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Fusarium oxysporum f. sp. *cubense* tropical race 4

A risk to the Brazilian banana
cultivation

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SUSTAINABLE
DEVELOPMENT
GOAL

2 ZERO
HUNGER



Fusarium oxysporum f. sp. *cubense* tropical race 4

a risk to the Brazilian banana cultivation¹

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Fusarium wilt (also known as Panama disease), caused by the fungus *Fusarium oxysporum* f. sp. *cubense* (Foc) is by far the most important disease of banana. The fungus inhabits the soil and colonizes the xylem vessels, leading to severe wilting and death of infected plants (Stover, 1962).

Detailed reviews of the disease epidemiology since it was first identified in Australia in 1876, until recently with the arrival of tropical race 4 in Colombia (Dita et al., 2018; Pegg et al., 2019) have been published. In the middle of the last century, due to the spread of Foc, the cultivar Gros Michel, which was used in plantations in Latin America to supply both foreign and local markets, was replaced by resistant cultivars from the Cavendish subgroup.

However, in 1990, a new variant of the fungus that severely affected Cavendish clones was reported.

The racial structure of Foc are currently divided into race 1, race 2 and race 4. Race 4 is further divided into Subtropical race 4 (SR4) and Tropical race 4 (TR4). Race 1 affects cultivars Gros Michel, Silk and Pome, among others, and race 2 attacks Bluggoe, known as 'Figo' in Brazil. TR4 affects cultivars in the Cavendish subgroup (Grande Naine, Valery, William, Nanica, etc.) and many others cultivars both resistant or susceptible to races 1 and 2 (Pegg et al., 2019). Race 3 attacks heliconias, but not banana, so it is no longer considered to be part of the racial structure of the Foc (Ploetz, 2006).

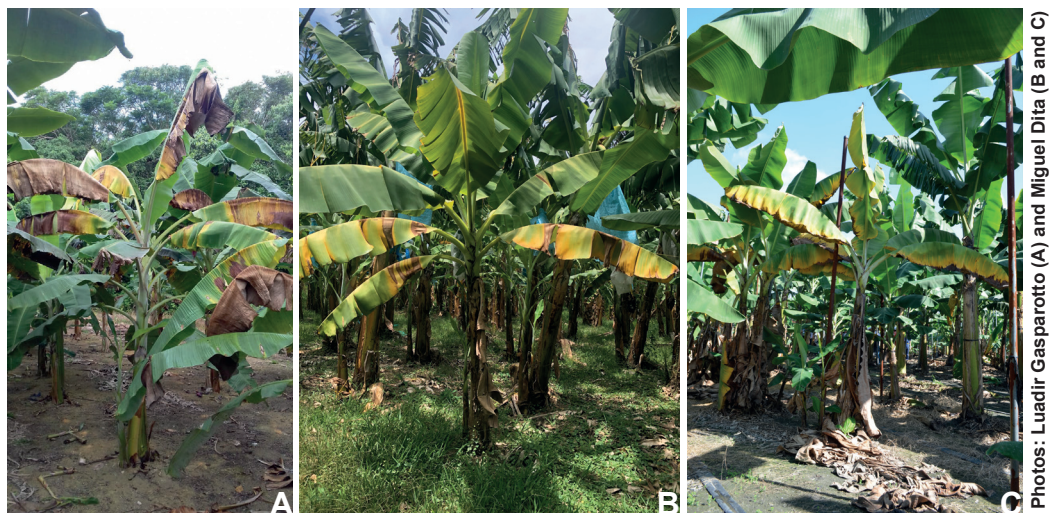
Tropical race 4 is currently is widespread to 20 countries and was recently found in Colombia (Promusa, 2020) and Peru (Ardiles, 2021), increasing risk to the Brazilian banana production.

This document is intended for producers, extension workers, technicians, and academics and has the objective to present detailed information about symptoms caused by Fusarium wilt to support monitoring and surveillance strategies of TR4 in Brazil. Symptoms of biotic (Moko, caused by *Ralstonia solanacearum*, race 2) and abiotic disorders such as abiotic wilt are presented making comparative analyses to facilitate its discrimination in the field. At the end of the document, the precautions to be observed during surveys and the procedures to be adopted in case of suspicious cases of TR4, are discussed.

Symptoms of races 1, 2 and TR4

The external symptoms caused by TR4 in banana are similar to those caused by races 1 and 2. Plants infected by the pathogen exhibit, externally, progressive yellowing from the older leaves to newer ones, starting at the edges and progressing towards the central vein (Figure 1) (Gasparotto et al., 2016).

Subsequently, the leaves wilt, dry and often split the petiole next to the pseudostem, hanging down, giving the appearance of a closed umbrella (Figure 2).



Photos: Luadir Gasparotto (A) and Miguel Dita (B and C)

Figure 1. External symptoms of Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense*, race 1 in Brazil (A) and TR4 in Colombia (B) and Taiwan (C).

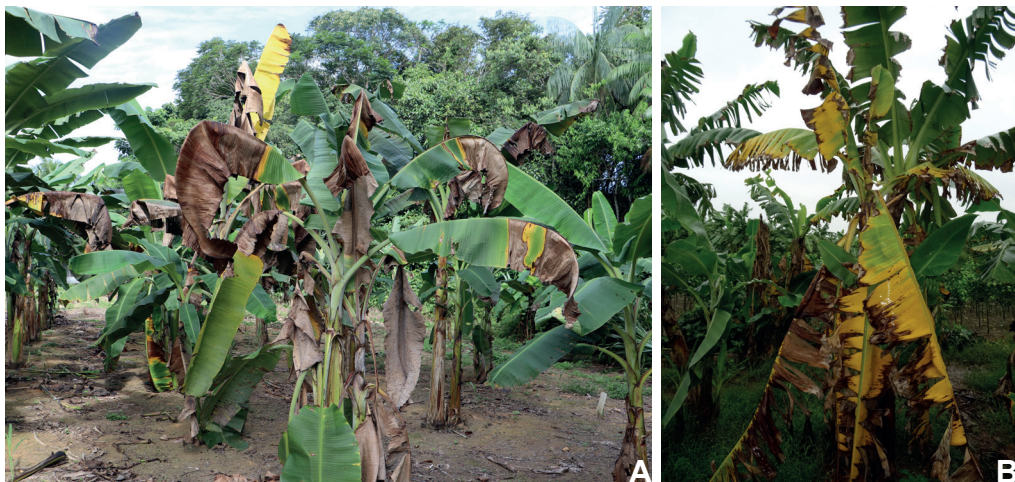


Figure 2. Banana plants affected by *Fusarium oxysporum* f. sp. *cubense*, showing wilted and broken leaves next to the pseudostem. A. Race 1 in Brazil. B. TR4 in Vietnam.

Commonly, the central leaves of the affected banana remain erect, even after the death of the older leaves. Pseudostems splitting may occur in the sheath bundle of the pseudostem close to the soil, the extent of which varies with the affected area in the rhizome.

Internal symptoms of Fusarium wilt, race 1 in Brazil and TR4 in Colombia, can be seen through a transverse or longitudinal section of the pseudostem, in which reddish-brown discoloration is observed. The typical affected area of the pseudostem appears as a necrotic ring surrounding the central cylinder (Figure 3).

Cross sectioning the rhizome of plants affected by panama disease Fusarium wilt shows the central necrotic area with a reddish-brown color (Figure 4).

Symptoms of moko and Fusarium wilt

Banana moko disease or bacterial wilt, caused by the bacteria *Ralstonia solanacearum* race 2, is a xylem vascular disease that affects all parts of the plant, including fruits. Symptoms of Fusarium wilt are restricted to the rhizome, pseudostem and leaves.

The initial symptoms of moko appear in plants of any stage and are characterized by a yellow-gold coloration of most leaves, including the emerging leaf (Figures 5A and 5B). In Fusarium wilt, external symptoms are mainly apparent in plants close to flowering stage, and suckers continue to growth normally (Figure 5C).



Photos: Fernando Goss (A) and Miguel Dita (B)

Figure 3. Internal symptoms of *Fusarium wilt* (*Fusarium oxysporum* f. sp. *cubense*), observed through transverse and longitudinal cutting of the banana pseudostem, showing a necrotic ring caused, race 1 in Brazil (A) and TR4 in Colombia (B).



Photos: Daniel A. Schurt (A) and Catalina Quinteros (B)

Figure 4. Internal symptoms of the disease observed through cross-section of the rhizome of banana affected by *Fusarium wilt* (*Fusarium oxysporum* f. sp. *cubense*), race 1 in Brazil (A) and TR4 in Colombia (B).

Photos: Luadir Gasparotto (A), Murilo R. Arruda (B) and Fernando Goss (C)



Figure 5. Symptoms of moko in young (A) and adult (B) banana, with leaves showing a golden-yellow color, and Fusarium wilt in plants close to flowering (C) with partial yellowing and dead leaves.

In areas affected by moko, all the plants in the mat die and commonly the plantation is completely decimated (Figure 6A). In areas affected by Fusarium wilt, only adult plants die, and

the clump continues to emit new suckers that will express symptoms usually from 5 to 6 months old close to the flowering stage (Figure 6B).

Photos: Murilo R. Arruda (A) and Fernando Goss (B)



Figure 6. Appearance of plants affected by moko in the field (A) and plants close to flowering affected by Fusarium wilt, with suckers apparently healthy (B).

When a transversal and longitudinal cuts are made in the pseudostem of plants affected by moko, reddish-brown spots are observed on the entire exposed surface including the central cylinder

(Figures 7A and 7B). In plants affected by Fusarium wilt, the typical necrotic ring is seen surrounding the central cylinder (Figure 7C).

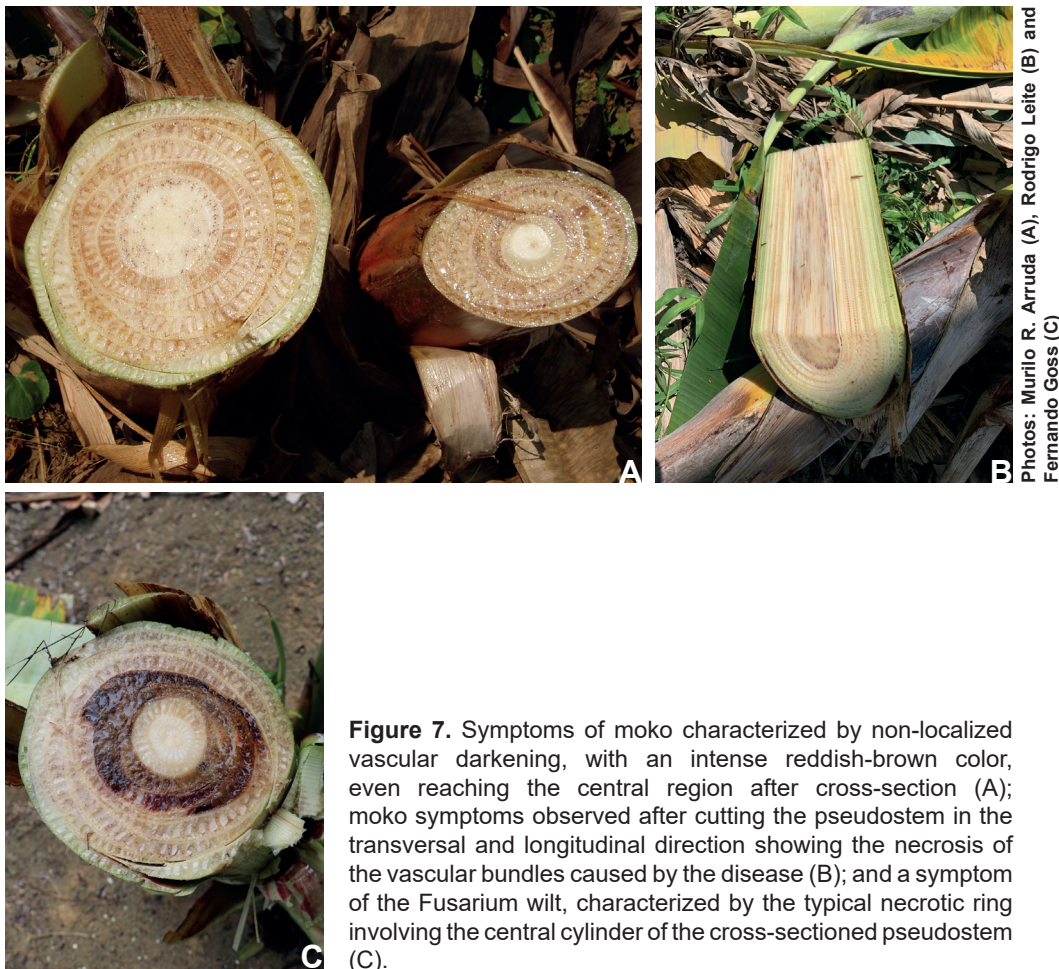


Figure 7. Symptoms of moko characterized by non-localized vascular darkening, with an intense reddish-brown color, even reaching the central region after cross-section (A); moko symptoms observed after cutting the pseudostem in the transversal and longitudinal direction showing the necrosis of the vascular bundles caused by the disease (B); and a symptom of the Fusarium wilt, characterized by the typical necrotic ring involving the central cylinder of the cross-sectioned pseudostem (C).

The rhizome of the plant affected by moko presents the central cylinder surrounded by a reddish-brown ring and with small specks of the same color scattered on the exposed surface (Figure

8A); the rhizome affected by Fusarium wilt is partially or totally necrotic (Figure 8B).

Unlike Fusarium wilt, symptoms of moko can be seen in the stalkrachis, with

vascular darkening in the form of reddish spots evenly arranged (Figure 9A). In the fruits, there is early yellowing, the darkening of the pulp followed by dry rot

(Figure 9B) and the an exudation of light pearly bacterial ooze after cutting the diseased organ is observed (Figure 9C).

Photos: Murilo R. Arruda (A)
and Daniel A. Schurt (B)



Figure 8. Internal symptoms of moko observed after the cross section of the rhizome, showing the central cylinder surrounded by a reddish-brown ring with small reddish dots scattered on the exposed surface (A); internal symptoms of rhizome affected by Fusarium wilt show the central cylinder partially or totally necrotic (B).

Photos: Murilo R. Arruda (A), Antonio Sabino
N. C. Rocha (B) and Luadir Gasparotto (C)

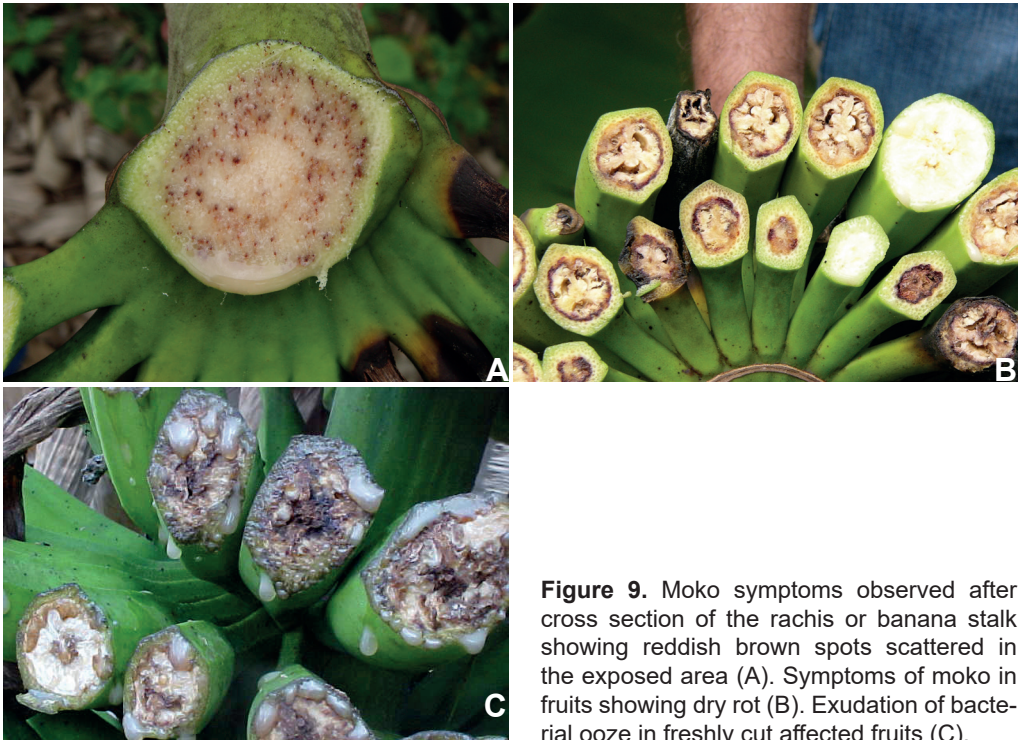


Figure 9. Moko symptoms observed after cross section of the rachis or banana stalk showing reddish brown spots scattered in the exposed area (A). Symptoms of moko in fruits showing dry rot (B). Exudation of bacterial ooze in freshly cut affected fruits (C).

To detect the presence of the bacterium in the tissues of the plant affected by the moko, the cup test can be performed. This test consists of inserting a slice of the affected part obtained from the suspect pseudostem or rachis, with the largest size cut in the longitudinal direction in a transparent glass with crystalline water. The presence of the bacteria is confirmed when a milky flow emerges from the plant tissue and decant towards the bottom of the glass (Figure 10). In approximately 1 minute, the bacterial flow begins to decrease.

Photo: Felipe S. Rosa

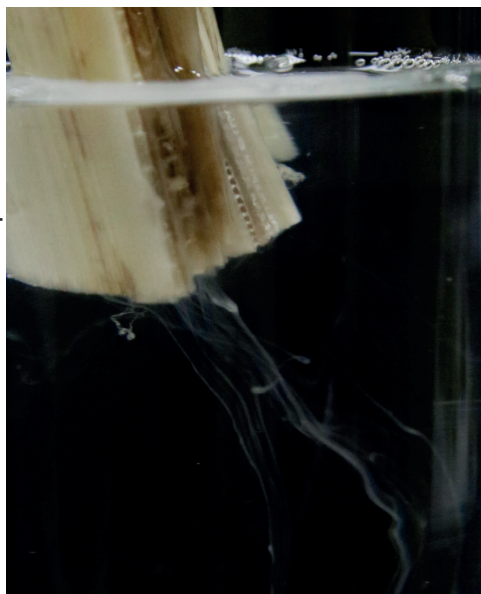


Figure 10. Cup test with milky flow of the bacteria *Ralstonia solanacearum* race 2 released from banana tissues affected by moko, also known as bacterial ooze.

Symptoms of abiotic wilt

The external symptoms of abiotic wilt, caused by acute potassium deficiency, can be confused with those of moko and especially with those of *Fusarium* wilt. For diagnosis it is recommended to make cross-sections of the pseudostem.

Abiotic wilt is characterized by the rapid yellowing of older leaves (Figures 11A and 11B). This chlorosis initially presents a yellow-gold color, then it dries and acquires an orange tint, almost uniform throughout the leaf blade. The main rib breaks about two thirds of its length, withering and rapid drying. The leaf has a characteristic shriveled appearance before bending, reminding the moko or *Fusarium* wilt symptom. The wilt progresses, reaching the youngest leaves, which can lead to the death of the entire leaf surface.

When making a cross section of the pseudostem, the spacing between the sheaths and the beginning of ocher browning in the leaf sheaths are seen (Figure 11C). This darkening is different from those presented by the pathogens that cause moko or *Fusarium* wilt. In abiotic wilt, it is the whole tissue that begins to necrotize, not the individualized vessels. Therefore, it does not present continuous vascular discoloration, being a entry point for microorganisms, which can speed up the tissue rotting

process (Figure 11D) and exhaling a characteristic sour odor. The central cylinder is soft and loose, separating from the other parts of the pseudostem. When tightening the tissues of the pseudostem, it is also noted that the tingle or stain found in malnourished

plants disappears, giving way to a liquid like water (Figure 11E).

The cluster of a plant with abiotic wilt is stunted and of poor quality, there is no complete filling of the fruits, which may appear curved with uneven maturation (Figure 11F).

Photos: Murilo R. Arruda



Figure 11. Symptoms of abiotic wilt: A and B) low leaves with a yellow-gold color and typical symptom of potassium deficiency; C) pseudostem showing spacing between the sheaths and beginning of ocher coloration of the sheaths; D) ocher browning is accentuated and the rotting of the tissues begins; E) rotting of tissues; F) plant with stunted bunch and fruits.

Recommendations

- Personnel involved on monitoring and surveillance of possible occurrence of *F. oxysporum* f. sp. *cubense*, TR4 must be well trained
- Suspects of the occurrence of TR4 can be indicated by observing symptoms on cultivars resistant to races 1 and 2, such as those of the

to not confuse *Fusarium* wilt with moko or abiotic wilt.

- Cavendish subgroup (Grand Nine, Valery, William, Nanica, etc.), Terra subgroup (D'Angola, known in the Amazon as Pacovan, Comprida, Farta Velhaco, Terra Anã, etc.) and Thap Maeo, Caipira, BRS Conquista, Pacovan Ken, BRS Japira, BRS Vitória, BRS Platina, BRS Caprichosa, BRS Pacoua and BRS Princesa.
- After careful analysis and reaching the conclusion that the disease symptoms in banana cultivars resistant to races 1 and 2, correspond to Fusarium wilt, technicians or growers should not collect samples or carry out any activity in that plantation and must isolate the area to avoid any access.
 - Contact, as soon as possible, the Federal Superintendence of Agriculture of the Ministry of Agriculture, Livestock and Food Supply (SFA/MAPA) and/or the Agricultural Defense Agency with headquarters in the state or municipality to take the appropriate measures such as collecting samples, sending it to the laboratory officially accredited by the MAPA, identifying the agent, and indicating the mitigation measures to prevent the spread of the pathogen to other plantations.
 - As there are no cultivars resistant to the tropical race 4, producers should pay attention to the ban on imports of banana and heliconia seedlings from countries where the pest occurs, especially Colombia and Peru.
 - Since the pathogen can remain in the soil for more than 30 years and moko for up to 2 years, banana producers should only use seedlings of safe and proven origin, preferably produced by tissue culture, to minimize the risk's introduction of pests in the production area.
 - If the producer observes symptoms that indicate the occurrence of one of the pests described in this work during the cultivation treatments, he should not use the same tool (machete, knives, deflowerers, etc.) on other plants before disinfecting it with sodium hypochlorite or quaternary ammonia.

Phytosanitary survey

Phytosanitary surveys are implemented to detecting a pest or delimiting its dissemination in a given area.

Detection surveys take place during routine monitoring in areas at risk, especially in the areas of production of host plants or under investigation, in case

of suspects, to confirm the presence of a pest.

The delimitation surveys are carried out after confirming the presence of the pest to verify the extent to which the spread has occurred and may cover more than one municipality or state. The result of these surveys is essential for decision making and the type of phytosanitary measures to be applied.

The preparation for the survey must consider the biology of the pest and the host culture as well as the geographic and socioeconomic characteristics of the region. Before acting, it is necessary to obtain information on the production area, size of properties, main cultivars, access to propagation material and the level of technification of the activity in the region. In addition, it is important to identify public and private agents able to locate properties and facilitate communication with local farmers such as technical officers, rural extension agents, representatives of associations, among others.

Phytosanitary surveys must address at least the following aspects:

- 1) The target of the survey must be well defined. In the case of Foc TR4, banana cultivars normally not affected by races 1 and 2 with typical symptoms of Fusarium wilt are priority. Avoid confusion

with symptoms caused by other diseases or biotic factor is key.

- 2) The area where the survey will be carried out must be previously defined. Banana plantations with a history of occurrence of *F. oxysporum* f. sp. *cubense*, race 1 and 2, could be considered as priority target sites because of the preconditions for disease occurrence.
- 3) The size of the area to be inspected will depend mainly on the commercial production area of the host in the region and the number of technicians and material available for the action. This definition is strategic and should be as representative as possible, which is why other factors must be considered, such as the level of technification of properties, the destination of production, risk routes identified in the region, among others. In addition, the number of plants to be inspected on each property and the walking route within the area need to be defined considering local characteristics.
- 4) Transparency and traceability are essential to give credibility to decision making process arisen from phytosanitary surveys.

Thus, all activities carried out on the properties visited must be recorded in a report, including the geographic coordinates that allow the elaboration of updated maps on the official actions for the prevention, surveillance, and control of the referred disease in Brazil.

- 5) Biosecurity is vital to avoid the disease spread since it is common that the agents survey several properties in the same day. Thus, due to dispersion over soil and water, all the equipment used (shoes, car tires, etc.) must be disinfected even with the use of footbaths before entering and before leaving each target area.
- 6) In case of suspicion of the occurrence of TR4 or when the typical symptoms of *Fusarium* wilt are observed in cultivars resistant to races 1 and 2, the biosecurity procedures should be reinforced mainly when collecting samples according to the procedures in Annex I.
- 7) The MAPA has official laboratories for this type of analysis. Currently, samples for detection of TR4 should be sent exclusively to the Federal Laboratory of Agricultural Defense of Goiás (LFDA/GO).

To avoid mistakes, in case of suspicion, the agent should consult the MAPA by email cgpp.dsv@agricultura.gov.br.

- 8) The plant under suspicion must be identified with yellow tape or another form that highlights it that indicates the restriction of access to the area within a radius of 5 meters. In this case, the georeferenced data specific to the plant being sampled should also be recorded.
- 9) The General Coordination of Plant Protection (CGPP/DSV/SDA) must be immediately informed about the suspicion, the monitoring procedures adopted and measures adopted or to be adopted.

Annex I – Adapted from Dita Rodríguez et al. (2013)

Methodology for tissue sampling from banana plants suspected of being affected by TR4.

- 1) Check the list of materials and utensils needed for sampling. Preferably use the list in Annex III and do a “check-list” before going to the sampling site.

- 2) Locate the suspected plant, check the symptoms and record on the form for the collection of field data. Make photographic records of the suspect plant and those around it and geo-reference the point with a GPS.
- 3) Place a portable footbath with its disinfectant at 1m to 1.5m from the base of the plant and ensure that anyone who enters or leaves the area of the suspected plant properly disinfects the shoes (wear rubber boots).
- 4) Wear surgical gloves and perform a longitudinal cut on the pseudostem, with previously disinfected tools, at a height of 50cm to 100cm from the base of the plant. Remove the pseudostem fragment about 15cm high x 10cm wide x 3cm to 5cm deep. Avoid taking samples from areas where there is advanced tissue decomposition. Make photographic records of the symptoms inside the plant after cutting.
- 5) Place the cut fragment of the pseudostem in a clean tray or plastic bag, avoiding contact with the soil. Using tweezers, remove 5 to 10 vascular bundles (from 3 cm to 10 cm in length) with typical symptoms of the disease (reddish-brown) from the pseudostem fragment and place them on a sterile paper towel.
- 6) Once the vascular bundles are removed, place the removed pseudostem fragment back on the plant in its original position. Apply insecticide and cover the area with moisture-resistant adhesive tape. This operation aims not to expose the plant tissues, to avoid or reduce the spread of the pathogenic agent via sporulation and by insects or other animals, as well as by the action of rain and wind.
- 7) Remove excess moisture from the collected vascular bundles with the aid of sterile paper towels.
- 8) Place the bundles wrapped in paper towels, already without excess moisture, in test tubes with screw cap and close the tube immediately. Wash the outside of the tube with 70% alcohol, dry completely and identify. Alternatively, a paper envelope can be used.
- 9) Proceed similarly with the rest of the samples.

Annex II – Adapted from Dita Rodríguez et al. (2013)

Biosecurity records and measures during sample collection.

1) Write down data for each sample (label), such as:

- a) Sample number or code (if several samples from the same plant are taken, it must be well identified).
- b) Collection date.
- c) Name of the cultivar of the host plant, including local names and, if possible, the genomic constitution of the host (for example: AA, AAA, AAB, ABB).
- d) If the sampled plant is in a garden, backyard, commercial plantation, or wild condition.
- e) Location and access facilities to the area, name of the property and owner, name of the city, town, municipality, state.
- f) Name of the collector.
- g) Other useful observations as a source of planting material, if the soil is flooded, how many

plants are affected, what other cultivars are planted on the property or in the neighboring areas. Evaluate and describe the agronomic management (good, weak, abandoned) of the property (planting) where sampling was carried out.

- 2) If the external symptoms are characteristic of the disease, but the internal symptoms are not seen in the cut made in the pseudostem, check for symptoms in the rhizome. In case of observation of typical symptoms in the rhizome, collect a piece of tissue (3cm x 3cm) and proceed as described from step 4 of the sampling methodology.
- 3) Whenever possible, avoid making large cuts over the suspect plant. Cover the exposed area and collect any portion of plant or material resulting from the operation and place them in a plastic bag (disposal bags). Rhizome sampling is not recommended if any rot is found.
- 4) Change the gloves whenever necessary and place them in disposal bags. Disinfect the surface of utensils used (with alcohol and fire, disinfectants based on quaternary ammonia or

sodium hypochlorite) whenever necessary, during the sampling process. The use of disposable materials may be an option. Collect all the material used for subsequent sterilization by autoclave after completing the sampling.

- 5) Do not allow samples to be exposed to high temperatures (e.g., direct sunlight or stay in car trunks), as heat reduces the success of subsequent fungi isolation.
- 6) If it is necessary to send samples to the LFDA/GO by post, to exceed the period of 5 days for the arrival, one should choose to send vascular bundles covered with sterile paper towels inside double envelopes of resistant paper and waterproof. Make sure to clearly identify the shipment and label fragile material.
- 7) If there is a possibility that samples have been mixed, sample details are confused or you are not sure if they were correct, the samples should be destroyed by incineration, autoclave, or other measures to ensure the destruction of the structures of the pathogen.

Annex III

List of materials and utensils needed for sample collection:

- Plastic bags for the collection and disposal of material.
- Rubber boots.
- Water-resistant adhesive tape.
- Yellow tape to delimit the radius (5 meters) of the suspect plant.
- Disinfectants [alcohol (70%-95%), quaternary ammonia (2,000 mg L⁻¹) and sodium hypochlorite (\geq 3,000 mg L⁻¹)].
- Georeferencing device (GPS).
- Adhesive labels.
- Form for data collection in the field.
- Surgical gloves (latex).
- Machete, knife, pocketknife, scissors.
- Overalls or disposable clothing (optional).
- Portable phytosanitary footbath.
- Tweezers.
- Container with water to prepare foot bath disinfectants and / or insecticide.
- Paper towels.
- Test tubes with their screw caps or paper envelopes.

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