Diseases Caused by Bacteria and Phytoplasmas

First Report of *Xanthomonas arboricola* pv. *pruni* Causing Leaf and Fruit Spot on Apricot (*Prunus armeniaca*) in Montenegro

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In July 2020, symptoms of leaf and fruit spot were observed on 2-year-old apricot plants (*Prunus armeniaca* L.), cultivar Rubista, in plantation covering approximately 0.5 ha near Podgorica, central Montenegro. The intensity of infection on leaves was more than 70%. Initially, leaf spots were mainly circular, 2 to 5 mm in diameter, water-soaked, and surrounded by a weak chlorotic halo, but they later became light to dark brown and necrotic. Eventually, the spots merged and necrotic tissue dropped out, leaving a “shot-hole” leaf appearance. On apricot fruits, small, dark brown, mainly circular superficial lesions were observed. The lesions merged and formed large necrotic areas, reducing the quality of fruits. Symptoms were not observed on woody parts, such as twigs or stems. A total of 10 bacterial strains, forming yellow, convex, and mucoid colonies on yeast extract–dextrose–CaCO₃ medium, were isolated from symptomatic leaf and fruit tissue. All strains induced hypersensitive reaction in tobacco leaves. They were gram-negative, strictly aerobic, oxidase negative, catalase positive, hydrolyzed gelatin and esculin but not starch, and did not grow at 37°C, showing similar biochemical properties as a reference strain *Xanthomonas arboricola* pv. *pruni* (*Xap*) (NCPBP 416) used in all tests as a positive control. Strains were further identified by PCR analysis, using primer pair XapY17-F/XapY17-R (Pagani 2004; Pothier et al. 2011), resulting in a single band of 943 bp, characteristic for *Xap*. Additionally, BOX-PCR with the BOX A1R primer (Schaad et al. 2001) showed 100% homology in

genetic profiles of all tested strains and the control strain. Amplification and partial sequencing of the *gyrB* gene of four representative strains was performed using a set of primers described by Parkinson et al. (2007). The obtained DNA sequences showed that the analyzed strains (GenBank nos. MW473770, MW473771, MW473772, and MW473773) share 99.44 to 99.57% *gyrB* sequence identity with *Xap* pathotype strain ICMP51. Pathogenicity of all strains was confirmed by spraying young apricot shoots using a hand-held sprayer, and by infiltration of apricot leaves (cv. Roksana) from the abaxial surface using a syringe without a needle, with the bacterial suspension (10⁷ CFU/ml in sterile distilled water), in three replicates. Sterile distilled water and the reference *Xap* strain (NCPBP 416) were used as negative and positive controls, respectively. The inoculated shoots and leaves were maintained at approximately 25°C under high-humidity conditions. Tissue necrosis appeared on all inoculated shoots 5 to 11 days and on leaves 5 to 9 days after inoculation. Koch’s postulates were completed by reisolation of the pathogen from inoculated tissue and identification by PCR using XapY17-F/XapY17-R primers. Based on pathogenic, biochemical, and molecular characteristics, the strains isolated from apricot leaves and fruits in Montenegro were identified as *Xap*, the causal agent of bacterial leaf spot and canker of stone fruits. This quarantine pathogen was previously reported on almond (Panić et al. 1998) and on peach (Popović et al. 2020) in Montenegro. This is the first report of *Xap* affecting apricot in this country. Therefore, strict phytosanitary measures have to be implemented to prevent spread of the pathogen in other areas and other susceptible hosts.

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