such as vegetative compatibility groups (VCGs), result in diverse evolutionary lineages recognized in global populations, with over 24 VCGs (Meldrum *et al.*, 2013). Contemporary genome sequencing data further detail the biological diversity of *Fusarium* spp. infecting bananas (Maryani *et al.*, 2019). The reduced costs of genome sequencing make VCG testing increasingly redundant for diversity analyses.
- ◆ **New nomenclature proposed:** In 2019, *Fusarium* spp. infecting bananas were found to be genetically diverse. According to Maryani *et al.* (2019), *Fusarium* TR4 showed a large clonal genomic identity, resulting in a proposal to change the nomenclature and reclassify as the new species *Fusarium odoratissimum*, representing the VCG01213/16. Other physiological races are distributed over several other genotypes that are currently proposed as new *Fusarium* species. However, Torres-Bedoya *et al.* (2021) reported they could not replicate the phylogeny and clades obtained by Maryani *et al.* (2019), and reported differences in the phylogeny. According to Westerhoven *et al.* (2022a), this modified nomenclature raised some controversy and therefore awaits additional conclusive data. Most experts, however, agree that *Fusarium* TR4 is a clonal lineage and genetically so dissimilar from other *Fusarium* spp. infecting banana that it can be justifiably recognized as a new species.

### 1.2.1 Morphology

*Fusarium* TR4 is an asexual (anamorphic) fungus without a known sexual stage (teleomorph). It produces three types of asexual conidia: microconidia, macroconidia and chlamydospores. (Ploetz, 2006). Figures 1, 2 and 3 show the reproductive structures:

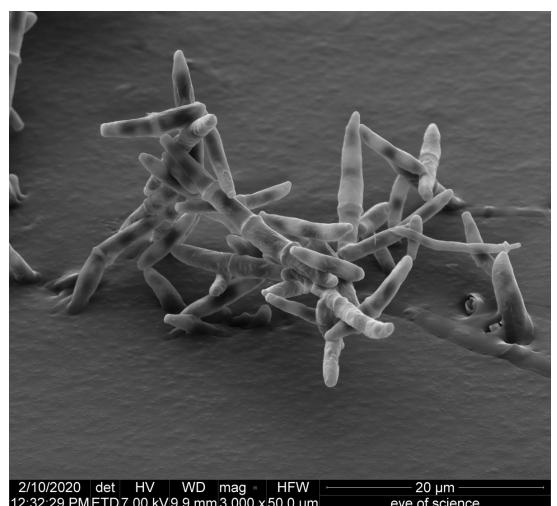
- ◆ **Microconidia:** (i) oval shaped, (ii) one- or two-celled, (iii) 5 µm to 16 µm × 2.4 µm to 3.5 µm, (iv) born in false heads (Ploetz, 2006)

**Figure 1: Electron microscope photography of *Fusarium* TR4 microconidia**



- ◆ **Macroconidia:** (i) sickle-shaped with foot-shaped basal cells, (ii) four- to eight-celled, (iii) 27 µm to 55 µm × 3.3 µm to 5.5 µm (Ploetz, 2006)

**Figure 2: Electron microscope photography of *Fusarium* TR4 macroconidia**



- ◆ **Chlamydospore:** (i) usually globose, (ii) 7 to 11 µm in diameter

**Figure 3: Electron microscope photography of Fusarium TR4 chlamydospore**



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### 1.2.2 The musaceous host and symptoms in suspect plants

- ◆ **Main host:** Fusarium TR4 is highly specialized and affects banana plants (*Musa* spp.). Weeds and grasses can be alternative hosts of Fusarium TR4 but usually do not exhibit disease symptoms (Ploetz, 2015a). Banana plants of the Cavendish cultivar suspected of being infected by Fusarium TR4 can be recognized in the field by the presence of the typical symptoms of the disease. However, it is necessary to undertake molecular (polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP), sequencing) or biological (VCG) tests to diagnose the pathogen's presence correctly.
- ◆ **Symptoms:** The noticeable primary symptoms of FWB are the common symptoms of all diseases that affect plant vascular systems. Figures 4, 5 and 6 show and describe external symptoms, such as yellowing of older leaves, collapsed older leaves hanging down the pseudostem and split pseudo stem. Figures 7 and 8 show and describe internal symptoms such as yellow, reddish or brown-black discoloration of the stem or corm's vascular tissue and vascular discoloration in the early and late stages of infection.
- ◆ **Leaf yellowing:** (i) Yellow leaf edges are observed in the early stages in older leaves, (ii) the yellowing progresses across the whole leaf, (iii) brown or black leaf edges can be present, (iv) young leaves may still be green and upright while older leaves collapse.

**Figure 4: Symptoms of leaf yellowing in banana**



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- ◆ **Wilting:** (i) The older leaves collapse, die and hang around the pseudostem, forming a skirt of dead leaves around the pseudostem, (ii) the yellowing typically progresses from older to younger leaves, (iii) younger leaves start to show typical symptoms.

**Figure 5: Symptoms of wilting in banana**



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- ◆ **Stem splitting:** (i) In some cases, a growth distortion may be manifested, which is difficult to detect in the field, however, commonly is revealed as a split into two or three layers on the pseudostem, (ii) the split appears later, deeper and higher up.

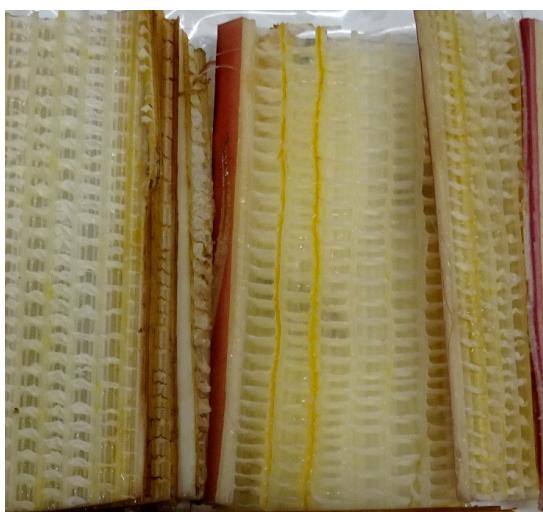
**Figure 6: Symptoms of stem splitting in banana**



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- ◆ **Yellow vascular discoloration:** Yellow vascular discoloration is an early symptom of the disease.

**Figure 7: Early internal symptoms of yellow vascular discoloration in banana**



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- ◆ **Dark vascular discoloration:** Advanced internal symptoms appear as dark red vascular discoloration.

**Figure 8: Advanced internal symptoms of dark red vascular discoloration in banana**



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### 1.2.3 Epidemiology

- ◆ **Survival structures:** Chlamydospores are believed to survive in the soil for a prolonged time (Pegg *et al.*, 2019), and evidence shows that they can also survive in non-host plants (Ploetz, 2015a; Postic *et al.*, 2012; Hennessy *et al.*, 2005). Chlamydospores in the soil are stimulated to germinate by nutrients in the exudates from banana roots and non-hosts. Those infecting the tips of secondary and tertiary roots of bananas penetrate the root cap and zone of elongation and establish an intercellular parasitic relationship in the root cortex before entering xylem vascular elements (Pegg *et al.*, 2019).
- ◆ **Spread of Fusarium TR4:** The spread of Fusarium TR4 at short and long distances, or from farm to farm or other locations locally or between countries or continents is mostly caused by anthropogenic factors such as movement of plant parts, biotic factors such as nematodes and weevils, natural factors such as water runoff, and movement of spore-bearing soil carried on vehicles, machinery, tools, equipment, infected plant propagation material and footwear (Mur *et al.*, 2016; Nasir *et al.*, 2003; Deltour *et al.*, 2017; Dong *et al.*, 2016; Peng *et al.*, 1999; Fortunato *et al.*, 2012a; Fortunato *et al.*, 2012b; Wang *et al.*, 2017; Domínguez *et al.*, 2001; Fernandez-Falcon *et al.*, 2004; Pegg *et al.*, 2019; Dita *et al.*, 2018).

♦ Life cycle

Figure 9: Representation of the Fusarium TR4 life cycle



Source: Authors' own elaboration

1. Spores are available in the soil. Fusarium TR4 chlamydospores may attach to banana roots and root hairs 72 hours after the plantlets are planted in a Fusarium TR4-infected soil and the root exudates stimulate chlamydospore germination (Dita *et al.*, 2018; Li *et al.* 2011).
2. Germ tubes penetrate banana roots (Dita *et al.*, 2018). Plant penetration may occur between three and ten days through the tips and elongation zones of lateral roots as well as natural wounds in secondary root bases (Li *et al.*, 2011; Xiao *et al.*, 2013). Rhizome infection is the most important step in disease development. Once the rhizome is colonized, the infection becomes systemic, reaching the pseudostem (Xiao *et al.*, 2013).
3. After penetrating the banana root cells, Fusarium TR4 produces thickened hyphae and microconidia inside the cells (Li *et al.*, 2011). Fungal hyphae and spores may fill vascular spaces in the rhizome and the surrounding tissue (Li *et al.*, 2011). If a few hyphae or spores enter the pseudostem through the rhizome, they spread rapidly and travel to the top of the pseudostem (Xiao *et al.*, 2013).
4. The thickened hyphae then develop into chlamydospores that are formed intra-intercellularly (Li *et al.*, 2011). Infections trigger a host defence response involving gels, tyloses and lignification that leads to vascular obstruction. In susceptible banana plants, after the pathogen has systemically invaded the xylem vessel elements with an appreciable invasion of the rhizome, a severe water shortage develops due to vascular plugging. This impaired water movement leads to reduced transpiration and the expression of external symptoms (Pegg *et al.*, 2019).
5. Colonization and destruction of vascular tissues provoke intense wilting (Dita *et al.*, 2018).
6. Once the vascular tissue has been fully colonized, the pathogen escapes from the xylem into the adjacent parenchyma and cortex to invade plant tissue weakened by water deficit. Then, when the deteriorated host tissue collapses, chlamydospores and conidia are produced and discharged into the environment (Pegg *et al.*, 2019).

## 2. Prevention and preparedness plan: when the pest is absent

Everyone involved in the banana industry is responsible for applying measures to prevent the introduction and spread of Fusarium TR4 to disease-free areas. This includes government agencies and everyone in the banana value chain, from farmers to transporters, traders, service and input providers, retailers and the public.

### 2.1 PHYTOSANITARY REGULATIONS

Phytosanitary regulations should be designed to maintain the absence of Fusarium TR4. By applying phytosanitary measures, the exclusion strategy helps to prevent its introduction, and requires ensuring that the NPPO has the authority to respond to outbreaks, with legislation that enables the NPPO to quarantine infested areas or farms in case of a Fusarium TR4 outbreak and regulate the movement of people, commodities, machinery and anything else that could spread Fusarium TR4.

Regulatory actions to undertake may include:

- ◆ conducting a pest risk analysis (PRA) in accordance with ISPM 11 (*Pest risk analysis for quarantine pests*) (see 2.2);
- ◆ updating the list of regulated pests based on the PRA pest categorization and include Fusarium TR4 as appropriate, following ISPM 19 (*Guidelines on lists of regulated pests*);
- ◆ establishing import phytosanitary requirements for relevant commodities according to the risk determined through a PRA<sup>1</sup> (see 2.2);
- ◆ developing and implementing a set of measures to prevent the introduction – or escape – of Fusarium TR4 at the farm level (see 2.3, 2.4 and 2.5);
- ◆ ensuring the availability of suitable methodologies and facilities through officially designated laboratories to diagnose Fusarium TR4 (see 2.6);

- ◆ implementing a surveillance programme for Fusarium TR4 following ISPM 6 (*Surveillance*) and supporting the programme through appropriate legislation that enables the NPPO to enter detection, delimiting and monitoring areas 2.7); and
- ◆ developing a contingency plan to respond to Fusarium TR4 outbreaks and testing its performance through simulation exercises, and ensuring the NPPO's regulatory and operational capacity to respond (see 2.8 and 2.9).

### 2.2 PEST RISK ANALYSIS

A pest risk analysis (PRA) evaluates biological, scientific and economic evidence to determine whether an organism is a pest, whether it should be regulated and what phytosanitary measures should be taken against it (ISPM 11) (IPPC Secretariat, 2013). A PRA evaluates the likelihood of introduction, spread and potential economic and environmental consequences of a given pest in a certain area. Each country's NPPO is responsible for conducting pest risk analyses.

Following the criteria established in ISPM 11 to initiate a PRA, NPPOs may initiate a new or revised PRA for Fusarium TR4, considering the fast international spread and introduction of Fusarium TR4 to new areas. The European Food Safety Authority (EFSA) conducted a pest categorization for the European Union and concluded that Fusarium TR4 satisfies the criteria to be regarded as a potential quarantine pest for the Union (EFSA, 2022). Ecuador initiated a PRA after a Fusarium TR4 outbreak in a neighbouring country, Colombia, concluding that the risk of entry, establishment and spread was high (Gallo-Lara, 2021). France has conducted a PRA for the French overseas areas of Guadeloupe, Martinique, French Guiana and Mayotte, finding that the risk of entry, establishment and spread

<sup>1</sup> Pest risk analysis (PRA): The process of evaluating biological or other scientific and economic evidence to determine whether an organism is a pest, whether it should be regulated, and the strength of any phytosanitary measures to be taken against it [FAO, 1995; revised IPPC, 1997; ISPM 2, 2007] (ISPM 5).

of Fusarium TR4 was high, and therefore constituted a candidate for pest risk management (Tassus *et al.*, 2018). Likewise, NPPOs may initiate a new or revised PRA for Fusarium TR4 for host and non-host commodities that have not been previously imported and that represent a potential Fusarium TR4 hazard, such as:

- ◆ planting material with or without roots;
- ◆ corms;
- ◆ *Musaceae* leaves;
- ◆ *in vitro* planting material using tissue culture technology;
- ◆ agricultural machinery, vehicles, farm equipment and tools contaminated with soil and/or plant debris;
- ◆ containers that have been contaminated externally with soil;
- ◆ travellers from countries where Fusarium TR4 is present and that carry soil-contaminated footwear (shoes or boots) in their luggage;
- ◆ handicrafts and other articles elaborated from musaceous plants or their parts; and
- ◆ soil and other regulated articles capable of transporting Fusarium TR4 propagules (chlamydospores) carried in soil and/or plant debris.

A regional PRA was conducted by the Regional International Organization for Plant Protection and Animal Health (OIRSA) for Fusarium TR4 in Central America, assigning high risk to planting material with or without roots, corms, *Musaceae* leaves, containers, and vehicles with soil and/or plant debris and soil. The OIRSA PRA assigned a low risk for *in vitro* planting material using tissue culture technology (OIRSA, 2019).

PRA conclusions will indicate whether Fusarium TR4 requires pest risk management measures and the relevant pathways to regulate. Any phytosanitary measure applied for import should be technically and scientifically justified, based on the PRA outcomes. Gallo-Lara (2021) and OIRSA (2019) identified some measures from the PRAs they conducted for their respective countries that may serve as a reference for countries initiating or revising a PRA for Fusarium TR4 or its pathways.

The import of *in vitro* banana planting materials should be subject to the performance and findings of a PRA, which should provide the basis to establish the import phytosanitary requirements to manage the identified risks.

## 2.3 PREVENTION MEASURES FOR NURSERIES

Certification schemes for planting materials need to be in place for large and small nurseries. One of the most promising prevention measures is tissue-culture-grown plantlets (Lule *et al.*, 2013). The import, export and local production of propagating plant material needs to comply with NPPO requirements. Nurseries should be registered, mapped by geographic information system (GIS) and inspected regularly. Nursery owners should commit to reporting any suspect symptoms to the NPPO.

## 2.4 PREVENTION MEASURES FOR LARGE-SCALE COMMERCIAL PLANTATIONS

Implementing prevention and exclusion measures is critical for protecting farms from the introduction and spread of Fusarium TR4. According to the State of Queensland (2020) and Kukulies and Veivers (2017) the following measures are suitable for such a purpose:

- ◆ use of certified planting material;
- ◆ use of exclusive tools on the farm for cultural practices;
- ◆ create zones with separate areas to restrict the movement between these zones to provide several layers to manage pathogen risk pathways onto and off a farm (see 2.4.2 farm zoning);
- ◆ construct and maintain a boundary fence (which may be a living fence), and control the movement of plants, soil and water entering and exiting the farm;
- ◆ establish specific access points for entry to the farm and post the protocols for entry, with each access point having appropriate infrastructure to ensure the effective exclusion of Fusarium TR4 (see 2.4.3 farm infrastructure requirements);
- ◆ restrict entry and movement of all non-essential visitors, vehicles and machinery from the outside;
- ◆ wash and then disinfect vehicles, equipment and tools (including ladders used for agronomic practices on the bunch), machinery and footwear before entering the farm, in an area established for this purpose, and manage water and soil residues;
- ◆ use the correct disinfectant in the right concentrations and with an appropriate replenishment schedule, and maintain a restricted area for dirty washing water (see 2.4.1 disinfectant solutions);
- ◆ ensure that disinfection units are adequately maintained with effective sanitization solutions, products and equipment.

#### 2.4.1 Disinfectant substances

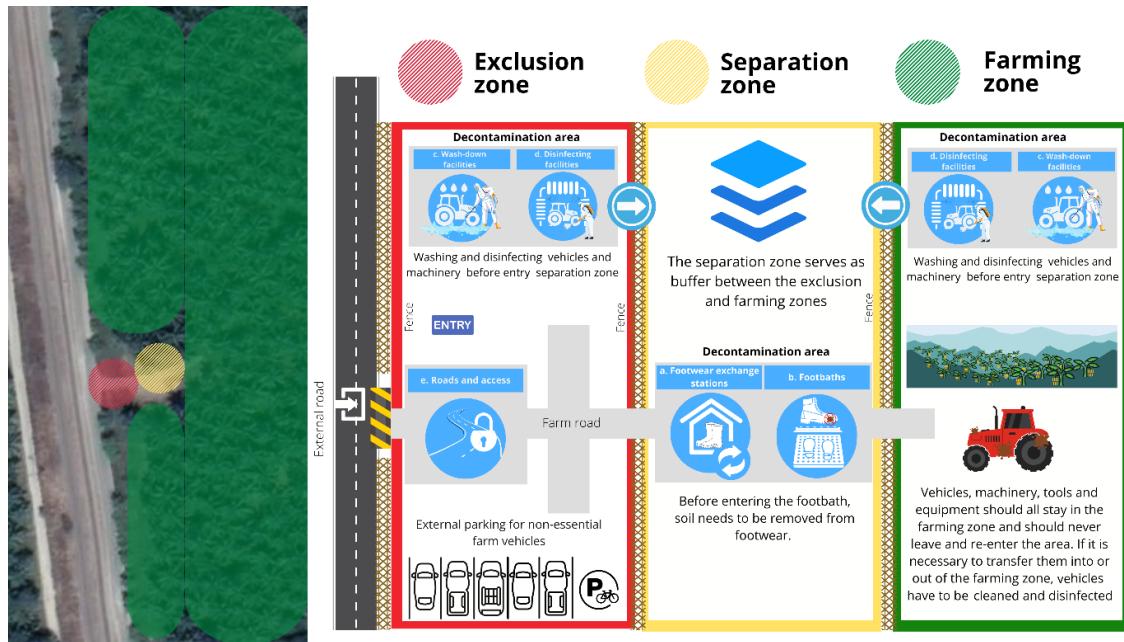
According to Lindsay (2018), quaternary ammonium (QA)-based products (120 g/L [12 percent] didecyldimethylammonium chloride [DDAC] applied at the recommended label rate [1200 ppm]) are frequently used to inactivate *Fusarium TR4* propagules (especially chlamydospores) that can be carried on footwear or other articles. Nguyen *et al.* (2019) evaluated the efficacy of 32 commercial disinfectants after ≤30 s, 5 min, 30 min and 24 h of contact with a suspension of chlamydospores of *Fusarium TR4*. As a result, they found that quaternary ammonium (10 percent) was the most effective. Salacinas *et al.* (2022) evaluated 13 disinfectants commonly used in the Philippines finding that the efficacy of these products depends on the type of fungal spores, the exposure time and the replenishment frequency of the disinfection units, and that the resting spores of TR4 were resistant to all but one – unfortunately corrosive – disinfectant.

The selection of the disinfectant solution is a critical decision; NPPOs and farmers are encouraged to select them according to their previous tests or to consult an expert. Before using any disinfectant substance, it is recommended to clean and remove all soil residues to avoid reducing the efficacy of the substance. The use of chemical disinfectants (in footbaths, for disinfection of vehicles, equipment and tools) in organic banana farms requires precautionary measures if they are to be used.

#### 2.4.2 Farm zoning

A set of measures required to protect farms from the introduction and spread of *Fusarium TR4* is a critical component of an on-farm system. For this purpose, it is necessary to create zones with separate areas to restrict movement between these zones that provide several layers to manage pathogen risk pathways onto and off the farm (see Figure 10).

Figure 10: Farm zoning areas and required infrastructure (see 2.4.2 and 2.4.3)



Source: Authors' own elaboration

Based on Kukulies and Veivers (2017), the following three-zone system is recommended:

**Table 1: Description of the farm's three-zone system**

<b>Exclusion zone</b>	<ul style="list-style-type: none"> <li>◆ The farm's main entry point needs an exclusion zone.</li> <li>◆ The exclusion zone is used as a place to reduce the probability of introducing Fusarium TR4 propagules carried by soil and plant debris through vehicles and machines. For instance, a car park for non-essential vehicles.</li> <li>◆ Before accessing and exiting different zones of the farm, essential vehicles that cannot be excluded from the farm need to be cleaned and/or disinfected.</li> <li>◆ It should be adequately surfaced (e.g. concrete, asphalt and/or reinforced with other measures such as fencing and barriers, with clear signage to inform personnel, staff and visitors about protocols before entering the farm).</li> <li>◆ Water used in this zone to clean and disinfect regulated articles needs to be carefully managed, treated and disposed of in a biosafe manner.</li> </ul>
<b>Separation zone</b>	<ul style="list-style-type: none"> <li>◆ The separation zone acts as a buffer between the exclusion and farming zones.</li> <li>◆ The zone is a part of the farm that provides access to vehicles crucial to farm operations.</li> <li>◆ Before being allowed to enter this zone, vehicles need to be clean, and they should be disinfected.</li> <li>◆ Farm vehicles and machinery should never cross or come into direct contact with the separation zone.</li> <li>◆ If farm vehicles and machinery move across the separation zone and into another zone, they need to be thoroughly cleaned and disinfected.</li> <li>◆ It should be adequately surfaced (e.g. concrete, asphalt or gravel), not be contaminated with soil, and reinforced with other measures such as fencing or barriers, and have clear signage to direct personnel, staff and visitors about protocols in this zone and the farm as a whole.</li> </ul>
<b>Farming zone</b>	<ul style="list-style-type: none"> <li>◆ The farming zone is the farm's production area, where staff conduct farming tasks, and vehicles, machinery, tools and equipment are used on a daily basis.</li> <li>◆ Requires managing footwear, fencing, barriers and signage, and recording these actions.</li> <li>◆ Vehicles, machinery, tools and equipment should all stay in the farming zone and should never leave and re-enter the area. When they are transferred into or out of the farming zone, they have to be cleaned and disinfected.</li> </ul>

Source: A Kukulies, T. & Veivers, S. 2017. Banana best management practices: on farm biosecurity. Agri-Science Queensland, Department of Agriculture and Fisheries, State of Queensland. [www.horticulture.com.au/globalassets/hort-innovation/resource-assets/ba14013-banana-on-farm-biosecurity-manual.pdf](http://www.horticulture.com.au/globalassets/hort-innovation/resource-assets/ba14013-banana-on-farm-biosecurity-manual.pdf)

### 2.4.3 Farm infrastructure requirements

Based on Kukulies and Veivers (2017), farm infrastructure requirements include:

**Table 2: Description of the required farm's infrastructure for zoning**

<b>Footwear exchange stations</b> 	<ul style="list-style-type: none"> <li>◆ Usually located adjacent to the exclusion zone or at crucial access points (for example, between the exclusion and separation zones, and between the separation and farming zones).</li> <li>◆ Footwear exchange stations offer another layer to the protection system by reducing the risk of introducing Fusarium TR4 propagules into the farm via contaminated footwear.</li> <li>◆ Ensure that footwear is clean before using footbaths.</li> <li>◆ Cleaning, disinfection and change of footwear are among the most important measures.</li> </ul>
<b>Footbath area</b> 	<ul style="list-style-type: none"> <li>◆ Footbaths should be located contiguous to the exclusion zone in designated decontamination areas.</li> <li>◆ Before entering the footbath, soil needs to be removed from footwear.</li> <li>◆ To clean footwear, clods of soil and mud must be removed with water and firm-bristled brushes in a separate area, being careful not to recontaminate the footwear before stepping through the footbath.</li> <li>◆ Soil and organic matter reduce the effectiveness of disinfecting products.</li> </ul>
<b>Wash-down facilities</b> 	<ul style="list-style-type: none"> <li>◆ Used for cleaning external vehicles and machinery that are contaminated with high levels of soil and plant debris, and require entry and exit to the different zones of the farm.</li> <li>◆ The wash-down facility should be located in an appropriate place.</li> <li>◆ The water used in this zone to clean vehicles and machinery needs to be carefully managed, treated and disposed of in a biosafe manner.</li> </ul>
<b>Disinfecting facilities</b> 	<ul style="list-style-type: none"> <li>◆ Adequate zoning is essential to ensure that these facilities are used effectively.</li> <li>◆ The best option is to clean all critical surfaces before entering and exiting the disinfecting facility.</li> <li>◆ Spray shuttles, spray grids and/or vehicle dips are widely used to apply disinfecting products to essential vehicles entering different zones of the farm.</li> <li>◆ Spray shuttles, spray grids and/or vehicle dips are not effective for cleaning dirty vehicles and machinery with clods of soil and plant debris.</li> </ul>
<b>Roads and access</b> 	<ul style="list-style-type: none"> <li>◆ The established roadways and access points should correspond to the distribution of the different zones (exclusion zone, separation zone and farming zone) on the farm.</li> <li>◆ A hard surface is required for the central access road (e.g. concrete, asphalt or gravel).</li> <li>◆ Appropriate fencing and physical barriers should be installed along the main access road to offer a layer and control for the movement of vehicles and machinery crossing into other zones.</li> </ul>

Source: A Kukulies, T. & Veivers, S. 2017. Banana best management practices: on farm biosecurity. Agri-Science Queensland, Department of Agriculture and Fisheries, State of Queensland. [www.horticulture.com.au/globalassets/hort-innovation/resource-assets/ba14013-banana-on-farm-biosecurity-manual.pdf](http://www.horticulture.com.au/globalassets/hort-innovation/resource-assets/ba14013-banana-on-farm-biosecurity-manual.pdf)

### 2.5 PREVENTION MEASURES FOR SUBSISTENCE AND SMALLHOLDER BANANA CULTIVATION

The prevention measures recommended for large-scale commercial plantations are also recommended

for smallholder banana cultivation. However, its implementation may require a territorial approach to zoning smallholder areas and building infrastructures such as wash-down and disinfecting facilities, roads and access paths.

Smallholders producing for export are often organized in producer associations or cooperatives. In these cases, the feasibility of territorial approaches in risk management and the application of preventive measures could be examined by grouping banana farmers into clusters. The zoning concept (exclusion zone, separation zone, farming zone) and economies of scale could be applied, realizing joint investments, e.g. a common fence around a group of adjacent farms, a joint footbath and a footwear exchange station at the entry of a shared access road.

In any case, investment in risk communication, awareness-raising and training should take place to prepare smallholder farmers and explain how Fusarium TR4 may be introduced and spread, how to recognize symptoms in the field, how to report suspicious plants to the NPPO and what to do in such a case, and to promote the use of clean, certified planting material.

## 2.6 SAMPLING AND DIAGNOSTICS OF FUSARIUM TR4

Each diagnostic phase involves critical steps, such as:

- ◆ sampling (see 2.6.1);
- ◆ submission of samples to diagnostic laboratory (see 2.6.2);
- ◆ fungal isolation (see 2.6.3);
- ◆ monosporic cultures (see 2.6.4);
- ◆ molecular identification (see 2.6.5);
- ◆ pathogenicity tests (Koch's postulates) (see 2.6.6);
- ◆ vegetative compatibility group (VCG) if the genome is not sequenced (see 2.6.7).

Running all the diagnostic testing for the first report of Fusarium TR4 can take several weeks. The molecular diagnostics can be run in days. Formal confirmation, Koch's postulates and required phenotyping in inoculation experiments, can take up to several months. However, precautions can be implemented right after positive molecular diagnostics.

### 2.6.1 Sampling

Usually, samples are taken from symptomatic plant material (see symptoms in 1.2.3). The following criteria for sampling suspect banana plants need to be considered:

- ◆ The sample consists of a pseudostem section (internal pseudostem or corm tissue) of wilted banana plants with evidently brown-black coloured vascular tissue.

- ◆ The sample should be taken from the lower part and close to the centre of the pseudostem, but not with advanced rotting.
- ◆ The obtained sample is wrapped in tissue paper, placed into a labelled non-polythene sample bag and transported to the laboratory.
- ◆ The plant should be flagged or marked in the field and the geographical coordinates recorded, indicating that samples have been taken. The location is recorded to enable tracking and follow-up from the point of collection in the field.
- ◆ The sampling methodology should ensure the sampling team can sufficiently capture internal symptoms.
- ◆ Sample information should be recorded on a submission form and include the banana variety or cultivar, sample type, location code, geographical coordinates, contact details of the sampler and date.
- ◆ NPPOs should establish measures to avoid the spread of Fusarium TR4 through sample movement and from farm to farm.

### 2.6.2 Submission of samples to diagnostic laboratory

- ◆ The samples should be quickly delivered to an officially designated laboratory for diagnostic processing or testing of TR4.
- ◆ Sample integrity should be maintained, and samples should be handled, prepared, packaged, labelled and stored, and pertinent details should be captured in a laboratory submission form.
- ◆ The samples should be dry before shipment and packed in paper bags or envelopes; never use plastic bags because a moist environment promotes bacterial growth.
- ◆ Upon arrival, the samples should be processed on the same day.

### 2.6.3 Fungal isolation

Upon arrival, plant samples are externally disinfected with 70 percent ethanol, cut into pieces, placed on wet filter paper in Petri dishes, and placed into a growing chamber under 25 °C. Filter paper is watered regularly, and samples are monitored for fungal development (Leslie and Summerell, 2006). In the case of fungal growth on plant samples, mycelium is transferred to the growing media. For morphological identification, Fusarium TR4 isolates

are grown on: potato dextrose agar (PDA) (Ujal *et al.*, 2021) to observe growth rate and mycelium colour; carnation leaf agar (CLA) to have better production of conidia (Fisher *et al.*, 1982); and Spezieller Nährstofffarmer agar (SNA) to promote branching of the conidiophores (Nirenberg *et al.*, 1981).

#### 2.6.4 Monosporic cultures

Since several fungal isolates can develop simultaneously on the same plant sample, it is necessary to obtain monosporic isolates, i.e. to isolate single spore/conidia to ensure the required purity for subsequent analyses. This can be done in several ways, usually involving isolation under a microscope with a special needle (Choi *et al.*, 1999; Zhang *et al.*, 2013a).

#### 2.6.5 Molecular identification

For molecular identification, a sample needs to complete a number of phases to determine whether *Fusarium TR4* is present. First, the sample needs to be subjected to DNA extraction. Then, the DNA sample with the extracted nucleic acids needs to be analysed through PCR or quantitative PCR (qPCR) protocols, which will amplify (copy) specific segments of DNA of *Fusarium TR4* (if present) using specific molecular

markers (primers). Several molecular markers are available, and using at least two sets of independent molecular markers is recommended to avoid false positives. For the first reports, it is recommended to complete the following three steps: (i) molecular tests, (ii) sequence the complete genome or conduct a VCG test, and (iii) conduct a pathogenicity test (see Figure 11).

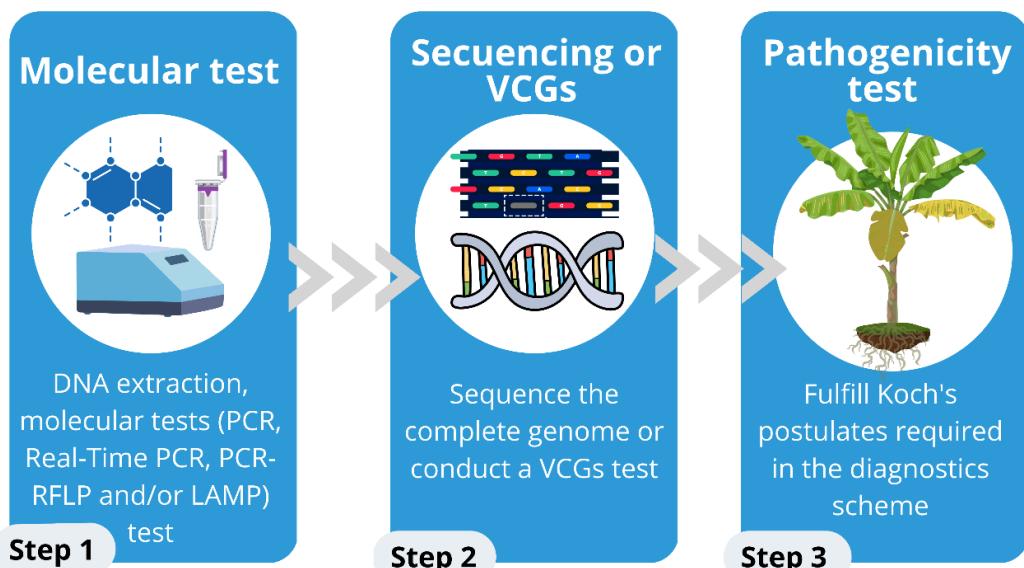
The following items describe relevant insights to complete a molecular identification from DNA extraction to sequencing.

a) **DNA extraction:** Genomic DNA can be extracted with commercial kits or with the Cenis protocol (Cenis 1992), cetyl trimethyl ammonium bromide (CTAB) method (Kalendar *et al.*, 2021). For molecular identification, isolates can be grown in liquid or solid media. High-quality DNA is essential for correct results in molecular identification.

b) **Molecular markers:**

- ◆ Dita *et al.* (2010) developed a PCR-based diagnostic tool to detect isolates from VCG 01213 (*Fusarium TR4*). A primer set was designed on the nuclear ribosomal intergenic spacer region (IGS rDNA). The *Fusarium TR4*

Figure 11: Main steps for *Fusarium TR4* diagnosis in a laboratory



Source: Authors' own elaboration

commercial kit that was developed is based on these primers, and provides a standard operating procedure required for (inter)national comparisons. The kit incorporated modifications to provide more specificity.

- ◆ Li *et al.* (2013) developed a VCG 01213/16-specific primer set, one forward (VCG01213/16 F1) and one reverse primer (VCG01213/16 R2), which were designed to generate a unique amplicon of 455 bp to detect isolates of Fusarium TR4 (VCG 01213/16).
- ◆ Aguayo *et al.* (2017) developed a Fusarium TR4-specific real-time PCR test that can simultaneously detect VCG 01213/16 and VCG 0121. This is significant, considering that VCG 0121 has proven to be genetically closely related to VCG 01213/16.
- ◆ Carvalhais *et al.* (2019) designed a molecular diagnostic assay based on *Foc* secreted in xylem (SIX) genes. These genes contain polymorphisms (two or more variant forms of a specific DNA sequence) that assist in distinguishing races and relevant VCGs of *Foc*. The method can detect R1, STR4, Fusarium TR4 and VCGs (0121 and 0122) causing Fusarium wilt in bananas.
- ◆ A real-time fluorescence loop-mediated isothermal amplification (RealAmp) assay was developed to rapidly and quantitatively detect Fusarium TR4 in soil. No cross-reaction with other relative pathogens was observed. The RealAmp assay was visual, with an improved closed-tube visual detection system. This method can potentially be used in the field to detect and monitor Fusarium TR4 (Zhang *et al.*, 2013b). Similarly, Ordóñez *et al.* (2019) developed a LAMP assay that is applicable under field and laboratory conditions to detect Fusarium TR4 in plants. The LAMP assay proves to be a powerful tool for identifying Fusarium TR4 in infected banana plants.

c) **Sequencing and phylogenetic analysis:** Generated sequences of purified PCR products need to be

analysed. Sequences are assembled and manually edited using appropriate sequence-editing software. Assembled sequences are then analysed by sequence alignment approach using Blastn search against publicly available sequences deposited in the GenBank database. The confirmation of Fusarium TR4 can be blasted/compared to all TR4 sequences in public databases. Similarly, in-house databases might be built using sequences of known races or those confirmed by VCGs. Phylogenetic analysis of obtained sequencing data can be based on Bayesian inference (BI), maximum likelihood (ML) and maximum parsimony (MP).

## 2.6.6 Pathogenicity Test

Pathogenicity tests with isolated *Fusarium* spp. need to be performed on the correct banana cultivar to determine what type of race is at play, fulfilling Koch's postulates required in the diagnostics scheme. Pseudostem symptoms are either recorded in qualitative scores or, ideally, in a quantitative way (percentage of affected corm tissues), and the results should be statistically processed. Garcia-Bastidas *et al.* (2019b) proposed a method that allows the inoculation of 250 plants per hour by one individual, thereby facilitating the phenotyping of large populations.

## 2.6.7 Vegetative compatibility groups (VCGs) of *Fusarium oxysporum* f. sp. *cubense*

*Fusarium oxysporum* f. sp. *cubense* (*Foc*) is also characterized by vegetative compatibility groups (VCGs). VCG-compatible strains can anastomose, form a stable heterokaryon and share genetic material (Fraser-Smith *et al.*, 2014). VCGs are therefore relevant evolutionary units that are genetically related. To date, 24 *Foc* VCGs have been described worldwide (Aguayo *et al.*, 2017). While *Foc* race 1, race 4 or STR4 includes several VCG groups, Fusarium TR4 is described as a single clone corresponding only to VCG 01213/16 (Groenewald *et al.*, 2006; Ordóñez *et al.*, 2015), which is highly aggressive to Cavendish bananas in the tropics (Li *et al.*, 2013). Currently, cumbersome VCG analyses are usually avoided and replaced by genome sequence analyses.

### 2.6.8 Resources that may guide NPPOs on the implementation of Fusarium TR4 diagnostics

	Technical Manual: Prevention and diagnosis of Fusarium Wilt (Panama disease) of banana caused by <i>Fusarium oxysporum</i> f. sp. <i>cubense</i> Tropical Race 4 (TR4) (Pérez-Vicente <i>et al.</i> , 2014). <a href="http://www.fao.org/publications/card/en/c/16426431-e2d6-499d-b1d9-530fa4fbc4dd">www.fao.org/publications/card/en/c/16426431-e2d6-499d-b1d9-530fa4fbc4dd</a>
	Andean guide for <i>Fusarium</i> Tropical Race 4 diagnostics (García-Bastidas <i>et al.</i> , 2020). (Spanish) <a href="https://bit.ly/3qhljQc">https://bit.ly/3qhljQc</a>
	Diagnostic Protocol: <i>Fusarium oxysporum</i> f. sp. <i>cubense</i> Tropical Race 4 (SENASICA, 2021). (Spanish) <a href="https://bit.ly/3x6bliS">https://bit.ly/3x6bliS</a>

### 2.7 SURVEILLANCE: DETECTION SURVEYS TO DETERMINE FUSARIUM TR4 CONDITION (PRESENCE OR ABSENCE)

National surveillance systems can be general or specific and require the availability of programmes and infrastructure to implement the system effectively. General surveillance involves collecting information from different sources. Specific surveillance includes

pest data collected by the NPPO or authorized entities through surveys over a defined period to determine pest condition, the boundaries of an area considered to be infested by the pest, and the characteristics (ISPM 6, 2018). Based on the surveillance results, the NPPO may determine the pest status in the area according to the appropriate descriptions provided in the ISPM 8, (*Determination of pest status in an area*).

	ISPM 6, provides guidelines for performing the surveillance process. <a href="http://www.ippc.int/en/publications/615">www.ippc.int/en/publications/615</a>
	The IPPC <i>Surveillance guide</i> helps to understand the principal requirements of surveillance programmes for NPPOs. <a href="https://doi.org/10.4060/cb7139en">https://doi.org/10.4060/cb7139en</a>

In countries where *Fusarium* TR4 is absent, surveillance for early detection purposes is usually carried out through detection surveys. However, early detection of *Fusarium* TR4 is challenging, considering the symptoms of the disease will be evident after a period of incubation, and there is no practical and effective way of discovering infected plants until

the symptoms are present (see epidemiology in item 1.2.4). Likewise, there is no practical way to detect *Fusarium* TR4 chlamydospores or conidia from the soil, healthy plants or the environment. Surveillance protocols for detecting *Fusarium* TR4 may consider:

- ◆ NPPO officers or authorized entities should be in charge of *Fusarium* TR4 surveillance.

- ◆ A surveillance protocol for Fusarium TR4 should include procedures to avoid potential spread during the survey or the movement of samples.
- ◆ Farm areas of the Cavendish subgroup, which is resistant to races 1 and 3 of *Foc*, might be prioritized for surveillance, considering that FWB symptoms in Cavendish bananas might suggest the presence of Fusarium TR4.
- ◆ Detection surveys may be conducted and prioritized in function of the areas of greater risk and warnings from stakeholders.
- ◆ Staff performing detection surveys need to identify suspicious banana plants (including other species and different varieties or cultivars belonging to the *Musa* genus), looking for external symptoms or signs of Fusarium TR4 (see external symptoms in item 1.2.2).
- ◆ When the disease's external symptoms or signs are found in banana plants, staff performing the survey should check for internal symptoms or associated signs, such as a yellow, reddish or brown-black discolouration of the stem or corm's vascular tissue (see internal symptoms in item 1.2.2).
- ◆ After checking the presence of external and/or internal symptoms, the staff performing detection surveys should take a sample as described in item 2.6.1. In parallel, the NPPO should initiate a contingency protocol.
- ◆ Plants with FWB symptoms should be marked and GPS coordinates recorded.
- ◆ While the officially designated laboratory analyses the sample, the NPPO should notify the farm owner, indicating the appropriate measures to contain the possible spread of Fusarium TR4.
- ◆ Staff performing detection surveys should be well trained to recognize symptoms caused by Fusarium TR4 in bananas and differentiate them from others caused by pests such as *Ralstonia* and *Xanthomonas*.



NPPOs may authorize entities to perform phytosanitary actions such as surveillance. In such a case, ISPM 45 establishes the *Requirements for national plant protection organizations if authorizing entities to perform phytosanitary actions*.  
[www.ippc.int/en/publications/89734](http://www.ippc.int/en/publications/89734)

## 2.8 CONTINGENCY PLANNING PREPARATION

A contingency plan for Fusarium TR4 should determine the technical basis, actions and procedures to respond to Fusarium TR4 outbreaks, and consider the roles and responsibilities of authorities and stakeholders. An appropriate legal framework and operative capacities should support contingency plan implementation. NPPOs preparing a contingency plan for Fusarium TR4 may include the following information:

- ◆ Consider the prevention and preparedness activities noted in this document and others, as appropriate.
- ◆ Create a national committee to coordinate outbreak response. The committee should involve relevant stakeholders and authorities. Stakeholder articulation is critical to achieving a timely and effective outbreak response.

- ◆ Detail the actions and procedures to perform phytosanitary surveillance for Fusarium TR4, including the required procedures and tools to conduct detection and delimitation surveys. Likewise, describing how the surveillance information should be processed, stored and reported to relevant actors.
- ◆ Identifying the geographical areas of the most significant risk of Fusarium TR4. It may involve proactive mapping of banana regions and indicating flood risks, waterways and logistics routes that are likely to facilitate Fusarium TR4 dissemination.
- ◆ Establish communication channels such as phone numbers, email or others to allow banana farmers to warn the NPPO about suspect banana plants in the field showing Fusarium TR4 symptoms. For this reason, training banana farmers to recognize symptoms is essential.

- ◆ Guiding NPPO staff and farmers about how to proceed when detecting a suspected banana plant by:
  - establishing procedures to mark suspected banana plants,
  - recording necessary information to avoid the movement of suspected banana plants or plant parts in the surrounding area,
  - establishing measures to enter or leave the suspected Fusarium TR4 areas,
  - identifying prevention measures to implement while the officially designated laboratory diagnoses the sample.
- ◆ Define the methodology and all necessary tools for sampling, considering measures to avoid the possible spread of Fusarium TR4.
- ◆ Describe the adopted diagnostic protocol and techniques, detailing all the required materials and procedures.
- ◆ Consider all administrative, operational, and legal actions and procedures to respond to a Fusarium TR4 outbreak and to declare a phytosanitary emergency.
- ◆ Assign roles and responsibilities to each actor involved in the response, creating an organizational chart for response implementation and the flow of information.
- ◆ Consider communication and dissemination of all contingency actions.
- ◆ Foresee training for NPPO staff, farmers and other relevant stakeholders.

The IPPC Secretariat is publishing a guide for contingency planning for quarantine pests, defining the main components and providing guidance on how NPPOs, in collaboration with relevant stakeholders, can effectively organize and allocate their resources for pest outbreak response. The guide incorporates

the criteria to establish and maintain pest free areas, the reporting of outbreaks and recovery, and includes eight case studies from around the world that illustrate various aspects of contingency planning for outbreaks of quarantine pests (FAO, 2023).

FAO has developed a Regional Strategy for the Preparedness, Prevention, Detection, Response and Recovery of Latin America and the Caribbean to Fusarium TR4 and a corresponding Regional Action Plan, Strengthening Regional Capacities for Surveillance, Prevention and Management of the Eventual Spread of Fusarium race 4 Tropical *Fusarium oxysporum* f. sp. *cubense* (Foc TR4). The FAO Regional Technical Cooperation Programme supports the Action Plan and the regional plant protection organizations (RPPOs) of Latin America and the Caribbean and the NPPOs of the countries that grow Musaceae in the region, as well as the Inter-American Plant Health Coordination Group (GICSV).

The strategy aims to strengthen coordination and focus efforts, actions and good practices to support understanding and risk reduction, strengthen the management of phytosanitary emergencies in Latin America and the Caribbean, as well as to improve the resilience of the banana and agricultural sectors and reduce damage and losses related to the introduction and spread of this important pest.

The strategy has taken into account the FAO Strategic Framework 2022-2031 (FAO, 2021), the priorities of the Sendai Framework for Disaster Risk Reduction 2015-2030 (UNISDR, 2015) and the Regional Strategy for Disaster Risk Management in the Agricultural Sector and Food and Nutrition Security in Latin America and the Caribbean (2018-2030) approved by the Community of Latin American and Caribbean States (CELAC) (CELAC, 2018), in addition to relevant ISPMs.



Regional strategy and action plan for the prevention, preparedness, response and recovery of Latin America and the Caribbean to Fusarium wilt of Musaceae TR4.  
[www.fao.org/documents/card/en/c/CB8674EN](http://www.fao.org/documents/card/en/c/CB8674EN)

Some regions and countries have elaborated contingency plans that may serve as a reference.

	<p>The NPPO of Ecuador elaborated a national contingency plan for Fusarium TR4 that includes a description of the full methodology for taking and managing a sample. (Spanish)</p> <p><a href="https://bit.ly/3QpSVpB">https://bit.ly/3QpSVpB</a></p>

## 2.9 SIMULATION EXERCISES

The World Organisation for Animal Health (WOAH) ad hoc Group on Veterinary Emergencies defines a simulation exercise as a controlled activity where a situation that could exist is imitated for training or assessment of capabilities and the testing of plans, procedures and mechanisms to respond to outbreaks and emergencies. Moreover, the World Health

Organization (WHO) (2017) described the concept of simulation exercise as a form of practice, monitoring or evaluation of the capabilities of emergency systems. Various fields such as agriculture, health, education and security, among others, apply simulation exercises. (PAHO, 2011; WHO, 2017). Lessons learned from a simulation exercise can lead to the revision/improvement of existing contingency plans.

	<p>FAO and OIRSA conducted simulation exercises for Fusarium TR4 in Belize, Costa Rica, Ecuador, El Salvador, Guatemala, Honduras, Nicaragua and Panama. The experience of the simulation exercise carried out in Nicaragua is documented in a video where the NPPO (<i>Instituto de Protección y Sanidad Agropecuaria [IPSA]</i>) explains the experience, available at:</p> <p><a href="https://youtu.be/Ee20Rlkzyog">https://youtu.be/Ee20Rlkzyog</a></p>
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FAO and OIRSA will publish a protocol to perform simulation exercises and simulacrum. To ask for information, you may contact [svegetal@oirsa.org](mailto:svegetal@oirsa.org).

The following references provide guidelines for preparing and implementing simulation exercises: Australian Institute for Disaster Resilience (2012); European Commission for the Control of Foot-and-Mouth Disease (EuFMD) (2020); OIE (2021); Murphy *et al.* (2012); PAHO (2011); PAHO (2011); PLANTPLAN (2022); USDA-APHIS (2010); WHO (2017).

## 2.10 COMMUNICATION AND INFORMATION SHARING WITH STAKEHOLDERS

An awareness programme for all relevant stakeholders is crucial to prevent the introduction and spread of Fusarium TR4. When stakeholders are aware, they can contribute to surveillance, reporting and managing the pest. An "early warning system" should be part of the communication plan, and simulation exercises can raise awareness of the risks of Fusarium TR4. When Fusarium TR4 is absent, the NPPO may need to communicate and share information with stakeholders for different purposes, such as to:

- ◆ increase awareness of Fusarium TR4 risks in bananas;
- ◆ inform measures to prevent the introduction and spread of Fusarium TR4;
- ◆ provide information on how to recognize the disease in the field and what to do when it is identified;
- ◆ inform the purpose of the surveillance programmes for Fusarium TR4;
- ◆ inform containment measures when symptoms of the disease are detected;
- ◆ inform quarantine measures when Fusarium TR4 is confirmed by the officially designated laboratory; and
- ◆ ask farmers and stakeholders to report any suspected case of Fusarium TR4 symptoms in bananas.

There are several web resources that NPPOs may consult as a reference to design specific communication materials:

	<p>The World Banana Forum launched the TR4 Global Network in 2020 as a platform to share written and audiovisual resources, organize webinars and link efforts worldwide.  <a href="http://www.fao.org/tr4gn/en">www.fao.org/tr4gn/en</a></p>
	<p>The <i>IPPC guide to pest risk communication</i> provides advice to NPPOs on communicating with stakeholders about pest risks.  <a href="http://www.fao.org/documents/card/en/c/ca3997en">www.fao.org/documents/card/en/c/ca3997en</a></p>
	<p>The <i>IPPC guide on Managing Relationships with Stakeholders</i> explains the organization and implementation of awareness-raising campaigns and extension materials.  <a href="http://www.fao.org/documents/card/en/c/ca6383en">www.fao.org/documents/card/en/c/ca6383en</a></p>
<p>Many organizations have created dedicated websites and published videos and communication materials about Fusarium TR4 to inform the broader public. See Appendix.</p>	

### 3. Response plan: when the pest is present (officially detected and confirmed)

Once Fusarium TR4 is officially detected and confirmed, a response plan should be implemented immediately, and prevention and preparedness activities should continue and be reinforced. Based on surveillance results, the NPPO may determine the pest status in the area according to the appropriate descriptions provided in ISPM 8 (*Determination of pest status in an area*).

Eradication of Fusarium TR4 is not technically feasible due to its long-term survival in soil. Therefore, the main role of the response plan is to contain and limit the spread of the pathogen. The response includes the following considerations:

- ◆ strengthening the set of prevention measures required to protect farms from the introduction and spread of Fusarium TR4 (see item 2);
- ◆ performing delimiting surveys to establish the boundaries of the affected area (see 3.1);
- ◆ zoning (destruction, dirty and clean zones) (see 3.2);
- ◆ applying phytosanitary measures to contain Fusarium TR4 in the affected area (see 3.3);
- ◆ applying safe destruction measures for Fusarium TR4 infected plants and plants presenting related risks (see 3.4); and
- ◆ communicating risks and measures to stakeholders, complying with National Reporting Obligations of the IPPC, especially the "pest reports" and "emergency action" obligations, and sharing outbreak information with the IPPC Secretariat and other relevant bodies (see 3.5).

#### 3.1 SURVEILLANCE: DELIMITING AND MONITORING SURVEYS

A delimiting survey is necessary to establish the boundaries of an infested area once the presence of Fusarium TR4 is officially confirmed in a specific location. Delimiting surveys will serve to:

- ◆ gather information to determine the incidence and distribution, determine how quickly Fusarium TR4 is spreading, and determine what environmental or other variables are contributing to it (estimation);

- ◆ identify as many infected sites as possible to undertake control and containment measures of new cases (targeting);
- ◆ zone areas into "destruction zone", "dirty zone" and "clean zone"; and
- ◆ establish the areas under quarantine.

According to ISPM 6, "if the objective of surveillance is to delimit an outbreak, the area selection should be focused on the immediate surroundings of the known infested area and on sites of the same habitat type". Aerial mapping with small airplanes or drones could be used as a tool for these purposes. Based on the surveillance results, the NPPO may determine the pest status in the area according to the appropriate descriptions provided in ISPM 8 (*Determination of pest status in an area*).

All types of production places should be included in the survey, from nurseries to smallholders and large plantations. Questionnaires should be used to obtain information about the location of the production site, source of planting material, movement of plant material and plant products, equipment and labour movement, and weather conditions during the plant production period, along with any other information necessary to aid in delineating the extent of spread.

According to ISPM 6, a monitoring survey will help verify a pest population's characteristics. To design an effective monitoring survey strategy for Fusarium TR4, it is necessary to understand when, where and how Fusarium TR4 is likely to occur. For this aim, epidemiological data on disease dynamics based on statistical surveillance models can provide answers. This precise data can also be used to design innovative and successful Fusarium TR4 control and containment approaches in new areas.

#### 3.2 ZONING (DESTRUCTION, DIRTY AND CLEAN ZONES)

Types of zones established and delimited following a survey of an affected area:

- a) *Destruction zones*: areas of land with symptomatic banana plants that pose a risk related to Fusarium TR4. All plants in these zones should be destroyed (when the presence of Fusarium TR4 has been officially confirmed). Access to these areas is permitted only if an authorized officer is notified.
- b) *Dirty zones*: areas of land adjacent to the "destruction zone" where in-field farm operations occur, and these are exposed to a pathogen risk. Dirty zones need to be signposted, fenced or have other natural barriers to restrict access to clean zones and land not subject to notice.
- c) *Clean zones*: areas of land adjacent to the "dirty zone". These areas are not subject to contamination by the pathogen, or no plants with symptoms have been observed. These areas are used as clean corridors to ensure that on-farm traffic remains within dirty zones. Clean zones include clean access roads that allow public access to the affected land. Clean zones need to be signposted, fenced or have other natural barriers to restrict access to dirty zones.

### 3.3 PHYTOSANITARY MEASURES TO CONTAIN FUSARIUM TR4 IN AN AFFECTED AREA

No chemical or biological substances are recognized for use in soil or banana plants for Fusarium TR4 suppression or eradication. Chemical control measures for Fusarium TR4 in countries where the pathogen has been reported have been shown to be ineffective, and the pathogen has continued to spread, presumably by the movement of infected plants for planting. (Dita *et al.*, 2018). The first response strategy is to contain Fusarium TR4 in affected areas and delay its spread. Therefore, early detection and prompt destruction of affected plants are essential for such a purpose.

Containment of Fusarium TR4 is challenging due to the difficulty of controlling all possible pathogen-spread pathways, which requires considering the different zones established through the delimiting survey: destruction zone, dirty zone and clean zone. Phytosanitary measures against a Fusarium TR4 outbreak may include restricting the movement of people, plants and equipment in the affected

area, which should be declared in quarantine. On-farm investments and joint investments as off-farm public investments are important for checkpoints and disinfection facilities for vehicles, containers and visitors at access roads or entry points (frontiers, ports, airports). Some containment measures may include:

- ◆ control points of entry and exit to the affected areas (see 3.2);
- ◆ destruction of affected and neighbouring plants (See 3.4.1 and 3.4.2); and
- ◆ prohibition of the movement of planting materials;
- ◆ regulation of agricultural practices, such as irrigation.

It is necessary to note that containment measures for Fusarium TR4 help to delay the outbreak's spread into a new territory. However, where Fusarium TR4 is present, disease management needs to be developed and implemented to learn to live with it. According to the Consultative Group for International Agricultural Research (CGIAR) programme on roots, tubers and bananas, long-term strategies for management include varieties with Fusarium TR4 resistance and consumer acceptance, soil health management practices, and cropping system and agronomic practices.

### 3.4 SAFE DESTRUCTION OF FUSARIUM TR4 INFECTED PLANTS AND PLANTS PRESENTING RELATED RISKS

If Fusarium TR4 is detected in planting material, the material should be immediately destroyed. If the pathogen is detected in the field, all banana plants in that field should be destroyed to minimize inoculum dispersal. Plant destruction should be carried out following official procedures set out by the NPPO, establishing the magnitude of the area to be destroyed.

The OIRSA contingency plan recommends destroying plants around 7.5 m from the infected plant. However, determining the size of the destruction zone requires case-by-case analysis. It requires the use of science and legislation, considering the size of the farm, the number of infected plants, if the plants overlapped, landscape conditions and an individual's rights against the public interest.

### 3.4.1 General considerations for plant destruction

According to the Queensland Government (2016), all banana plants that present a risk related to Fusarium TR4 are required to be destroyed as follows:

- ◆ Entry to the destruction zone needs to be restricted only to those conducting the destruction activities or any destruction zone maintenance required under the notice.
- ◆ Prior to destruction, an authorized (NPPO) officer should be informed of all destruction activities.
- ◆ Records of destruction activities need to be created, maintained and be made available to an authorized NPPO officer upon request.
- ◆ Keeping the farm tools, equipment, footwear (shoes or boots), vehicles or any other high-risk items inside the destruction zone is essential. It is necessary to avoid taking these tools out of the zone unless they receive cleaning and decontamination treatment with effective disinfectants against Fusarium TR4 propagules.
- ◆ All agricultural chemicals need to be registered and regulated by the relevant national authority and used under the instructions on the approved label.
- ◆ As soon as the results of the diagnostic report confirm the presence of Fusarium TR4, banana growers (stakeholders) should destroy all banana plants within the destruction zone under the supervision of the NPPO.

### 3.4.2 Destruction protocol

According to the Queensland Government (2016), for banana plants that have wilted, have been cut down or are still standing in the field (up to 1 metre tall or over 1 metre tall), the following protocol should be considered:

- "i. cut off pseudostems at 10 cm above the growing point without disturbing the soil; and
- ii. chop up pseudostems and leaf material into 60–80 cm pieces; and
- iii. place all pieces of the infected banana plant(s) in heavy duty plastic bags; and
- iv. place 1 kg of urea in each bag, seal bags securely and leave in the destruction zone; and
- v. gouge out the surface of each remaining corm to create a hollow; and
- vi. inject each stool with 5 mL prepared solution containing 90 g glyphosate active constituent

per litre of water, following the relevant ... permit requirements for the destruction of banana plants; and

- vii. inject each stool with 18 mL of prepared solution containing 200 mL of a 350 g/L imidacloprid product per 100 mL water, following the relevant ... permit requirements for the destruction of banana plants; and
- viii. spray each stool, and a 30 cm band around each stool, with 500–750 mL (depending on stool size) of prepared solution containing 66 g bifenthrin active constituent per 100 L water following the relevant ... permit requirements for control of banana weevil borer in banana crops that pose a biosecurity risk related to Panama disease tropical race 4. Do not remove trash from around the base of infected banana plants prior to application of bifenthrin; and
- ix. apply 200 g of urea evenly to the gouged surface of each corm; and
- x. apply urea at the rate of 1 kg per m<sup>2</sup> evenly around each infected stool."

Immediately after completing the steps previously described, growers (stakeholders) need to cover the destruction zone, including the bags of banana plant material, with high-grade plastic sheeting, and secure the plastic sheeting in place.

### 3.5 COMMUNICATION AND INFORMATION SHARING WITH STAKEHOLDERS

Stakeholder communication is essential when pest presence has been officially confirmed. Before Fusarium TR4 is detected, a dedicated communication team and a spokesperson from the NPPO should be appointed to draft a communication plan.

Communication allows the exchange of information about Fusarium TR4 risks between the NPPO and stakeholders (such as farmers, industry and universities), because these actors may have different perceptions and views on the risk situation and how to manage it. As a result, a pest risk communication strategy is critical for achieving a common understanding of pests to develop consistent regulations and harmonized phytosanitary measures that reduce pest spread and socioeconomic impact, increase stakeholder confidence in regulatory systems for plant health and improve regulatory

decision-making. An awareness programme for stakeholders should be developed, including information on how to recognize disease symptoms and what should be done after the disease has been

confirmed. At the international level, the NPPO should inform all relevant international and national bodies and comply with the National Reporting Obligations (NROs) under the framework of the IPPC.



For more information on pest reporting, please refer to ISPM 17 (*Pest reporting*).  
[www.ippc.int/en/publications/606/](http://www.ippc.int/en/publications/606/)

### 3.6 CASE STUDY (PREVENTION AND CONTAINMENT PROGRAMME FOR FUSARIUM TR4 IN COLOMBIA)

In June 2019, the NPPO of Colombia (Colombian Agriculture Institute [ICA]) reported their first detection of Fusarium TR4 in La Guajira Department and, in 2021, they reported a second detection in Magdalena Department. Consequently, the NPPO activated a prevention and containment programme called "From theory to action", which consists of six core actions and draws on solid governance to respond to the threat that Fusarium TR4 represents in Colombia. The core activities involve:

- ◆ **Programme supervision:** a Unified Command Post (UCP) was created at the behest of the president of Colombia and the minister of agriculture. National agriculture authorities, banana farmers' associations, the military and police forces formed the command to coordinate interinstitutional actions for the containment of Fusarium TR4 in La Guajira, Colombia.
- ◆ **Articulation and coordination with stakeholders:** the programme involved action by the Colombian Farmers' Association (SAC), local banana farmers' associations and other actors in the supply production, commercial and logistics chains.
- ◆ **Budget:** the NPPO managed a specific budget to respond to the Fusarium TR4 outbreak.
- ◆ **Empowerment and mandate:** The NPPO led the response to contain the outbreak, was accountable for the programme execution, and acted as technical secretariat of the UCP.
- ◆ **Take advantage of national capacities:** activities drew on the capacities of the NPPO, the national research institution of Colombia (AGROSAVIA), academic institutions and other institutions dedicated to agricultural technology transfer. These institutions proposed sustainable alternatives based on scientific evidence and appropriate risk communication to respond to the presence of Fusarium TR4.
- ◆ **Cooperation and technical assistance:** recognized organizations and world experts in Fusarium TR4 provided sustained support, cooperation and technical assistance.

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# Appendix

## COMPILATION OF COMMUNICATION MATERIALS ABOUT FUSARIUM TR4

	Fusarium wilt: an ongoing battle (QAAFI) <a href="https://youtu.be/dc2hEknAinl">https://youtu.be/dc2hEknAinl</a>
	Masterclass on Fusarium wilt of banana (Promusa) <a href="https://www.youtube.com/channel/UCsxm3SYT-7ZGOJYx5N9Ahww">www.youtube.com/channel/UCsxm3SYT-7ZGOJYx5N9Ahww</a>
	Panama disease (Wageningen UR) <a href="https://www.youtube.com/playlist?list=PLXv9pwN0ObKUwfKGATz6MGQT6ivNCbhK3">www.youtube.com/playlist?list=PLXv9pwN0ObKUwfKGATz6MGQT6ivNCbhK3</a>
	Panama tropical race 4 (Biosecurity Queensland Government) <a href="https://www.youtube.com/playlist?list=PLpiCDHV-IjhHhQEteJfg5xpOJDkvOZEpu">www.youtube.com/playlist?list=PLpiCDHV-IjhHhQEteJfg5xpOJDkvOZEpu</a>
	The science of stopping Panama Disease (Australian Centre for International Agricultural Research) <a href="https://www.youtube.com/watch?v=GAZGa1v7u8A">www.youtube.com/watch?v=GAZGa1v7u8A</a>
	CABI (Centre for Agriculture and Bioscience International) has a web page dedicated to <i>Fusarium</i> TR4 with news constantly being released <a href="https://www.cabi.org/isc/tr4">www.cabi.org/isc/tr4</a>
	Government of Trinidad and Tobago published a fact sheet about <i>Fusarium</i> TR4 to introduce farmers to the most relevant information <a href="https://agriculture.gov.tt/target/farmers/">https://agriculture.gov.tt/target/farmers/</a>
	The Belize Agricultural Health Authority issued a press release in 2019 about <i>Fusarium</i> wilt of banana, addressing stakeholders of the banana industry, government agencies, agricultural partner organizations and the public in general <a href="https://baha.org.bz/2019/08/02/press-release-3-19-fusarium-wilt-of-bananas/">https://baha.org.bz/2019/08/02/press-release-3-19-fusarium-wilt-of-bananas/</a>

	An article titled "The 'pandemic' destroying the world's favourite fruit" was published on the BBC website about the devastating effect of Fusarium TR4 on world banana production, comparing it to the current COVID pandemic <a href="http://www.bbc.com/future/bespoke/follow-the-food/the-pandemic-threatening-bananas.html">www.bbc.com/future/bespoke/follow-the-food/the-pandemic-threatening-bananas.html</a>
	Webinar on Fusarium TR4 diagnostics (part 1) <a href="http://www.youtube.com/watch?v=-kGk8u1MMbY&amp;t=10s&amp;ab_channel=ferchuckygarcia">www.youtube.com/watch?v=-kGk8u1MMbY&amp;t=10s&amp;ab_channel=ferchuckygarcia</a>
	Isolation of Fusarium Tropical Race 4 from plant tissue <a href="http://www.youtube.com/watch?v=XnKO3qXvJfs&amp;t=149s&amp;ab_channel=ferchuckygarcia">www.youtube.com/watch?v=XnKO3qXvJfs&amp;t=149s&amp;ab_channel=ferchuckygarcia</a>
	Fusarium TR4 spore production <a href="http://www.youtube.com/watch?v=6EWf_d6902Y&amp;t=7s&amp;ab_channel=ferchuckygarcia">www.youtube.com/watch?v=6EWf_d6902Y&amp;t=7s&amp;ab_channel=ferchuckygarcia</a>
	A comprehensive video on TR4 made by <i>Business Insider</i> that went viral <a href="http://www.youtube.com/watch?v=nhPEErJnErU">www.youtube.com/watch?v=nhPEErJnErU</a>
	Website of the Costa Rican banana growers' association <a href="http://www.corbana.co.cr/fusarium">www.corbana.co.cr/fusarium</a>









## **IPPC**

The International Plant Protection Convention (IPPC) is an international plant health agreement that aims to protect global plant resources and facilitate safe trade. The IPPC vision is that all countries have the capacity to implement harmonized measures to prevent pest introductions and spread, and minimize the impacts of pests on food security, trade, economic growth and the environment.

## **Organization**

- ◆ There are over 180 IPPC contracting parties.
- ◆ Each contracting party has a national plant protection organization (NPPO) and an official IPPC contact point.
- ◆ Ten regional plant protection organizations (RPPOs) have been established to coordinate NPPOs in various regions of the world.
- ◆ The IPPC Secretariat liaises with relevant international organizations to help build regional and national capacities.
- ◆ The secretariat is provided by the Food and Agriculture Organization of the United Nations (FAO).

## **Did you read this guide?**

Please send an email to [ippc@fao.org](mailto:ippc@fao.org) and share your feedback.

Your responses will help the IPPC Secretariat and the IPPC Commission on Phytosanitary Measures (CPM) Implementation and Capacity Development Committee (IC) strengthen this and other guides and training resources.

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With the financial support of

