PREVENTING *XYLELLA FASTIDIOSA* INTRODUCTION IN SERBIA – CHALLENGES IN PATHOGEN DETECTION

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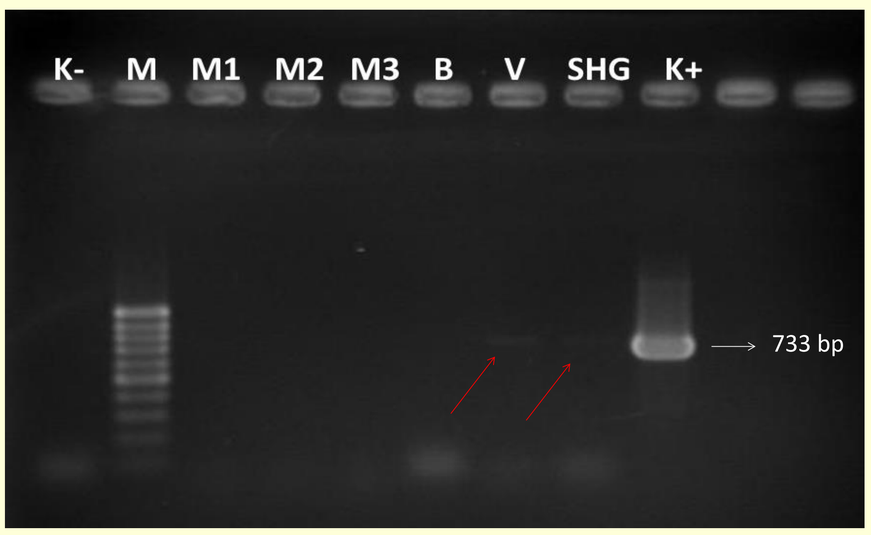
Recent interceptions of *Xylella fastidiosa* in asymptomatic ornamental plants imported from Central America into Europe and the introduction and establishment of the bacterium in some European countries caused a major concern of plant bacteriologists, and imposed restrictions in international trade of live plant material. Serbia is considered a Xylella-free country and the status has been checked periodically by surveying and sampling of potential host plants mainly of the external origin.

- Since the establishment of *X. fastidiosa* in Italy and later in France, and due to the findings of new host species in the recent outbreaks, a number of plant species inspected and sampled for the analysis by the national plant quarantine inspectors at the border crossings have increased rapidly.
- Received plant samples were tested first by conventional PCR according to Minsavage *et al*. (1994). DNA was extracted from the xylem tissue taken from various parts, depending on the sample material, by using the DNeasy plant mini kit (Qiagen). DNA from *X. fastidiosa* subsp. *pauca* strain CoDiRo was used as a positive control.
- No pathogen was detected in 173 samples in 2016 and 126 samples in 2017.

However, apart from the unambiguous negative results, a few reactions produced a faint band of similar size as the expected product (ca. 700 bp), indicating positive detection of the pathogen by the applied PCR procedure. This was repeatedly associated with testing of sweet cherry samples and thus challenged the pathogen detection accuracy.

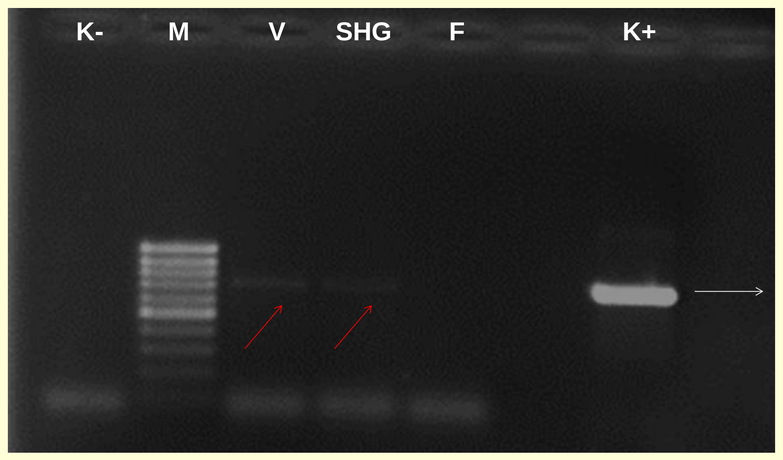
Picture 1. Sweet chery leaf scorch caused by *X. fastidiosa.* Photo: D. Boscia

- Sweet cherry has been recently reported as Xf host in Italy and Spain (Photo 1). It represents a fast growing production and therefore very important fruit species in Serbian agriculture.
- On the Gel 1, the two tested sweet cherry samples (V and SHG) produced a faint bend, similar size as positive control.



Gel 1. K-: negative control; M: marker; M1, M2, M3, B, V, SHG: tested samples; K+: positive control

- In order to exclude a technical error, the analysis was repeated following the same procedure, starting from the DNA extraction of the tested plant material, followed by the preparation of the reaction mix using newly opened reagents, and it again resulted in the same faint signal appearance (Gel 2).



Gel 2. K-: negative control; M: marker; V, SHG, F: tested samples; K+: positive control

To exclude false positive results, the samples were additionally tested by real-time PCR (qPCR, Applied Biosystems StepOne™Real-Time PCR System), following the assay designed by Harper *et al*. (2010, erratum 2013), suggested as a more sensitive alternative validated for *X. fastidiosa* detection (EPPO, 2016).

The qPCR results were negative for the presence of the targeting gene (*rim M*). Consequently, the samples were considered pathogen free.

The experience with the conventional PCR (Minsavage *et al*. 1994) resulting in a false positive signal, the most likely produced by an unspecific amplification from these particular DNA samples, confirmed the necessity for constant checking of the laboratory routine and continuous improvement of the practice and expertise facilitated by the interlaboratory cooperation within the POnTE project (GA 635646).