



IPPC WORKSHOP SERIES

**Fusarium TR4 Diagnostic,
Surveillance, Inspection and
Simulation Exercises**

The first session of the workshop series on *Fusarium* TR4 Diagnostic, Surveillance, Inspection, and Simulation Exercises

24 March 2022

Questions & Answers

Session on *Fusarium* TR4 diagnostics

This document compiles Questions & Answers from the first session of the workshop series on ***Fusarium* TR4 Diagnostic, Surveillance, Inspection and Simulation Exercises** held on 24 March 2022, 11:00 – 13:30 (CET). About three hundred (300) participants from 125 countries worldwide attended the session.

Questions are organized by technical topic: 1) symptoms, 2) sampling and sample management, 3) tools for diagnostic, and 4) other matters. Some questions and answers were further edited for enhanced clarity.

The speakers' profile, agenda, presentations, recording of the sessions can be found [here](#).

Symptoms caused by *Fusarium* TR4 in bananas

1. Is there any way to differentiate the races of *Fusarium oxysporum* f. sp. *cubense* (*Foc*) based on the symptoms?

Answer: There are four races of *Fusarium oxysporum* f. sp. *cubense* (*Foc*). Race 1 attacks Lady Finger, Sugar, and Ducasse, but not Cavendish bananas. Race 2 attacks cooking bananas like Bluggoe and Blue Java bananas. Race 3 attacks only Heliconia species, not bananas. Finally, race 4 attacks nearly all varieties of bananas, including the main commercial variety, Cavendish. Recently *Fusarium* spp. infecting bananas were found to be genetically diverse, but race 4 showed a largely clonal genomic identity, resulting in a change in nomenclature. The species representing race 4 is now re-classified as *Fusarium odoratissimum* (Maryani *et al.*, 2019¹).

The symptoms caused by the different races are practically the same in all banana materials. That means that races cannot be differentiated by symptoms it causes in bananas. If symptoms related to the disease are found on Cavendish materials, an alert should be sent immediately, as this is most likely due to TR4.

¹ Maryani, N., Lombard, L., Poerba, Y. S., Subandiyah, S., Crous, P. W., and Kema, G. H. J. (2019). Phylogeny and genetic diversity of the banana *Fusarium* wilt pathogen *Fusarium oxysporum* f. sp. *cubense* in the Indonesian centre of origin. *Studies Mycol.* 92, 155–194.

Before 1990, isolates classified as race 4 only caused severe yield losses in Cavendish genotypes in the subtropical areas of Australia, the Canary Islands and Taiwan, particularly during abiotic stress. However, the incidence and severity of the disease increased independently of abiotic stress and resulted in an unforeseen yield loss in commercial Cavendish plantations in Southeast Asia, particularly in Taiwan. The new race identified as the causative agent was called tropical race 4 (R4T), while the milder variant that caused the disease in the subtropics after abiotic stress was called subtropical race 4 (R4ST).

2. Which part of banana plants first and most expresses *Fusarium* TR4 symptoms?

Answer: The very first symptoms are expressed in younger leaves. The primary noticeable disease symptoms are the common symptoms of all diseases that affect the plant vascular system: wilting of the older leaves. Chlorosis is also common, but not with all cultivars. After a while, younger leaves start to show disease symptoms, forming a skirt of dead leaves around the pseudostem, and finally, the plant collapses.

The first symptoms become visible from a few months to one or more years after infection of the roots. In general, internal symptoms are not observed in the fruits (Ploetz, 2015)².

3. Does *Fusarium* TR4 only affect the Cavendish variety?

Answer: No. *Fusarium* TR4 affects many other banana varieties, including plantain. Thus, the recommendation is to collect samples and undertake molecular diagnostics whenever the symptoms of the disease are observed in any banana plant variety.

4. TUMAINI app was mentioned during a presentation. Could you please provide more details about this app?

Answer: TUMAINI is an app created by the International Center for Tropical Agriculture (CIAT) Bioversity, which helps to identify some of the *Fusarium* TR4 symptoms in the field. However, the app does not provide 100% accurate results, as symptoms of *Fusarium* TR4 are very alike to other biotic and abiotic issues. More information about the TUMAINI app can be found at <https://ciat.cgiar.org/phenomics-platform/tumaini/>

Sampling and sample management of *Fusarium* TR4

5. Should systematic samples be taken, or only samples of plants presenting suspicious chlorosis?

Answer: First, samples should be taken from affected plants, and then, samples from surrounding healthy plants should be taken, as there is a likelihood that these plants will show symptoms of the disease at later stages. Therefore, once TR4 is confirmed, larger surrounding areas should be quarantined.

6. Should sample collection be conducted in a specific phase of vegetative growth?

Answer: Sample collection can be conducted at any phase of vegetative growth.

² Ploetz, R. C. (2015). *Fusarium* wilt of banana. *Phytopathology*, 105(12), 1512-1521.

7. Approximately at what height of the pseudostem a sample must be taken?

Answer: Samples for isolation of TR4 should consist of a section of the pseudostem of wilted banana plants with evidently colored vascular tissue. The sample should be taken from the lower part and close to the center of the pseudostem, but not with advanced rotting.

8. Can a plastic material be used to transport the samples?

Answer: Yes, plastic material can be used to transport samples, but the risk of bacterial contamination is high because banana tissue is very humid. The samples can rot rapidly particularly in hot humid weather conditions. Therefore, it is better to use paper material to prevent the sample from rotting.

9. Besides drying vascular tissues or samples on tissue or paper at room temperature, can the tissues be desiccated in calcium chloride or silica gel before transferring them to laboratories for diagnosis?

Answer: No, there is no need to desiccate the tissues before sending the sample to the laboratories.

10. In countries where the *Fusarium* TR4 disease has not yet been identified, is it useful for banana producers to start taking random samples for early detection and prevention of the disease?

Answer: Surveillance and sampling is the responsibility of the National Plant Protection Organization to avoid the spread of the pathogen through sample mobilization, and to achieve a reliable and accurate diagnosis.

Tools for diagnostic of *Fusarium* TR4

11. Are there any detection kits available for *Fusarium* TR4?

Answer: Yes, there is a commercial kit that allows everybody to use the same protocol and conduct comparative analyses. In non-commercial kits, there are other molecular markers that allow the lab diagnosis through PCR or qPCR. For more information consult Li *et al.* (2013)³, Carvahails *et al.*, (2019)⁴, and Aguayo *et al.* (2017)⁵. Also, a loop-mediated isothermal amplification assay (LAMP) is available, consult Zhang *et al.*, (2013)⁶. Ordonez *et al.* (2019)⁷.

12. Is the LAMP protocol specific to detecting *Fusarium* TR4?

Answer: Yes, it is specific.

13. What is the level of sensitivity of each of the diagnostic techniques for *Fusarium* TR4?

Answer: The commercial kit has been very effective to diagnose TR4 until now. It is easy to use and has the same protocol for every user. LAMP is also very specific to TR4, as well as the other kits. It is suggested

³ Li C, Mostert G, Zuo C, Beukes I, Yang Q, Sheng O, et al. Diversity and distribution of the banana wilt pathogen *Fusarium oxysporum* f. sp. cubense in China. *Fungal Genomics & Biology*. 2013.

⁴ Carvahails, L. C., Henderson, J., Rincon-Florez, V. A., O'Dwyer, C., Czislowski, E., Aitken, E. A., & Drenth, A. (2019). Molecular diagnostics of banana Fusarium Wilt targeting secreted-in-xylem genes. *Frontiers in plant science*, 10, 547.

⁵ Aguayo, J., Mostert, D., Fourrier-Jeandel, C., Cerf-Wendling, I., Hostachy, B., Viljoen, A., & Ios, R. (2017). Development of a hydrolysis probe-based real-time assay for the detection of tropical strains of *Fusarium oxysporum* f. sp. cubense race 4. *PLoS One*, 12(2), e0171767.

⁶ Zhang, X., Zhang, H., Pu, J., Qi, Y., Yu, Q., Xie, Y., & Peng, J. (2013). Development of a real-time fluorescence loop-mediated isothermal amplification assay for rapid and quantitative detection of *Fusarium oxysporum* f. sp. cubense tropical race 4 in soil. *PLoS one*, 8(12), e82841

⁷ Ordóñez, N., Salacinas, M., Mendes, O., Seidl, M. F., Meijer, H. J. G., Schoen, C. D., & Kema, G. H. J. (2019). A loop-mediated isothermal amplification (LAMP) assay based on unique markers derived from genotyping by sequencing data for rapid in planta diagnosis of Panama disease caused by Tropical Race 4 in banana. *Plant Pathology*, 68(9), 1682-1693.

to use at least two diagnostic methods, but the genomic targets should be different to ensure that one diagnostic is confirmed by another.

14. What culture media should be used to grow *Fusarium* TR4 in the lab?

Answer: Potato Dextrose Agar works well. The Komada medium should be used when samples come from the soil.

15. Do anaerobic conditions speed up or delay *Fusarium* TR4 growth?

Answer: Anaerobic conditions delay the growth of the fungus or fully inhibit it.

16. Will the Andean community guide on TR4 diagnosis, of which Fernando Garcia-Bastidas is the principal author, be translated into English for non-Spanish speakers' use?

Answer: The principal author is looking for support to get the guide translated. You can access the guide at this link in Spanish:

<https://www.comunidadandina.org/StaticFiles/202072181721Guia%20Andina%20Final.pdf>

17. Is there any support offered to send samples for the TR4 diagnosis for countries where there are no lab facilities to diagnose the pathogen?

Answer: You can photograph samples, according to the instructions provided, and send the images to Wageningen University & Research (WUR). In case there is a genuine concern about likely TR4 infection, samples can be sent to WUR for further analyses and support. For that purpose, contact Gerrit H.J. Kema at gert.kema@wur.nl.

18. Are videos about the isolation of *Fusarium* TR4 from plant tissue publicly available? How can these be accessed?

Answer: You can access one of the videos at this link: <https://youtu.be/XnK03qXvJfs>. Contact Fernando García-Bastidas at fernando.garcia-bastidas@keygene.com, or Gerrit H.J. Kema at gert.kema@wur.nl for more video materials.

Other matters

19. Can *Fusarium* TR4 be spread through the banana fruits from the infected banana plants, planting materials and infected plant leaves? Is there any ongoing research to find out if fruits can transmit the disease?

Answer: There is currently no experimental evidence of fruits spreading *Fusarium* TR4. WUR is working on a manuscript on colonization throughout banana plants.

20. How long can the inoculum of *Fusarium* TR4 remain viable and infective in the soil after the eradication of infected banana plants?

Answer: Banana plants eradication from fields does not mean fungus eradication. *Fusarium* TR4 may stay in the soil and alternative hosts such as weeds even for decades.

21. How long is the occupation period of *Fusarium* TR4 in Cavendish banana clones?

Answer: The length of the occupation period depends on many factors, but on average it ranges from 6 to 12 months.

22. What steps do you recommend to prevent *Fusarium* TR4?

Answer: Firstly, implementation of Pest Risk Assessment and phytosanitary measures for pathways of concern according to the risk, is important. Next, it is necessary to execute active surveillance and implementation of appropriate diagnostic tests for fungus identification. Lastly, aerial monitoring works very well to identify suspect plots for more detailed analysis.

23. Is it possible to reduce the inoculum load of *Fusarium* TR4 in soil by increasing the levels of endemic and beneficial microorganisms in the soil?

Answer: Reducing the *Fusarium* TR4 load is possible at considerable costs. However, reduction is epidemiologically not enough, as it allows the fungus to continue its dissemination.

24. Can the infected land by *Fusarium* TR4 incursion be used again for plantation after different treatments?

Answer: There are ways to decontaminate infected areas, but it is very complicated in practice. The inundation method conducted using a commercial product may reduce *Fusarium* TR4 inoculum in the soil, but not eradicate it.

25. How effective are microbial control agents to treat the TR4 inoculum in soil? Has this method been tested with successful replantation of banana plants in treated soil?

Answer: It has been tested but has not been proved to be effective. Panelists discourage treating the infected soils with this method as it supports further development and, most importantly, disease dissemination. Thus, replanting/continuation of banana production in infected soil is not possible unless the used variety of the plant is resistant to fungus. It worked once Cavendish replaced Gros Michel since the former has an adequate resistance level to *Fusarium* race 1. Currently, no replacement for Cavendish is available with adequate resistance to *Fusarium* TR4.

26. What can you say about material GCTCV 218? Is it resistant to *Fusarium* TR4?

Answer: It is not. It is only less susceptible, and panelists, therefore, discourage growing it on infected plots. The disease will develop again, and most importantly, the disease will continue to disseminate.

27. Have you tested some synthetic chemicals like Carbendazim or Benomyl against *Fusarium* TR4?

Answer: The dogma that *Fusarium* spp. cannot be controlled with fungicides is incorrect. WUR has tested many chemicals to treat infected plots and the fungus is very sensitive to fungicides. WUR has ongoing projects on fungicide efficacy where the infected soil is drenched. However, the application of chemicals is problematic, as controlling a soil-borne disease is extremely difficult. Moreover, we have increasing evidence that *Fusarium* strains easily survive in other hosts. The fungus also produces persistent chlamydospores, but its ability to survive in other hosts, such as weeds, is even more important. Therefore, even after abandoning the field, the inoculum will be maintained.