



Paper: Tunisian population of *Phyllosticta citricarpa* is clonal

In Tunisia, the CBS disease has impacted the citrus industry since it was first reported in 2019. So far *Phyllosticta citricarpa*, the causal agent of CBS is mostly observed on lemon (*Citrus lemon*) and rarely observed on sweet orange (*Citrus sinensis*) (Boughalleb-M'Hamdi et al. 2020).

The goals of the study performed by a group of scientists from Tunisia, Spain and France, were i) to confirm the identity of *P. citricarpa* as the causal agent of CBS in Tunisia, ii) to determine the frequency of the mating types present and iii) to explore the possibilities of sexual reproduction and subsequent dispersal via ascospores. Over 216 *Phyllosticta* strains were collected between 2021 and 2023 from lemon orchards in seven citrus-producing regions in Tunisia. All fungal strains were characterized by both morphological and molecular means. Moreover, mating type characterization and microsatellite genotyping was performed.

The structure of the population of the CBS fungus in Tunisia suggests a single introduction event. In addition, only one mating type was found in the population analyzed (Mat 1-1-1). These two together are indicators of the clonal nature of *P. citricarpa* population in Tunisia despite this fungus is considered heterothallic. Sexual reproduction may occur when two strains of compatible mating type encounter. In this case the absence sexual reproduction and the production of ascospores that are actively discharged and air borne may limit the spread of the fungus. If you want to read the publication

you can click on the link below:

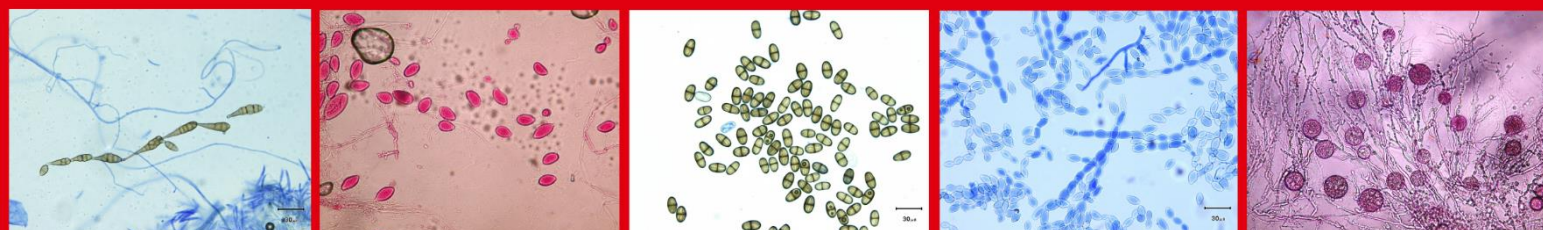
<https://www.sciencedirect.com/science/article/pii/S1878614624000497?via%3Dihub>.

Introducing EU quarantine fungal pathogens – CIR (EU) 2019/2072

We would like to introduce the second fungal pathogen which name starts with the letter “P”, *Phyllosticta solitaria*.

Phyllosticta solitaria is a fungal plant pathogen primarily affecting apple (*Malus domestica*) but other species of *Malus* and *Crataegus* have been reported (CABI 2022, EFSA 2018). The pathogen is native to North America and causes a disease known as apple blotch. Despite the disease is rarely found in commercial orchards nowadays, a different scenario was observed at the beginning of 1900s where disease incidence went up to 90% in susceptible untreated cultivars. Symptoms associated to this disease are characterized by dark, irregular spots on leaves, fruit, and twigs. Infection at the petioles may cause defoliation.

The fungus overwinters in cankers on twigs and releases spores in the spring, which are spread by rain splash. Long distance spread might happen by the movement of cankerous planting material (McClintock, 1930). The disease is most severe in regions with frequent rainfall, as moisture is crucial for spore dispersal and infection. Infected fruits often develop sunken, dark lesions, reducing their market value (EPPO ----, EFSA 2018). Control measures include pruning infected twigs, applying fungicides, and selecting resistant apple varieties. Effective management is essential



to prevent significant crop losses and maintain the health of apple orchards.

According to a Pest Categorization of *P. solitaria* made by EFSA, 2018, the identification of the pathogen was mainly based on host association and symptoms along with the study of diagnostic morphological features. However, it should be noted that the identification may be difficult due to the fact that other pathogens attacking apples cause similar symptoms. To our knowledge, there is no species-specific molecular-based detection assays reported in the literature.

EURL ACTIVITIES

Upcoming proficiency test: *Phyllosticta citricarpa*

The EURL is working on launching the campaign for the last proficiency test of the 2023 – 2024 work program. As per the request of the EC, the target species for the 2024 was changed to *P. citricarpa*.

This will be the second proficiency test organized by the EURL on this species. The EURL will provide two panels to all the participating laboratories. One panel is intended for the identification of *P. citricarpa* using macro-morphological features and sequences from common barcoding regions. The second panel seeks that the NRLs implement detection assays published in international standards such as the PM 7/017 (3) *Phyllosticta citricarpa*. The panels will be shipped at the end of November (exact date will be informed in the coming weeks). Considering the fungus has a low growth rate and that sequencing might be involved in the identification process, the participating laboratories will have two months to submit the results. The EURL will offer a virtual training on October 22nd from 15:00 to 17:00 (Paris Time). Please note that the virtual training is intended for EU-NRLs only. The main purpose of this training is to give an overview of the most relevant aspects of the biology, epidemiology and

detection of *Phyllosticta citricarpa*, the causal agent of Citrus Black Spot (CBS). We will also have a time slot allocated to discuss technical and logistical aspects of the upcoming proficiency test. We will send you the official agenda and the link to join the meeting soon.

EURL Collection: Reference material requests

We invite you to process your requests for reference material through the EURL website (<https://eurl-fungi.anses.fr/>). You will find the request form on the EURL Reference Collection tab. Once you fill the information in the request form, the EURL team will process your request and contact you if additional information is required. Please remember that living cultures can only be shipped once the EURL receives a LOA and requests its endorsement to local authorities. DNA and positive control plasmids are not subject to this regulation. In order to improve the response time and the traceability of the reference material, we will only process requests submitted through the website. If you experience problems while processing your request do not hesitate to contact us at eurl.fungi@anses.fr.

2024 EURL Fungi workshop in Greece

Last 28th of May, the Benaki Phytopathological Institute (BPI) hosted the fifth EURL for Fungi workshop. Over 40 in-person participants from 19 different NRLs and 69 online attendees actively participated in their workshop. The invited speakers shared their experience and research on the impact of *Ceratocystis platani* in South-east Europe and the biology and epidemiology of *Geosmithia morbida* in the US. Additionally, a talk on how official surveys are implemented in Greece provided participants with information about regulatory and logistical aspects of surveillance programs for quarantine organisms. You may find the presentations of the EURL workshops in the restricted access section of the EURL Fungi website (<https://eurl-fungi.anses.fr/>).



The hosts at the BPI gave participants a guided visit to the facilities including glasshouses and laboratories. The closing events consisted of a guided visit to historical sites of Athens and a dinner where all participants enjoyed a wonderful view of the Acropolis.

We appreciate if you take a few minutes to fill out this satisfaction survey by following the link below:

https://survey.anses.fr/SurveyServer/s/LRUE_SV/Workshop_2024_Fungi/questionnaire.htm

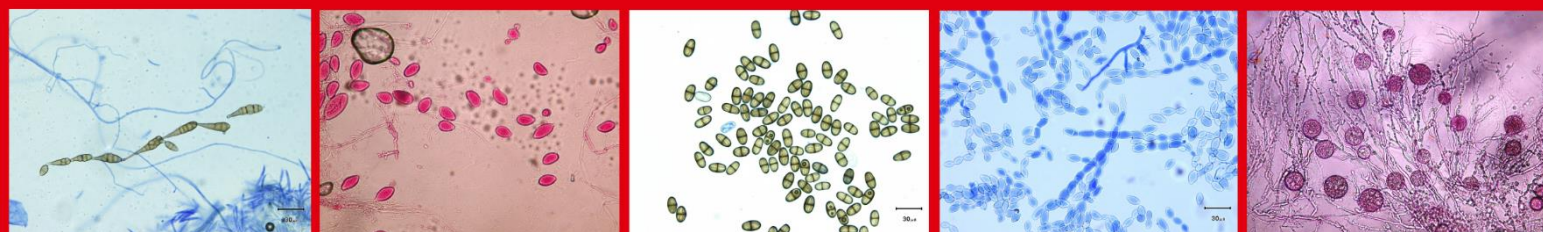
Cause analysis and follow-up actions

During the EURL workshop, our colleagues from ILVO (Belgium) presented relevant information and the rationale behind the cause analysis and corrective actions proposed by them. Several participants expressed their interest in having

the documentation generated by their team. After further consideration, it has been decided that the most convenient way to make available this information is to directly contact the team at ILVO. They will be happy to provide the information you consider pertinent for your to implement in your laboratory.

Changes in the EURL organization

After five years as Deputy Director of the EURL Fungi, Jaime Aguayo will refocus on his research activities. Jaime will still be part of the EURL Team and continue to support the development and validation of detection assays as well as the planning and execution of activities within the EURL mission. From now on, Pablo Parra will assume the duties as Deputy Director as He performs as manager of the EURL.



References

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