



New EPPO diagnostic protocols published

The European and Mediterranean Plant Protection Organization (EPPO) has recently published new diagnostic protocols on plant pests. Among the newly published documents you can find the protocols for two species in the list of quarantine organisms (UE) 2019/2072 regulation: PM 7/1 (2) *Bretziella fagacearum*, and PM 7/93 (2) *Melampsora medusae*. You can access these documents in the Volume 53, Issue 3 of the EPPO Bulletin by following the link: <https://onlinelibrary.wiley.com/toc/13652338/2023/53/3>.

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

We would like to introduce the second fungal pathogen which name starts with the letter “n”, *Neocosmospora euwallaceae*. This is one of the two species in *Neocosmospora* listed in the QO (UE) 2019/2072 regulation.

Neocosmospora euwallaceae formerly known as *Fusarium euwallaceae*, is a species in the Nectriaceae and is the nutritional symbiont of *Euwallacea fornicatus* (Scolytinae). It is morphologically similar to *N. ambrosia* (Kasson et al. 2013) and other closely related ambrosia species. The fungus is considered invasive in the United States, Mexico and South Africa. It has been isolated from avocado trees (*Persea americana*) in the State of California from ambrosia beetles of the genus *Euwallacea*, as well as from at least species in seven genera of plants in Israel including: *Acer negundo*, *Dispyros kaki*, *Ficus carica*, *Ricinus communis*, *Platanus*

orientalis, *Quercus ithaburensis*, *Quercus pedunculifolia*, *Quercus robur*, and *Ricinus communis*.

Host associations (e.g. avocado) or morphological features are not a good mean to provide a reliable identification of the species. In consequence, molecular-based identification is the best approach to distinguish *N. euwallaceae* from other phylogenetically related fungi (Freeman et al. 2013). The phylogenetic placement of this species was done through the combination of sequences from the ITS + LSU, RPB1, RPB2 and the EF1- α . *Neocosmospora euwallaceae* can be distinguished from its sister species (*N. ambrosia*) through the phylogenetic reconstruction of any of the four loci (Freeman et al. 2013). There is a plethora of sequences from informative loci available in public repositories such as the NCBI.

Conventional PCR detection includes taxon-specific primers targeting a hypothetical protein similar to cutinase transcription factor 1 alpha. Specificity of the primers was assessed by using DNA extracted from taxa within the ambrosia fusaria clade (AFC) and the *Fusarium solani* species complex (FSSC) (Short et al. 2017). More recently, Jager and Roets (2022), used the primers developed by Short et al. (2017) to further optimized the protocol for the direct detection of *N. euwallaceae* from symptomatic plant tissue. Moreover, real time PCR assays targeting the β -tubulin gene have also been developed to detect and distinguish several species of fungal symbionts (including *N. euwallaceae*) associated with the polyphagous



shot hole borer (*Euwallacea whitfordiodendrus*) and the Kuroshio shot hole borer (*Euwallaceae kuroshio*) (Carrillo et al. 2022).

EURL ACTIVITIES

Registration for the EURL Workshop in Greece

Registration for the fifth EURL workshop is open. In-person participation to this workshop is highly recommended, but online attendance will also be possible. If you want to present your research or want to share research activities of your laboratory, there is a session dedicated to this. Send us an email letting us know the title of your presentation. The workshop will give us the opportunity to review ongoing EURL activities in the context of our 2023 – 2024 work program. This includes the development of new diagnostic tools and validation of methods. If you have not received the registration form, do not hesitate to send us an email to eurl.fungi@anses.fr.

EURL reference collection and reference material

Three additional strains of *Geosmithia morbida* are included in the EURL reference collection. The strains were collected in Lyon in the surrounding areas where the pathogen was detected for the first time in France. In addition, two strains of *Ceratocystis platani* will be also part of the EURL collection. The EURL Team is currently drafting the technical sheets and obtaining sequence data that will be made available when you request the reference material.

Validation of detection assays: *Bretziella fagacearum*

As we progress in the validation of the detection assay targeting *Bretziella fagacearum*, the EURL produced a positive control plasmid (PCP) that includes the region targeted in the assay developed by Bourgault et al. 2022. We hope to

share the outcome of the validation assays during the EURL workshop in Greece. This will be a favorable scenario to discuss and contrast the results obtained during the 23Bfag proficiency test.

Upcoming events 2024

European Mycological Network Meeting. The 26th EMN annual meeting will take place in Riga this year between the 13th and 15th of May. Registration open.

EURL for Fungi and Oomycetes workshop. This year, the workshop will take place in Kifissia, Greece on the 28th of May. Registration deadline: 26th of April, 2024.

International Mycological Congress. The 12th IMC will take place in Maastricht, Netherlands between the 11th and 15th of August. Registration deadline: 21st of May, 2024.

References

Bourgault É, Gauthier MK, Potvin A, Stewart D, Chahal K, Sakalidis M, Tanguay P. 2022. Benchmarking a fast and simple on-site detection assay for the oak wilt pathogen *Bretziella fagacearum*. *Front. For. Glob. Change* 5:1068135. doi: 10.3389/ffgc.2022.1068135.

Carrillo JD, Mayorquin JS, Stajich JE and Eskalen A. 2020. Probe-Based Multiplex Real-Time PCR as a diagnostic tool to distinguish distinct fungal symbionts associated with *Euwallacea kuroshio* and *Euwallacea whitfordiodendrus* in California. *Plant Disease* 2020 104:1, 227-238. doi.org/10.1094/PDIS-01-19-0201-RE.

EPPO, 2023. PM 7/1 (2) *Bretziella fagacearum* (formerly *Ceratocystis fagacearum*). EPPO Bulletin. 2023; 53:505–517. doi.org/10.1111/epp.12944.

EPPO, 2023. PM 7/93 (2) *Melampsora medusae*.
EPPO Bulletin. 2023; 53:580–593.
doi.org/10.1111/epp.12959.

Freeman S, Sharon M, Maymon M, Mendel Z,
Protasov A, Aoki T, Eskalen A, O'Donnell K. 2013.
Fusarium euwallaceae sp. nov.—a symbiotic
fungus of *Euwallacea* sp., an invasive ambrosia
beetle in Israel and California. *Mycologia*, 105(6),
pp. 1595-1606. doi:10.3852/13-066.

Jager M and Roets F. 2022. Rapid and cost-
effective detection of *Fusarium euwallaceae* from
woody tissues. *Plant Pathology*. 2022;71:1712–
1720. doi: 10.1111/ppa.13600.

Kasson MT, O'Donnell K, Rooney AP, Sink S,
Ploetz RC, Ploetz JN, Konkol JL, Carrillo D,
Freeman S, Mendel Z, Smith JA, Black AW, Hulcr J,
Bateman C, Stefkova K, Campbell PR, Geering
ADW, Dann EK, Eskalen A, Mohotti K, Short DPG,
Aoki T, Fenstermacher KA, Davis DD, Geiser DM.
2013. An inordinate fondness for *Fusarium*:
phylogenetic diversity of fusaria cultivated by
ambrosia beetles in the genus *Euwallacea* on
avocado and other plant hosts. *Fungal Genet Biol*
56:147-157,doi:10.1016/j.fgb.2013.04.

Short DPG, O'Donnell K, Stajich JE, Hulcr J,
Kijimoto T, Berger MC, Macias AM, Spahr EJ,
Bateman CC, Eskalen A, Lynch SC, Cognato AI,
Cooperband MF, Kasson MT. 2017. PCR
Multiplexes discriminate *Fusarium* symbionts of
invasive *Euwallacea* ambrosia beetles that inflict
damage on numerous tree species throughout
the United States. *Plant Disease*. 2017.
101(1):233-240. doi:10.1094/PDIS-07-16-1046-
RE.