

Results of official annual survey for *Xylella fastidiosa* in Montenegro

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SURVEILLANCE AND EARLY DETECTION TOOLS

- ✓ *Xylella fastidiosa* (Xf) is one of the most important bacterial pathogens worldwide.
- ✓ The bacterium is listed as a quarantine organism in the EU as well as in Montenegro.
- ✓ In order to detect potentially infected material, monitoring was carried out since 2015.
- ✓ During 2019, survey of Xf host plants was conducted in places with an increased phytosanitary risk, such as the Montenegrin coast, including areas near the port of Bar and along main roads, plant production sites and urban areas, covering especially the highest risk host plants (olive trees and ornamental plants in nurseries). The potentially Xf infected plants were inspected and sampled during the period of their active growth.
- ✓ A total of 40 samples of suspected plant material (*Olea europaea*, *Myrtus communis*, *Nerium oleander*, *Lavandula* spp., *Rosmarinus officinalis*, *Laurus nobilis*) were collected (Figure 1). Symptoms on these plants varied and included marginal leaf chlorosis and necrosis, wilting of leaves and defoliation, twig dieback.



Figure 1. The symptoms resembling presence of *Xylella fastidiosa*. Olive leaf tip necrosis (a, b) and defoliation (c), wilting and necrosis of lavender canopy (d), marginal leaf scorch of laurel (e), necrosis and scorch of rosemary leaves (f). (Foto: J. Todorović)

SURVEILLANCE AND EARLY DETECTION TOOLS

- ✓ Xf is a vector-transmitted bacterium.
- ✓ *Philaenus spumarius* is widely present potential Xf vector in Montenegro.
- ✓ Five adults of *Philaenus spumarius* were collected from two sites in Montenegro and tested for the presence of Xf (Figure 2).



Figure 2. Adults of *Philaenus spumarius*. (Photo: T. Popović)

SURVEILLANCE AND EARLY DETECTION TOOLS

- ✓ Real-time loop-mediated isothermal amplification (real-time LAMP) (Harper et al., 2010; Yaseen et al., 2015) was applied for detection of Xf in the samples of plant and insect material.
- ✓ During the analysis, only positive control developed distinctive amplification curve.
- ✓ No positive samples were detected among the tested material (Figure 3 and 4).
- ✓ Results of the 2019 survey and LAMP laboratory analyses confirmed that Xf was not present in Montenegro.

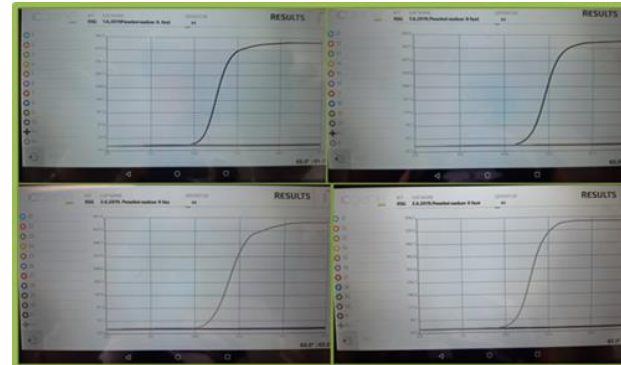


Figure 3. Results of Real-time LAMP analysis. All tested samples of plant material showed negative reaction. (Photo: T. Popović)

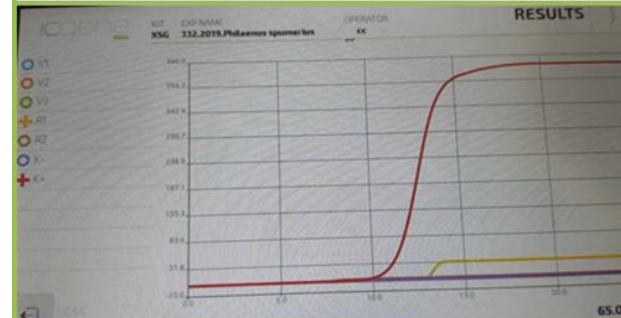


Figure 4. Results of Real-time LAMP analysis. All tested samples of insect material were negative for Xf presence. (Photo: T. Popović)

SURVEILLANCE AND EARLY DETECTION TOOLS

- ✓ LAMP system consisted of a portable ICGENE mini device (Enbiotech S.r.L., Italy) (Figure 5) and a ready to use diagnostic kit "Xylella Screen Glow".
- ✓ The advantages of real-time LAMP method were easy handling and rapid results of the analysis.
- ✓ The disadvantage is logging to the ICGENE system with the provided operator badge, which sometimes requires a lot of patience and time.
- ✓ Overall impression is that this test is suitable for the rapid detection of Xf in plant and insect vector material.

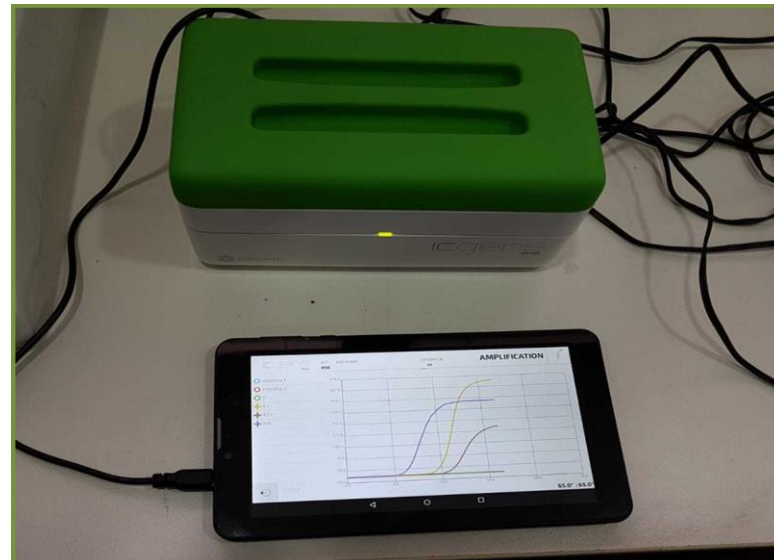


Figure 5. ICGENE mini device and tablet for real-time visualization of results. (Photo: T. Popović)