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DETECTION OF STOLBUR PHYTOPLASMA ON BLACKBERRY - A NEW NATURAL HOST IN SERBIA

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During the late summer of 2007, a severe phytoplasma-like disease was observed for the first time in blackberry plants (*Rubus fruticosus*), commercial cv. Čačanska beztrna. Redness and downward rolling of leaves were symptoms observed in three localities in Central Serbia. The presence of Stolbur phytoplasma, belonging to the taxonomic subgroup 16SrXII-A, in diseased samples was confirmed by the PCR – RFLP analysis of 16S rDNA genes and elongation factor Tu (*tuf*) gene. A sequence analysis of the *tuf* gene confirmed homology with phytoplasmas stolbur tuf-type II detected previously in Italian grapevines and red clovers in the Czech Republic. This

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is the first report of Stolbur phytoplasma 16SrXII-A group tuf-type II on blackberries in Serbia.

Key words: elongatoin factor Tu (*tuf*) gene, 16S rDNA genes, RFLP, *Rubus fruticosus*, Stolbur phytoplasma

INTRODUCTION

The plant pathogenic phytoplasmas are cell wall-less prokaryotic organisms grouped in the class Mollicutes. Phytoplasmas live as parasites in the phloem tissues of infected plant hosts and are transmitted by insect vectors. They are important plant pathogens causing more than 700 diseases in several hundred plant species. The phytoplasmas induce an array of symptoms that suggest profound disturbances in the normal balance of plant hormones or growth regulators. Usually, plants infected by the phytoplasmas exhibit symptoms like virescence, phyllody, yellowing, leaf roll, flowers sterility, axiliary buds proliferation, resulting in appearance of the "witches broom" as well as the shoot stunting and generalized decay of diseased plants (BERTACCINI and DUDUK 2009).

Identification of the phytoplasmas was based on symptomatology, host range and vector specificity, but these methods are not suitable to establish the genetic relatedness among different phytoplasmas (BONNET *et al.*, 1990; AHRENS and SEEMÜLLER, 1992). DNA-based molecular techniques are being used to identify and differentiate between the different phytoplasmas. The phytoplasmas are classified on the basis of the restriction fragment length polymorphism (RFLP) and sequence analysis (SEEMULLER *et al.*, 1998; LEE *et al.*, 1998, 2000) of the PCR-amplified ribosomal DNA. At present, more than 900 sequences have been deposited in the GenBank. Several years ago, it was proposed that the phytoplasmas should be placed within the novel genus "*Candidatus* (Ca.) Phytoplasma" (IRPCM, 2004).

Recently the phytoplasma - like symptoms were observed on the blackberry (*Rubus fruticosus*) in three localities in central part of Serbia starting from 2007.

Blackberry, a perennial shrub popular for its fruit, grows very quickly and is tolerant of poor soils, qualities that have made it an invasive species in some parts of the world (REEDER *et al.* 2009). Production of blackberries is concentrated in the central part of Serbia. The yearly production of fruits is 28.000 t, which represent 69% of all European production (NIKOLIC *et al.*, 2009).

Blackberries infected by the phytoplasma belonging to the 16SrIII (X disease) have been well documented in the United Kingdom (DAVIES, 2000). The following types of pytoplasma have been described in blackberries: Elm yellows phytoplasma group in North America (VAN DER MEER 1987), United Kingdom (DAVIES 2000), Italy (VINDIMIAN *et al.*, 2004), Turkey (SERTKAYA 2004), X disease (16SrIII) in the United Kingdom (DAVIES 2000), as well as "*Candidatus* Phytoplasma asteris" in the United Kingdom (REEDER *et al.*, 2009) and Pakistan (FAHMEED *et al.*, 2009).

Besides the phytoplasmas in the 16SrXII-A taxonomic subgroup ('*Candidatus* Phytoplasma solani'), associated with Stolbur disease in a wide range of wild and cultivated plants in Europe, no data for Stolbur occurrence on blackberries has been reported so far. Our preliminary results suggested that the Stolbur

phytoplasma are the causal agents of blackberry reddening. The objective of this study was to detect and determine the phytoplasma on blackberry using nested-PCR, RFLP of 16S rDNA and a sequence analysis of the *tuf* gene.

MATERIALS AND METHODS

Disease incidence (DI). Disease incidence was monitored in three localities in Serbia (Sićevo, Tuleš and Ljubava) in the period 2007-2009. The symptomatic plants were recorded and the percentage of infected plants (DI) was calculated. The increase of disease appearance was expressed as disease index, which represented a quotient of DI in the current and previous year. During the third year of investigation, the blackberry fruit yield loss or reduction was estimated, compared with fruit yield from healthy plants.

Samples collection. The samples of blackberry leaves from 15 year old plants (cv. Čačanska beztrna) were collected for PCR analysis in all three localities during 2007 and 2008: 15 symptomatic and 5 asymptomatic samples from Sićevo (Ks), 12 symptomatic and 3 asymptomatic samples from Tuleš (Kt) and Ljubava (Klj) for each year.

Amplification and RFLP analysis of 16S rDNA. The total DNA was isolated from 1g of each leaf collected from the infected and healthy tissues following the procedure earlier described by ANGELINI *et al.*, (2001). Two sets of primers were used to amplify phytoplasma DNA: the primer pair P1/P7 in direct-PCR (DENG and HIRUKI 1991; SCHNEIDER *et al.*, 1995) followed by the primer set R16F2n/R16R2 (GUNDERSEN and LEE 1996) in nested-PCR. The amplifications were carried out using the Dream Taq Green Master mix (Fermentas, Lithuania) in Eppendorf MastercyclerPerssonal thermocycler. The PCR parameters consisted of 35 cycles of denaturation at 94°C for 1,5 min (3 min for the first cycle), annealing at 58°C for 1 min, and extension at 72°C for 2 min (final extension for 7 min). Diluted products (1:10) of the direct-PCR were reamplified by the nested-PCR in the same condition as described for direct-PCR, using R16F2n/R2 primers. Reaction mixture without the DNA templates was used as a negative control. An aliquot of 8 µl of each PCR product was analyzed by electrophoresis in a 1.2% agarose gel, stained with ethidium bromide in TBE and visualized on an UV transilluminator.

Nested PCR products (1.2 kb) of the phytoplasma 16S rDNA sequence were subjected to RFLP analysis. Flavescence dorée (FD) and Stolbur phytoplasma (STOL) from Serbian grapevines and aster yellow (AY) phytoplasma were used as reference strains. Five µl aliquots of each PCR product were separately digested overnight with restriction endonucleases *Alu*I (at 37°C) and *Tru*I (at 65°C) (Fermentas, Lithuania). The digested products were analyzed by electrophoresis using 2.5% agarose gel, stained with ethidium bromide and DNA bands were photographed under UV light.

Amplification of tuf genes. All Stolbur phytoplasma isolates obtained were subjected to genotyping on the non-ribosomal gene *tuf*. The *tuf* gene was amplified in the nested PCR using the primers Tuf1f/r, followed by TufAyf/r (LANGER and MAIXNER 2004). The amplified products were analyzed by electrophoresis using 1.5% agarose gel, ethidium bromide stained and visualized under UV light. The product of

TufAyf/r amplification (~940 bp) of representative isolate Ks1 was purified using the PCR purification kit (Fermentas, Lithuania) and sequenced using service facilities of IMGGE, Belgrade.

RESULTS

Symptoms. The first symptoms appeared at the beginning of August with discoloration and mild chlorosis of the leaves, which later developed into a reddish shade. The leaf veins became intensly red on the underside. On the upper side the tissue between the veins was discoloured. During September, the leaves became completely red and slightly bent downward (Fig.1a).

The smaller leaves were formed on the infected shoots by the end of September. The shoots as well as leaves became dark red, while the healthy plants still had green shoots and leaves. Fruit production was reduced and fruit ripened unevenly or not at all, so they were not suitable for consumption (Fig. 1b).

The plants with a severe infection had sparse leaves and bore no fruit. Some shoots dried out during the autumn, and immature shoots died due to frost in winter. These plants were vigourless and leaf formation was delayed, therefore they started the next winter unprepared for the frost. Most of them dried and died during the winter.

Figure 1. *Rubus fruticosus* plants infected with Stolbur phytoplasma. Redness and downward rolling of leaves (a). Uneven maturity of blackberry fruits (b).

Disease incidence (DI). In 2007, when the first symptoms were observed, DI of infected plants was between 5% (Tuleš) and 50% (Sićevo). In subsequent year the DI rose from year to year, so in 2009 DI varied from 20% (Tuleš) to 90% (Sićevo). The disease index ranged from 1,3 times in Sićevo from 2008 to 2009 to 2,5 times in Ljubava, from 2007 to 2008 (Tab. 1). The estimates of yield reduction were between 20% to 50%. The blackberry plantation in Sićevo was not commercially viable any more and was erradicated in 2010.

Table 1. The Disease incidence (disease index) and the yield reduction of blackberries in three localities in Serbia

Locality	No. of Plants	Disease incidence * (Disease index)**			Yield reduction(%)
		2007	2008	2009	2009
Sićevo	440	50	70* (1,4)**	90 (1,3)	50
Tuleš	220	5	10 (2,0)	20 (2,0)	20
Ljubava	340	10	25 (2,5)	40 (1,6)	30

*Disease incidence = the percentage of diseased plants

**Disease index = quotient of DI in current and previous year

*Amplification and RFLP analysis of 16S rDNA***.** A primer pair P1/P7 was employed in the direct-PCR, to prime a DNA fragment of 1.8 kb expected size, and positive reaction showed 16 samples from blackberry tissue with typical phytoplasma symptoms (Tab. 2). No amplicons were obtained from the 18 out of 22 symptom-less plants. All samples from Sićevo (Ks) with the symptoms and some of asymptomatic samples yielded a nested PCR product of 1.2 kb in size by using the R16F2n/R2 universal primers. Nested PCR amplicons of representative samples from each of the three localities are shown on Figure 2.

RFLP profiles obtained by using *Alu*I and *Tru*I restriction enzymes on 1.2 kb products showed that the phytoplasmas belonging to the Stolbur group (16SrXII-A) were present in symptomatic blackberry samples.

Table 2. Phytoplasma detection using direct and nested PCR

Locality	Samples' symbols	No. of samples (s+as)*		Products in direct (A) and nested (B) PCR			
				2007		2008	
		2007	2008	A	B	A	B
Sićevo	Ks	15+5	15+5	5+0	15+1	6+0	15+2
Tuleš	Kt	12+3	12+3	0+0	4+0	1+0	7+0
Ljubava	Klj	12+3	12+3	0+0	5+0	4+0	10+1
Total		39+11	39+11	5+0	24+1	11+0	32+3

* samples from symptomatic (s) + asymptomatic (as) plants

Amplification of the tuf genes. Fragments of the *tuf* gene encoding the phytoplasma elongation factor Tu (EF-Tu), were amplified using the primers Tuf1f/r, followed by TufAyf/r. Amplicons of ~ 940 bp in size were obtained from all symptomatic blackberry samples. After nucleotide sequence determination of the TufAyf/r amplification product (919 bp) of representative Ks1 isolate, and its alignment with sequences of other phytoplasmas elongation factor Tu (*tuf*) genes (which are available in NCBI GenBank), phytoplasma of Stolbur group tuf-type II was confirmed. GenBank accession number for 666 bp coding region of elongation factor Tu (*tuf*) genes for isolate Ks1 is JN808109.

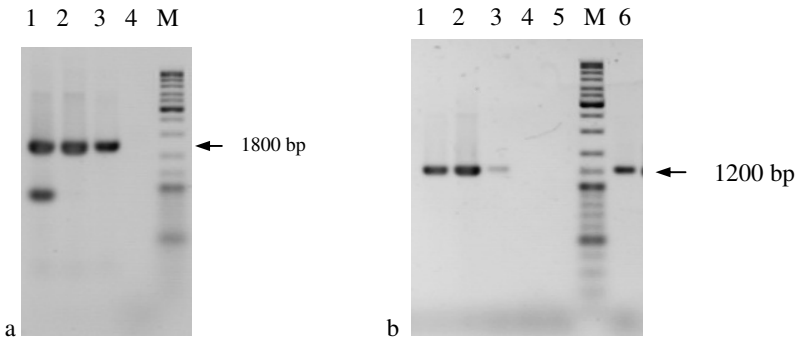

Figure 2. PCR amplification of 16S ribosomal DNA using (a) P1/P7 primers: lane 1-3. symptomatic blackberry samples Ks1, Kt12, Klj7, lane 4. asymptomatic blackberry sample Ks16, lane 5. Marker; (b) R16F2n/R2 primers: line 1-3: symptomatic blackberry samples Ks1, Kt12, Klj7; lane 4. asymptomatic blackberry sample Ks16; line 5. negative control; line 6. Marker: GeneRuler DNA Ladder mix SM0331 (Fermentas, Lithuania); line 7. control 16SrXII group (STOL)

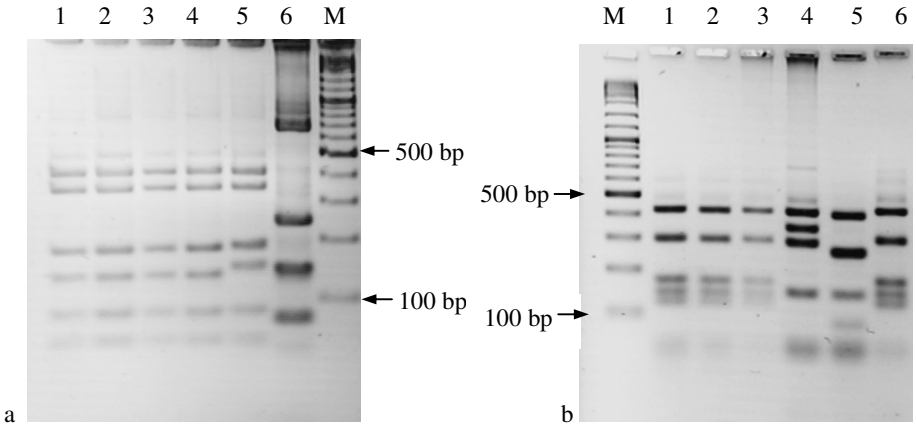

Figure 3. RFLP analysis of the 16S rDNA 1.2-kb PCR product (R16F2n/ R2) digested by a) *Alu*I: lane 1-3. symptomatic blackberry samples Ks1, Kt12, Klj7; lane 4. control 16SrXII group (STOL); lane 5. control AY phytoplasma; lane 6. control FD-C; lane 7. Marker; b) *Tru*I: lane 1. Marker; lane 2-4. symptomatic blackberry samples Ks1, Kt12, Klj7; lane 5. control AY phytoplasma; lane 6. control FD-C; lane 7. control 16SrXII group (STOL); Marker: GeneRuler DNA Ladder mix SM0331 (Fermentas, Lithuania)

DISCUSSION

The digestion of 1.2 kb 16S rDNA amplified products using *Alu*I and *Tru*I restriction enzymes showed that all phytoplasma positive isolates from symptomatic blackberries belong to the Stolbur - 16SrXII-A phylogenetic group. This phytoplasma type was detected in earlier investigations on grapevines in different vineyards (KUZMANOVIC *et al.*, 2003, 2008; JOSIC *et al.*, 2010a). Some of these vineyards are located near the investigated blackberry fields. In Serbia, Stolbur phytoplasma was detected on different cultivated and weed plants: corn (DUDUK and BERTACCINI 2006), plantain (JOSIC *et al.*, 2010b) and purple coneflower (PAVLOVIC *et al.*, 2011). RADONJIC *et al.*, (2009) confirmed the presence of two types of Stolbur phytoplasma in grapevine samples from Montenegro.

Partial sequence of elongation factor Tu (*tuf*) gene, obtained in this study and deposited in GeneBank as JN808109 showed high similarity (99.85%) with FJ394552.1 *Candidatus* Phytoplasma solani isolate R47/5 (BERGER *et al.*, 2009), EU552455.1 *Candidatus* Phytoplasma solani isolate 1-38-40 (FRANOVA *et al.,* 2009) and GU220565.1 - GU220562.1 Phytoplasma sp. (strains BN-Si238, BN-Ma202, BN-Op40 and BN-Op437, respectively) (QUAGLINO *et al.*, 2010).

The sequence FJ394552.1 represents Stolbur tuf-type II phytoplasma infected grapevines and was deposited by BERGER *et al.*, (2009). Sequence EU552455.1 (FRANOVA *et al.*, 2009) represents the phytoplasma stolbur tuf-type II detected in red clover *(*Trifolium pretense*)* plants exhibiting dwarf symptoms.

The presence of Stolbur phytoplasma 16SrXII-A group tuf-type II in blackberry (*Rubus fruticosus*) plants is confimed in Serbia for the first time. The fact that the blackberries with Stolbur type phytoplasma were found so close to the vineyards in which the cultivated grapevines expressed the same symptoms, and with the same type of phytoplasma identified, suggested that the pathogen was probably transmitted by vectors from the grapevines to the blackberry crop.

The results of field surveys revealed that the Stolbur phytoplasma became a very serious disease affecting blackberries. In the light of the economical importance of blackberry crop, and the significant yield reduction resulting from the phytoplasma infection, further investigations concerning epidemiological aspects of the disease and identification of vectors need to be undertaken.

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REFERENCES

AHRENS, U., W. SEEMÜLLER (1992): Detection of DNA of plant pathogenic mycoplasma-like organisms by a polymerase chain reaction that amplifies a sequence of the 16S rRNA gene. Phytopathology, *82*: 828-832.

ANGELINI, E, D CLAIR, M BORGO, A BERTACCINI, E.BOUDON-PADIEU (2001): Flavescence dorée in France and Italy - Occurence of closely related phytoplasma isolates and their near relationships to Palatinate grapevine yellows and an alder yellows phytoplasma. *Vitis*, *40*: 79-86.

BERGER, J., J DALLA VIA, S.BARIC (2009): Development of a TaqMan allelic discrimination assay for the distinction of two major subtypes of the grapevine yellows phytoplasma Bois noir. Eur J Plant Pathol, *124* (3): 521-526

BERTACCINI, A, B. DUDUK (2009): Phytoplasma and phytoplasma diseases: a review of recent research. Phytopathol Mediterr, *48*: 355-378.

BONNET, F., C. SAILLARD, A. KOLLAR, SEMÜLLER, JM. BOVÉ (1990): Detection and differentiation of the mycoplasma-like o E.rganism associated with apple proliferation disease using cloned DNA probes. Mol Plant Microbe In, *3*: 438-443.

DAVIES DL. (2000): The occurrence of two phytoplasmas associated with stunted *Rubus* species in the UK. Plant Pathol 49: 86-88.

DENG, S., C. HIRUKI (1991): Amplification of 16S rRNA genes from culturable and nonculturable Mollicutes.- J Meth Mic, *14*: 53-61.

DUDUK, B., A.BERTACCINI (2006): Corn with symptoms of reddening: new host of stolbur phytoplasma. Plant Dis, *90*: 1313-1319.

QUAGLINO, F, Y. ZHAO, PA BIANCO, W. WEI, F.GAFFURI, N. MORI, P.CASATI , RE.DAVIS (2010): Biological complexity among populations of 'Candidatus Phytoplasma solani'-related strains in Italy is plausibly associated with molecular markers in genes tuf and hlyC. In: Bertaccini A, Laviña TA,Torres E. (eds) Abstract book of COST Action Combined meeting of Work Groups 1-4: Current status and perspectives of phytoplasma disease research and management, 1-2.February 2010. Sitges, Spain, pp 34.

FAHMEED, F., AY ROSETE, KA PÉREZ, BOA E, J. LUCAS (2009): First Report of `*Candidatus* Phytoplasma asteris' (Group 16SrI) Infecting Fruits and Vegetables in Islamabad, Pakistan. J Plant Pathol , *157*(10): 639-641(3).

FRÁNOVÁ, J, M. NAVRÁTIL, H.JAKEŠOVÁ (2009): Molecular Identification of Stolbur Phytoplasma Associated with Red Clover Dwarf Disease Symptoms. J Phytopath,157 (7-8): 502–506.

GUNDERSEN, DE, IM.LEE (1996): Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. Phytopathol Mediterr, *35*: 144-151.

IRPCM (2004) "Candidatus Phytoplasma", a taxon for the wall-less, non-helical prokaryotes that colonize plant phloem and insects. Int J Syst Evol Microbiol *54*: 1243-1255.

JOŠIĆ,, D., S. KUZMANOVIĆ, S. STOJANOVIĆ, G. ALEKSIĆ, S. PAVLOVIĆ, M. STAROVIĆ (2010a): Detection of XIIA Phytoplasma group on Cultivar Zupljanka in Zupa vineyard region by RFLP analysis of 16S rDNA sequences. Genetika, Belgrade, *42*(1): 146-153.

JOŠIĆ, D., S. PAVLOVIĆ, S. STOJANOVIĆ, S. KUZMANOVIĆ, V .GAVRILOVIĆ, M.STAROVIĆ (2010b): *Phytoplasma disease* on *Plantago major* in Serbia. In: Mueen A. (ed) Abstracts Book of 6th CMPSEEC, 18-20 April 2010. Antalya, Turkey. *Pharmacognosy Magazine,*6 (22): S170-S171.

KUZMANOVIĆ, S., M. STAROVIĆ, M. TOŠIĆ, S .STOJANOVIĆ, T.TOMIĆ (2003): Phytoplasmas on grapevine in Serbia. In Martelli GP, Bovey R, Gugerli P. (eds) Extended abstract of 14th Meeting of ICVG, 12-17 September 2003. Locorotondo, Italy. pp. 93-94.

KUZMANOVIĆ, S., M .MARTINI, F. ERMACORA, F. FERRINI, M. STAROVIĆ ,L. CARRARO, R. OSLER, M.TOŠIĆ (2008): Incidence and molecular characterization of *Flavescence doree* and *stolbu*r phytoplasma in grapevine cultivars from different viticultural areas of Serbia. Vitis, *47*(2): 105-111.

LANGER, M, M. MAIXNER (2004): Molecular characterisation of grapevine yellows associated phytoplasmas of the stolburgroup based on RFLP-analysis of non-ribosomal DNA. Vitis *43*: 191-199.

LEE, IM, DE GUNDERSEN, RE DAVIS, IM BARTOSZYK (1998): Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. Int J Syst Bacteriol*, 48*: 1153-1169.

LEE, IM, RE DAVIS, DE.GUNDERSEN-RINDAL (2000): Phytoplasma: Phytopathogenic mollicutes.- Annu Rev Microbiol**,** *54:* 221-255.

NIKOLIĆ, M., J. MILIVOJEVIĆ, A.LEPOSAVIĆ (2009): Berry production in Serbia – present and future. Programme and abstracts, COST863 Workshop „The new biotechnology applied in berry fruits", pp.16.

PAVLOVIC, S, M.STAROVIĆ, S. STOJANOVIC, T. POPOVIC, G.ALEKSIC, S .DRAZIC, D.JOSIC (2011): *Echinacea purpurea*- a host of 16Sr XIIA phytoplasma group in Serbia. Phytopatogenic Mollicutes, *1* (1): 35-39

RADONJIĆ, S., S. HRNČIĆ, J .JOVIĆ, T .CVRKOVIĆ, O. KRSTIĆ, S. KRNJAJIĆ, I.TOŠEVSKI (2009): Occurrence and Distribution of Grapevine Yellows Caused by Stolbur Phytoplasma in Montenegro. J Phytopath, *157:*682–685.

REEDER, R. PL KELLY, Y.AROCHA (2009): "Candidatus Phytoplasma asteris" identified in blackberry (Rubus fructicosus agg.) in the United Kingdom. New Dis Rep, *19*: 66.

SCHNEIDER, B., E. SEEMÜLLER, CD SMART, BC.KIRKPATRICK (1995): Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas. In: Razin S,Tully JG. (eds) Molecular and Diagnostic Procedures in Mycoplasmology, Academic Press, San Diego, CA, California, *1*, pp. 369-380.

SEEMÜLLER, E., C. MARCONE, U. LAUER , A. RAGOZZINO, M.GÖSCHL (1998): Current status of molecular classification of the phytoplasmas. J Plant Pathol*, 80*: 3-26.

SERTKAYA, G., R. OSLER, R .MUSETTI, P. ERMACORA, M MARTINI (2004): Detection of phytoplasmas in *Rubus* spp. by microscopy and molecular techniques in Turkey. In Martin RR (ed) X International Symposium on Small Fruit Virus Diseases, 25 September 2004. Valencia, Spain. Acta Horticulturae, pp.656.

VAN DER MEER, FA. (1987): Rubus stunt. In: Converse R. (ed) Virus Diseases of Small Fruits. Washington, D.C.: US Department of Agriculture, Handbook No. *631*: 197–203.

VINDIMIAN, ME, A. GRASSI, A. CICCOTTI, CP POLLINI, F.TERLIZZI (2004): Epidemiological studies on Rubus Stunt (RS) in Blackberry orchards located near Trento (Italy). In Martin RR.(ed) X International Symposium on Small Fruit Virus Diseases, 25 September 2004. Valencia, Spain. Acta Horticulturae, pp 654.

DETEKCIJA STOLBUR FITOPLAZME NA KUPINI KAO NOVOM DOMAĆINU U SRBIJI

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I z v o d

Simptomi nalik fitoplazmi prvi put su uočeni tokom leta 2007 g. na kupini (*Rubus fruticosus*) sorte Čačanska beztrna. Crvenilo i uvrtanje listova kao najčešći simptomi primećeni su na 3 lokaliteta u centralnoj Srbiji. Prisustvo Stolbur fitoplazme, koja pripada 16SrXII-A taksonomskoj podgrupi, utvrđeno je PCR – RFLP analizom 16S rDNK gena i gena za elongacioni faktor Tu (*tuf*) u uzorcima sa izraženim simptomima. Potvrđena je homologija dobijene sekvence *tuf* gena sa fitoplazmama Stolbur tuf- II tipa detektovanim na vinovoj lozi u Italiji i crvenoj detelini u Češkoj Republici. Ovo je prvi nalaz Stolbur fitoplazme 16SrXII-A grupe tuf- II tipa na kupini u Srbiji.

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