STUDYING *XANTHOMONAS ARBORICOLA* PV. *CORYLINA* STRAINS FROM SERBIA FOR STREPTOMYCIN AND KASUGAMYCIN RESISTANCE AND COPPER COMPOUNDS SENSITIVITY *IN VITRO*

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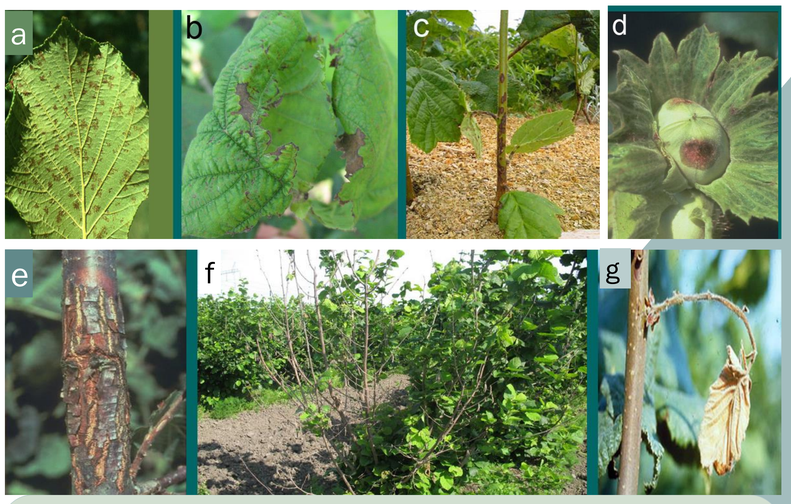
Introduction

Bacterial blight of hazelnut, caused by a Gram-negative bacterium *Xanthomonas arboricola* pv. *corylina*, is one of the widespread and economically most important bacterial diseases affecting commercial hazelnut cultivars, and resulting in significant losses worldwide (Figure 1).
Different strategies have been used in controlling this disease, including phytosanitary measures, cultural practices and chemical control. Antibiotics (streptomycin, oxytetracycline and kasugamycin) and copper derivatives (copper sulfate, copper oxychloride, copper hydroxide) have shown various efficacy in control of the disease. They are recommended as preventive treatments in order to eliminate or to inactivate the pathogen before it penetrates the host plant tissue.



Picture 1. Xanthomonas arboricola pv. *corylina* disease symptoms: leaf and fruit spotting (a, b, d), bud and twig necrosis and dieback (c, e, f, g) (Foto: a, d, f - www.atlasplantpathogenicbacteria; d, g, - L. Gardan, source: http://www.forestryimages.org; c - M. Redpath, Waitrata, source: Forest Farm, New Zealand; b, e - A. Obradović).

Aim of the research

Since use of antibiotics in plant protection is restricted in most EU countries, as well as in Serbia, the control of plant bacterial diseases is most commonly based on preventative application of copper bactericides. However, continuous use of copper-based treatments and antibiotics in plant protection may result in occurrence of resistant bacterial strains. Therefore, it is necessary to monitor population of the pathogen and determine potential changes in sensitivity to these bactericides. In this research we studied *in vitro* effect of different concentrations of these bactericides on 46 *Xanthomonas arboricola* pv. *corylina* strains.

Table1. Sensitivity of 46 *X. a.* pv. *corylina* strains to different concetration of bactericides *in vitro*. Legend: + normal growth; (+) reduced growth; - no growth.

Strain	Isolated	Origin	Streptomycin sulfate		Kasugamycin 2%		Copper sulfate		Copper oxychloride		Copper hydroxide		Control (SPA)
			25 ppm	50 ppm	50 ppm	100 ppm	100 ppm	200 ppm	100 ppm	200 ppm	100 ppm	200 ppm	
KFB 275	2008	Serbia	-	-	+	-	+	-	(+)	-	(+)	-	+
KFB 276	2008	Serbia	-	-	+	-	-	-	-	-	(+)	-	+
KFB 277	2008	Serbia	-	-	+	-	+	-	(+)	-	(+)	-	+
KFB 278	2008	Serbia	-	-	+	-	+	-	(+)	-	(+)	-	+
KFB 279	2008	Serbia	-	-	+	-	+	-	(+)	-	(+)	-	+
KFB 280	2008	Serbia	-	-	+	-	+	-	(+)	-	(+)	-	+
KFB 281	2009	Serbia	-	-	+	+	+	-	(+)	-	(+)	-	+
KFB 282	2008	Serbia	-	-	(+)	-	+	-	+	-	+	-	+
KFB 283	2008	Serbia	-	-	+	(+)	+	-	+	-	+	-	+
KFB 284	2008	Serbia	-	-	+	(+)	+	-	(+)	-	+	-	+
KFB 285	2008	Serbia	-	-	+	(+)	+	-	(+)	-	(+)	-	+
KFB 0128	2007	Poland	-	-	(+)	-	+	-	+	-	+	-	+
KFB 0130	2007	Poland	-	-	(+)	-	+	-	+	-	+	-	+
KFB 0132	2007	Poland	-	-	(+)	-	+	-	+	-	+	-	+
KFB 0134	2009	Poland	-	-	(+)	-	+	-	+	-	+	-	+
KFB 0135	2009	Poland	-	-	(+)	-	+	-	(+)	-	-	-	+
KFB 0136	2009	Poland	-	-	+	-	+	-	+	-	+	-	+
KFB 0137	2009	Poland	-	-	(+)	-	+	-	+	-	+	-	+
KFB 286	2010	Serbia	-	-	+	(+)	+	-	(+)	-	(+)	-	+
KFB 287	2010	Serbia	-	-	(+)	-	+	-	+	-	+	-	+
KFB 288	2010	Serbia	-	-	+	-	+	-	+	-	+	-	+
KFB 289	2010	Serbia	-	-	+	-	+	-	+	-	+	-	+
KFB 290	2010	Serbia	-	-	+	+	+	-	-	-	(+)	-	+
KFB 291	2010	Serbia	-	-	+	+	+	-	(+)	-	(+)	-	+
KFB 292	2010	Serbia	-	-	+	(+)	+	-	(+)	-	(+)	-	+
KFB 293	2010	Serbia	-	-	(+)	(+)	+	-	+	-	+	-	+
KFB 294	2010	Serbia	-	-	(+)	-	+	-	+	-	+	-	+
KFB 295	2010	Serbia	-	-	+	-	+	-	+	-	+	-	+
KFB 296	2010	Serbia	-	-	+	(+)	+	-	+	-	+	-	+
KFB 297	2010	Serbia	-	-	+	-	+	-	+	-	+	-	+
KFB 298	2010	Serbia	-	-	+	-	+	-	+	-	+	-	+
KFB 299	2010	Serbia	-	-	+	-	+	-	+	-	+	-	+
KFB 300	2010	Serbia	-	-	+	-	+	-	+	-	+	-	+
KFB 301	2010	Serbia	-	-	+	-	+	-	+	-	+	-	+
KFB 302	2010	Serbia	-	-	+	-	+	-	+	-	+	-	+
KFB 303	2010	Serbia	-	-	+	(+)	+	-	+	-	+	-	+
KFB 304	2010	Serbia	-	-	(+)	-	-	-	+	-	+	-	+
KFB 305	2010	Serbia	-	-	(+)	-	-	-	-	-	-	-	+
KFB 306	2010	Serbia	-	-	(+)	-	-	-	-	-	-	-	+
KFB 307	2010	Serbia	-	-	+	-	+	-	-	-	-	-	+
KFB 308	2010	Serbia	-	-	+	-	(+)	-	-	-	-	-	+
KFB 309	2010	Serbia	-	-	+	-	(+)	-	-	-	-	-	+
KFB 310	2010	Serbia	-	-	+	-	-	-	-	-	-	-	+
KFB 311	2010	Serbia	-	-	-	-	-	-	-	-	-	-	+
KFB 312	2010	Serbia	-	-	-	-	-	-	-	-	-	-	+
KFB 313	2011	Serbia	-	-	+	-	-	-	-	-	(+)	-	+
E-3	1960	Florida	+	+	+	(+)	+	+	+	+	+	+	+

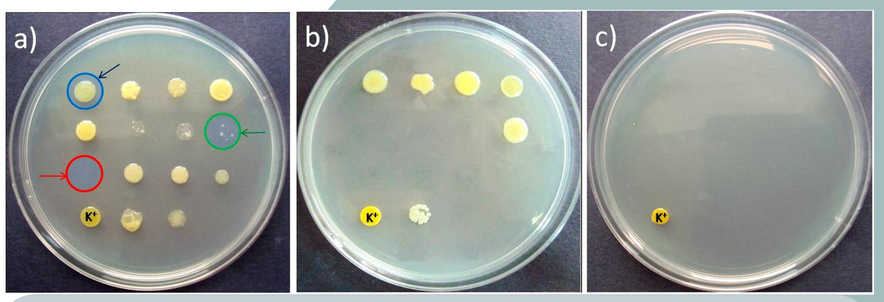


Figure 2. *X. a.* pv. *corylina.* Development of the strains on media amended with a) 100 ppm of copper sulfate; b) 100 ppm of copper oxychloride, c) 25 ppm of streptomycin sulfate. Different growth is indicated with an arrow: normal growth – blue, reduced growth - green and no growth – red arrow, K^+ control strain.

Conclusions

This study suggests the possibility of resistance development to copper ions in *X. a.* pv. *corylina* population from Serbia that may be due to intensive use of this compound in disease control. Bactericidal effect of streptomycin and kasugamycin indicated that treatments based on these substances can potentially improve control of the disease, especially in conditions of high inoculum pressure. However, antibiotics are not allowed in plant protection in Serbia. Therefore, a permit for limited and controlled application of antibiotics could be taken in consideration in Serbia, in order to prevent spread of copper-resistant pathogen population and potential epidemics.

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Materials and methods

The bacterial growth, on Nutrient agar (NA) for 48 h, was suspended in sterile distilled water and concentration was adjusted to approx. 1×10^8 CFU/ml.. Droplets of each strain suspension were spotted on the surface of sucrose peptone agar (SPA) plates amended with either $CuSO_3 \times 5H_2O$, $Cu_2(OH)_3Cl$, $Cu(OH)_2$ (100, 200 ppm), streptomycin sulfate (25, 50 ppm) or kasugamycin (50, 100 ppm) or on SPA without any of the bactericides. *Xanthomonas euvesicatoria*, strain E-3, resistant to these compounds was used as a positive control. Plates were incubated for 48 h at 28 °C and observed for bacterial growth.

Results

All *X. a.* pv. *corylina* strains from this study were sensitive to the lowest concentration of streptomycin sulfate (25 ppm). Kasugamycin inhibited growth of two strains at 50 ppm, while 44 were able to grow. Two times higher concentration of kasugamycin inhibited growth of 35 strains while 11 strains showed reduced growth. Control strain *X. euvesicatoria* E-3 was not affected by any of the bactericides used, and produced expected growth and appearance on the media (Table 1).
On the other hand, studied strains showed different susceptibility to copper compounds (Figure 2). None of the strains developed on SPA amended with 200 ppm of copper compounds. However 38, 37 and 35 strains showed tolerance to 100 ppm of Cu sulfate, hydroxide and oxychloride, respectively, indicating development of copper tolerance.