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**SURVEY ON THE PRESENCE OF QUARANTINE BACTERIA OF
GRAPEVINE *XYLOPHILUS AMPELINUS* IN REPUBLIC OF
SRPSKA 2019-2022**

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ABSTRACT

Xylophilus ampelinus, the causal agent of bacterial necrosis in grapevine is listed in Annex II, Part A, Section II of the Directive 2000/29/EC. The only known host of these quarantine bacteria is grapevine. Considering that *X. ampelinus* is listed on quarantine I A list in Bosnia and Herzegovina, and due the high direct and indirect impacts in yield reduction, death of host plants and specific disease management procedures survey in Republic of Srpska (RS) was approved and financed by the Ministry of Agriculture, Forestry and Water Management of RS since 2019. Sampling of host plants was carried out in seven regional units of RS in registered nurseries and seedling production places, farms, as well as at border crossings in RS. Laboratory analyses were carried out in accordance with the EPPO diagnostic protocols: PM Bulletin 39, 403–412. Protocols include extraction from symptomatic and asymptomatic plant material, followed by isolation or two tests based on a different principle: DAS-ELISA for the serological and a conventional PCR test for the molecular detection. Manceau *et al.* (2005) protocol was used for DNA extraction and a set of primers Xa TS1 5'-TGC GTA GTT CAA CAC CAA AGT-3', Xa TS2 5'-TAT GAC CCT CTT TCC ACC AGC-3' or Xa TS2 BIO 5'-Biotine-TAT GAC CCT CTT TCC ACC AGC-3' was used for the detection of *X. ampelinus*. During 2019, 2020, 2021 and 2022, 65, 58, 40 and 30 samples were analyzed, respectively. All tested samples were *X. ampelinus* negative, but considering consequences if bacteria occur, surveillance program is continued in 2023.

Keywords: *Xylophilus ampelinus*, grapevine, survey, Republic of Srpska.

INTRODUCTION

Xylophilus ampelinus (Panagopoulos) Willems *et al.* is the plant pathogenic bacterium causing 'bacterial blight' of grapevine. The disease was originally described in Greece (Crete) and was named *Xanthomonas ampelina* (Panagopoulos, 1969). It was transferred to the new genus *Xylophilus* (Willems *et al.*, 1987) on the basis of DNA and RNA studies.

The bacterium infects only grapevine (*Vitis vinifera* and *Vitis* spp. used as rootstock). It is recorded in Mediterranean country, (Italy) by Garovaglio and Cattaneo (1879), Baccarini (1893) and Macchiati (1897), from the end of the 19th century. However, recent years is present sporadically in Sicily and Sardinia, but also in other EU countries (northern France) (EFSA, 2014). Sampayo *et al.* (1981), Lopez, (1983), Gomez and Lopez (1986), Gracia *et al.* (1988) and Cambra Álvarez (1997) recorded bacteria in Spain. Panagopoulos and Psallidas (1983) and Panagopoulos (1987) recorded *X. ampelinus* in Greece, while Dreo *et al.* (2005a, b) confirmed present of bacteria in Slovenia, even it is observed since the late 1950s in western Slovenia (Seljak *et al.*, 2005). Apart from the mentioned countries, according to EPPO (2023) *X. ampelinus* was detected also in 1994 in Moldova, Japan (2009 in Hokkaido and 2014 in Honshu), 2016 in Jordan, 2020 in Russia and 2022 in Ukraine. Considering that the pest has a limited distribution in the EU it is listed in Annex II, Part A, Section II of the Directive 2000/29/EC. The only known host of this quarantine bacteria is grapevine (EPPO, 2009). Considering that *X. ampelinus* is in Bosnia and Herzegovina (B&H) listed on quarantine I A1 list (Trkulja *et al.*, 2012), and due the high direct and indirect impacts in yield reduction, death of host plants, their distribution in Republic of Srpska (RS) and specific disease management procedures the survey in RS was approved and financed by Ministry of Agriculture, Forestry and Water Management of Republic of Srpska from 2019.

MATERIALS AND METHODS

Survey on the presence of quarantine bacteria of grapevine *Xylophilus ampelinus* in RS was approved and financed by Ministry of Agriculture, Forestry and Water Management of RS during 2019-2022. Sampling of host plants was carried out in seven regional units of RS (Prijedor, Banja Luka, Gradiška, Doboj Bijeljina, Istočno Sarajevo and Trebinje) in registered nurseries and seedling production places, production farms and agricultural households, as well as on border crossing during their import. From Table 1, it can be seen the number of samples and places where host plant samples were taken for laboratory analysis of the presence of quarantine bacteria *X. ampelinus* in the territory of the Republic of Srpska in the period 2019-2022.

Table 1. Sample number and locations where host plant samples were taken for laboratory analysis of the presence of bacteria *X. ampelinus* in the period 2019-2022

Locations where host plant samples were taken for laboratory analysis	Sample number for laboratory analysis per year			
	2019	2020	2021	2022
Registered nurseries and producers of seedlings of agricultural plants	5	0	0	0
Production farms and agricultural households	55	55	37	28
Host plants from import	5	3	3	2
Total:	65	58	40	30

According to the “Special surveillance program for the presence of quarantine pest *X. ampelinus* (Panagopoulos) Willems *et al.* – bacterial necrosis in grapevine in Republic of Srpska” (Official Gazette of the Republic of Srpska 56/19, 34/20, 41/21 and 33/22) every inspection and sampling were mapped, recorded and digitized using FITO GIS software (Photo 1-4).

Laboratory analysis were carried out in accordance with the EPPO diagnostic protocols: PM Bulletin 39, 403–412. Protocols include extraction from symptomatic and asymptomatic plant material, followed by isolation or two tests based on a different principle: DAS-ELISA for the serological and a conventional PCR test for the molecular detection. Manceau *et al.* (2005) protocol was used for DNA extraction and set of primers Xa TS1 5'-TGC GTA GTT CAA CAC CAA AGT-3', Xa TS2 5'-TAT GAC CCT CTT TCC ACC AGC-3' or Xa TS2 BIO 5' Biotine-TAT GAC CCT CTT TCC ACC AGC-3' was used for the detection of *X. ampelinus*.

RESULTS AND DISCUSSION

Regarding Commission for plant health protection proposal at Ministry of Agriculture, Forestry and Water Management of RS, survey on the presence of quarantine bacteria of grapevine *X. ampelinus* started in 2019. Program survey was published in Official Gazette of RS 56/19. Sampling of host plants was carried out in a registered nurseries and seedling production places, production farms and agricultural households in six regional units of RS (Prijedor, Banja Luka, Doboje, Bijeljina, Istočno Sarajevo and Trebinje), as well on crossings border in RS. During 2019, total 65 samples were analyzed in Laboratory for plant protection, seed and biotechnology in PI Agricultural Institute of Republic of Srpska, Banja Luka. 55 samples were analyzed from production farms and agricultural households, while 5 samples were analyzed from nurseries and seedling production places, as well 5 samples from crossings border. Also according Official Gazette of RS in case of suspected samples, sampling of larger number from nurseries, seedling production places and crossings border was predicted for laboratory analysis. All tested samples were negative for the presence of the quarantine bacterium *X. ampelinus*.

During 2020, Ministry of Agriculture, Forestry and Water Management of RS published “Program survey on the presence of quarantine bacteria of grapevine *X. ampelinus* in RS” in Official Gazette of RS 34/20. Sampling of host plants was carried out in a production farms and agricultural households in six regional units of RS (Prijedor, Banja Luka, Doboje, Bijeljina, Istočno Sarajevo and Trebinje), as well on crossings border in RS. During 2020, total 58 samples were analyzed in Laboratory for plant protection, seed and biotechnology, PI AIRS, BL. 55 samples were sampled and analyzed from production farms and agricultural households, while 3 samples were analyzed from crossings border. As previous year, according Official Gazette of RS in case of suspected samples, sampling of larger number from nurseries, seedling production places and crossings border was predicted for laboratory analysis. The presence of the quarantine bacterium *X. ampelinus* was not

detected in any of the tested samples. The geographic distribution of the host plant samples taken in the territory of the Republic of Srpska during 2019 and 2020 are shown in Photo 1 and 2.

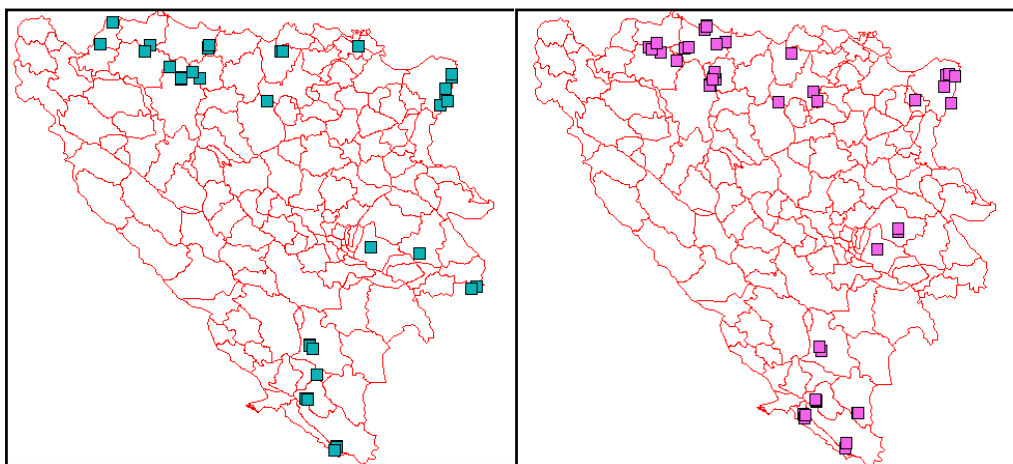


Photo 1. Fito GIS mapping – *X. ampelinus*, visual inspection and sampling in Republic of Srpska,2019
 Photo 2. Fito GIS mapping – *X. ampelinus*, visual inspection and sampling in Republic of Srpska,2020

In 2021, „Program survey on the presence of quarantine bacteria of grapevine *X. ampelinus* in RS” was published in Official Gazette of RS 41/21. Sampling of host plants was carried out in a production farms and agricultural households in seven regional units of RS (Prijedor, Banja Luka, Gradiška, Doboj Bijeljina, Istočno Sarajevo and Trebinje), as well on crossings border in RS. During 2021, total 40 samples were analyzed in Laboratory for plant protection, seed and biotechnology, PI AIRS, BL. 37 samples were sampled and analyzed from production farms and agricultural households, while 3 samples were analyzed from crossings border. According Official Gazette of RS in case of suspected samples, sampling of larger number from nurseries, seedling production places and crossings border was predicted for laboratory analysis. All laboratory tested samples were negative for the presence of bacteria *X. ampelinus*.

During 2022, „Program survey on the presence of quarantine bacteria of grapevine *X. ampelinus* in RS” was published in Official Gazette of RS 33/22. Sampling of host plants was carried out in a registered nurseries and seedling production places and farms in seven regional units of RS (Prijedor, Banja Luka, Gradiška, Doboj Bijeljina, Istočno Sarajevo and Trebinje), as well on crossings border in RS. During 2022, total 30 samples from production farms and agricultural households were analyzed in Laboratory for plant protection, seed and biotechnology, PI AIRS, BL, while samples from nurseries, seedling production places and crossings border were planed according Official Gazette of RS in case of suspected

samples. All tested samples were negative for the presence of the quarantine bacterium *X. ampelinus*. The geographic distribution of the host plant samples taken in the territory of the Republic of Srpska during 2021 and 2022 are shown in Photo 3 and 4.

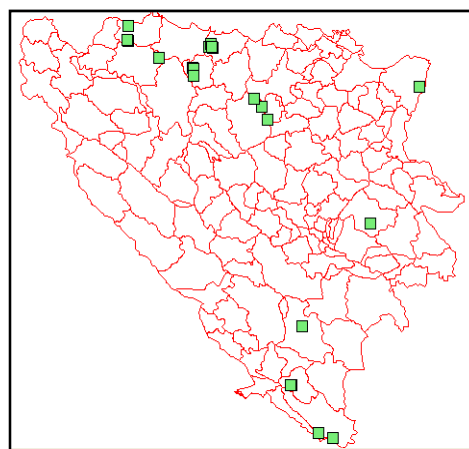
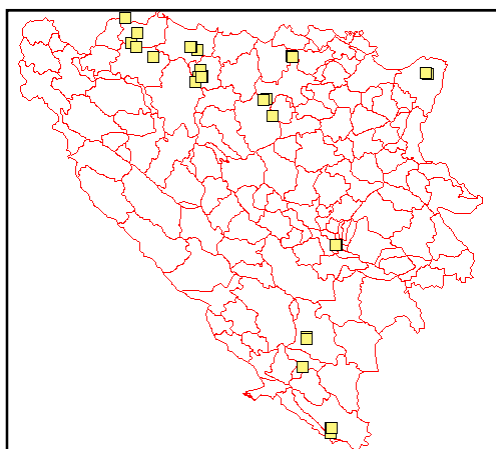


Photo 3. Fito GIS mapping – *X. ampelinus*, visual inspection and sampling in Republic of Srpska,2021

Photo 4. Fito GIS mapping – *X. ampelinus*, visual inspection and sampling in Republic of Srpska,2022

CONCLUSION

Based on the conducted laboratory analysis during 2019-2022, all tested samples were negative for the presence of quarantine bacteria of grapevine *X. ampelinus*, whose surveys was carried out under the Program approved and financed by Ministry of Agriculture, Forestry and Water Management of RS. Considering consequences if bacteria occurs, program survey is also continued in 2023, in order to ascertain and monitor the presence status of this quarantine pathogen in the RS, so in case of its occurrence, that emergency measures can be implemented and further spreading could be prevented.

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