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XANTHOMONAS ARBORICOLA PV. *PRUNI* ASSOCIATED WITH LEAF AND FRUIT SPOT AND TWIG NECROSIS OF PEACH, APRICOT AND SWEET CHERRY IN MONTENEGRO

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INTRODUCTION

Bacterial spot of stone fruits and almond, caused by *Xanthomonas arboricola* pv. *pruni* (Xap) is one of the most important bacterial diseases of *Prunus* spp. worldwide. The bacterium is listed as a quarantine organism in Montenegro.

In Montenegro, the disease was first described on almond trees in 1998. However, this pathogen was not studied in details since then. In order to check the status of this pathogen we carried out survey of stone fruit orchards.

Figure 1. *Xanthomonas arboricola* pv. *pruni*: leaf spot on *P. armeniaca* (a, b) and *P. persica* (c, d, e); twig cankers on *P. persica* (f, g) and *P. avium* (h, i); fruit spot on *P. persica* (j, k) and *P. armeniaca* (l, m). Natural infection. (Foto: T. Popović)

SYMPTOMS

During 2017-2020, peach and apricot leaf and fruit spot and twig necrosis were observed near Podgorica, while sweet cherry twig cankers were observed on trees near Ulcinj. The leaf lesions were initially small, angular, water-soaked, surrounded by a halo. As the disease progressed, the necrotic areas dropped out, leaving a 'shot-hole' leaf appearance. Eventually, infected leaves turned yellow and dropped off. On fruits, small, circular, water-soaked or dark brown spots were observed. Cankers on young twigs were dark, elongated, sunken, and accompanied by gummosis (Figure 1).

Figure 2. *Xanthomonas arboricola* pv. *pruni*. Colony morphology on YDC medium (Foto: T. Popović)

ISOLATION

From diseased leaves, fruits and twigs bacterial strains were isolated. They formed yellow, convex, and mucoid colonies on yeast extract–dextrose–CaCO3 agar medium (YDC) (Figure 2).

Based on their growth characteristics and preliminary pathogenicity testing (hypersensitivity in tobacco) 47 strains were selected for identification.

PATHOGENICITY TESTS

Pathogenicity in host plants was tested by spraying young shoots and infiltrating leaves and fruits with bacterial suspension (10^7 CFU/ml SDW) of all 47 strains and Xap reference strains. Lesions appeared on all inoculated shoots, leaves and fruits within a week after inoculation (Figure 3).

DNA CHARACTERIZATION

Specific primers XapY17-F/XapY17-R, detecting *ftsX* gene characteristic for Xap (Pagani, 2004; Pothier et al., 2011), were used for identification of the strains. As a result of this PCR, a product of 943 bp was amplified in all 47 and Xap reference strain.

BIOCHEMICAL TESTS

All strains were Gram-negative, catalase positive, oxidase negative and obligate aerobic, hydrolyzed esculin and didn't grow at 37°C. Out of all, three strains hydrolyzed starch and two strains didn't hydrolyze gelatin. Xap strains NCPPB 416 and KFB 0104 were used as a positive control. Based on the biochemical tests, the strains possess characteristics similar to Xap, in spite of the variation in some phenotypic properties.

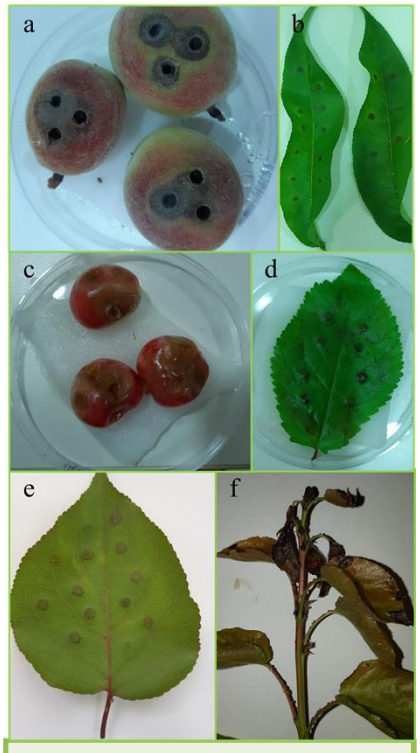

Figure 3. Pathogenicity tests: (a) necrosis of *P. persica* fruits; (b) necrotic spots on *P. persica* leaves; (c) necrosis of fruits of *P. avium*; (d) necrotic spots on *P. avium* leaf; (e) necrotic spots on *P. armeniaca* leaf; (f) shoot necrosis on *P. armeniaca* (Foto: T. Popović).

SEQUENCING OF *gyrB* GENE

Amplification and sequencing of *gyrB* gene of 14 representative strains was performed using primers described by Parkinson et al., 2007. Obtained partial DNA sequences showed that 12 strains (GenBank nos. MN 092937, MN092938, MN092940, MN092941, MN092942, MN092944, MN092945, MN092946, MW473770, MW473771, MW473772, MW473773), share 98.97 to 99.71% of *gyrB* sequence identity with Xap pathotype strain ICMP51. The remaining two strains (GenBank nos. MN092939 and MN092943) showed 100% identity with Xap strains originating from peach and apricot in Hungary and peach in Italy.

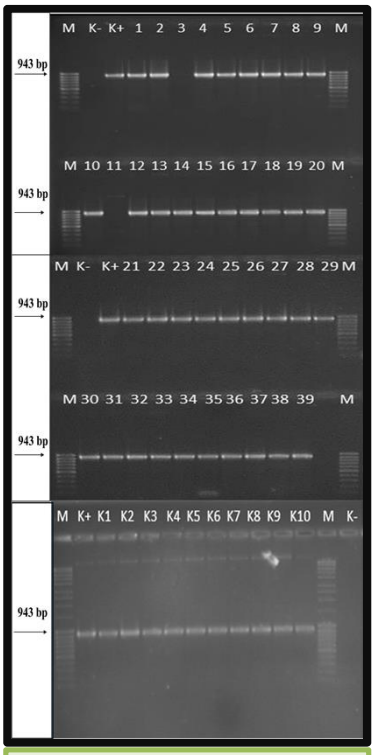

Figure 4. PCR detection *ftsX* gene in 47 tested strains. K – negative control, K + positive control Xap (69VR - CFBP3892), M- molecular marker (MassRuler Low Range DNA Ladder, Fermentas, Litvanija).

CONCLUSION

The results of this study indicated that Xap is present and spread in peach, apricot and sweet cherry commercial orchards in central and southern part of Montenegro. This could indicate spread of the local population of the pathogen previously detected in almond in Montenegro. Favorable climate and increase in stone fruit production probably contributed to that. However, stone fruit planting material is intensively imported and increases the risk of introduction of latently infected material. Therefore, strict phytosanitary measures have to be implemented to control this risk and prevent either import or spread of the pathogen in other areas and other susceptible hosts.

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