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DISTRIBUTION OF ALDER YELLOWS PHYTOPLASMA ON COMMON AND GRAY ALDER (*ALNUS GLUTINOSA* AND *ALNUS INCANA*) IN SERBIA

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Alder yellows (AldY) phytoplasma associated with common alder (*Alnus glutinosa*) and grey alder (*A. incana*) belongs to the ribosomal RNA group16SrV. This phytoplasma is closely related to the *Flavescence dorée* (FD) phytoplasma, a quarantine pathogen of economic importance that affects vineyards of southern Europe including Serbia. To date, alder yellows phytoplasma has been reported in many European countries including France, Germany, Switzerland, Austria, Italy and the Baltic region. Infected alders are exhibiting symptoms such as leaf yellowing, small leaves, reduced foliage, or sometimes they remain symptomless. To investigate occurrence and distribution of this phytoplasma, a survey was conducted on a wide territory of Serbia. Results confirmed wide distribution of alder yellows phytoplasma in Serbia in both symptomatic and asymptomatic trees. From the 72 plants sampled, 54 were positive for the presence of phytoplasmas. RFLP profiles of the 16S rRNA gene indicated presence of 16SrV-C phytoplasma subgroup. Further characterization by PCR-RFLP analysis of the ribosomal protein gene operon of all phytoplasma positive isolates tested confirmed presence of the 16SrV-C phytoplasma subgroup. Implication of the wide distribution of AldY phytoplasma to the epidemiology of FD phytoplasma as well as disease management are discussed.

Key words: 16S rRNA, disease epidemiology, *Flavescence dorée*, PCR-RFLP, *rpl22-rps3*, symptoms.

INTRODUCTION

Phytoplasmas are wall-less, phloem-limited, non-culturable prokaryotes of the class Mollicutes that are associated with diseases in several hundred plants species. Because they cannot be cultured on an artificial medium and lack measurable phenotypic characters, classification of phytoplasmas has been based primarily on molecular analyses of highly conserved 16S rRNA gene sequences (Lee et al., 1998).

Alder yellows is a disease caused by phytoplasma genetically closely related to the *Flavescence dorée* phytoplasma, that affects vineyards in many south European countries. To date, severe outbreaks caused by *Flavescence dorée* (FD) have been reported from France, Italy, Spain (Daire et al., 1997; Martini et al., 1999; Angelini et al., 2001; Arnaud et al., 2007), including Serbia (Krnjajić et al., 2007). Recently this phytoplasma has been reported from Portugal, Switzerland, Austria and Croatia (De Sousa et al., 2009; Schaerer et al., 2007; Reisenzein and Steffek, 2011; Škorić et al., 2011) showing trend of continuous spreading throughout vineyard regions across southern Europe. In general, the outbreaks of the *Flavescence dorée* in Europe are caused by two genetic entities, where both belong, in a wider sense, to elm yellows phytoplasma or 16SrV group (Lederer and Seemüller 1991; Maurer et al., 1993). In east Europe, including northern parts of Italy, the vineyards are affected with phytoplasma belonging to the 16SrV-C subgroup (FD-C), while in France and western part of Italy with phytoplasma belonging to the 16SrV-D subgroup (FD-D). Both phytoplasmas are quarantine diseases of the grapevine, epidemically transmitted by the leafhopper *Scaphoideus titanus* Ball (Angelini et al., 2003; Arnaud et al., 2007). However, occasionally grapevine could be inoculated with alder yellows phytoplasma by leafhopper *Oncopsis alni* (Schrank), which causes Palatinate grapevine yellows (PGY) disease, known from Palatinate region in South West Germany (Maixner and Reinert, 1999; Maixner et al., 2000). In addition, finer molecular and phylogenetic studies strongly support hypothesis that phytoplasmas from ribosomal 16SrV-D subgroup affecting vineyards in west Europe are originating from alder trees (Malembic-Maher et al., 2007; Arnaud et al., 2007).

The importance of alder yellows phytoplasma in epidemiology of grapevine yellows disease becomes obvious after reported great diversity within field infected alders. Cvrković et al. (2008) reported the presence of alder yellows phytoplasma from two sites in Serbia. Even though 16SrV-C phytoplasmas affecting vineyards in Serbia were found in *Clematis vitalba* (Filippin et al., 2009), different genotypes associated with *Alnus glutinosa* and *Alnus incana* may represent potential threat in epidemiology of grapevine yellows diseases caused by phytoplasmas from 16SrV group. Thus, in this paper, we performed a study to determine incidence of alder yellows phytoplasma associated with alder trees in Serbia.

MATERIALS AND METHODS

The material for this study was collected between 2006 and 2010. Leaves with petioles from *Alnus glutionsa* and *Alnus incana* trees were collected on the forests margins and riverbanks in several districts of Serbia. A total of 12 sites were surveyed (Picture 1). Leaves with petioles from six randomly selected trees per site, expressing symptoms of yellowing or being symptomless were sampled for phytoplasmas detection by PCR-RFLP analysis. Fresh midribs and petioles were dissected, distributed in 1 gram aliquots and stored at -20ºC prior to analyses. DNA was extracted according to previously reported protocols (Angelini et al., 2001).

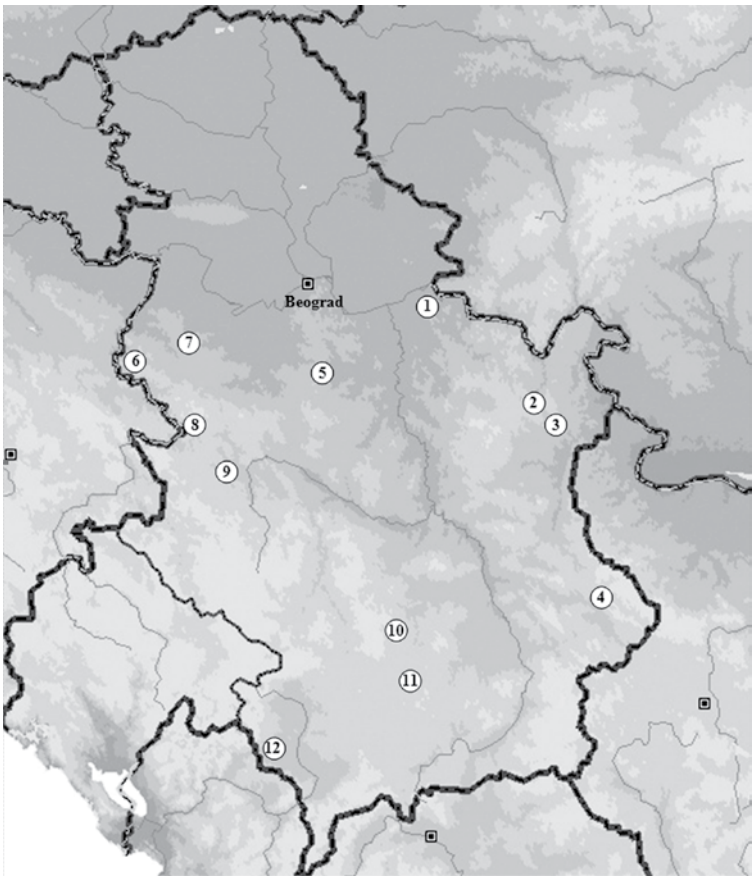
Initial phytoplasma identification was conducted using a nested PCR assay on the 16S ribosomal gene with P1/P7 (Deng and Hiruki, 1991; Smart et al., 1996) and 16r758f/M23Sr (Gibb et al., 1995; Padovan et al., 1995) primers according to Angelini et al. (2001). PCR products were separated on 1% agarose gels, stained with ethidium bromide and visualized under UV transilluminator. Samples producing an amplicon in nested PCR were subjected to restriction digestion with *Taq*I endonuclease (Fermentas) according to the manufacturer's instructions. The restriction digestion products were subsequently separated electrophoretically on 13% polyacrylamide gels in TBE buffer (Tris-Borate 90mM, EDTA 1mM), stained with ethidium bromide and visualized under UV light.

Diversity of phytoplasmas associated with alders in Serbia was estimated by characterization of ribosomal protein gene operon comprising *rpl22* and *rps3* genes, using the primer pair rp(V)F1/rpR1 in direct PCR followed by nested PCR with rp(V)F1A/rp(V)R1A primers (Lee et al., 2004). The reaction mixture (3mM $MgCl_2$, 0.3mM each dNTPs, 0.6µM each primer, 0.75 U of Ampli*Taq* Gold polymerase (Applied Byosistem)) was subjected to 34 cycles with the following steps: 10 min at 95ºC for enzyme activation, 1 min (90 s for the first cycle) at 94ºC for denaturation, 2 min at 50ºC for annealing and 3 min (10 min for the last cycle) at 72ºC extension. All parameters were identical in direct and nested PCR. PCR products were separated in 1% agarose gel, stained with ethidium bromide and visualised under a UV transilluminator, then subjected to RFLP analyzes with *Mse*I restriction enzyme (Fermentas). The restriction products were separated in 13% polyacrylamide gel, stained and visualized as described above.

RESULTS

Survey and collection. Common alder (*Alnus glutinosa*) is a wide distributed and common tree in Serbia, often found at river and stream banks and mesic foothills sites. To estimate incidence of alder yellows phytoplasma in Serbia we have sampled alder trees from twelve sites: Šuvajić (near Veliko Gradište) (1),

Debeli Lug (near Majdanpek) (2), Jabukovac (near Negotin) (3), waterfall Bigar (Monastery St. Onufrije near Temska) (4), vicinity of Topola (5), Mt. Radalj (near Loznica) (6), Zavlaka (near Osečina) (7), Okletac (near Bajina Basta) (8), Potpeći (near Užice) (9), Aleksandrovac (10), Kraljevo (11) and Prijepolje (12) (Picture 1).



Picture 1 – Map of Serbia showing localities surveyed for alders with Alder yellows phytoplasmas between 2006-2010: (1) Šuvajić (near Veliko Gradište); (2) Debeli Lug (near Majdanpek); (3) Jabukovac (near Negotin); (4) Waterfall Bigar (Monastery St. Onufrije near village Temska); (5) vicinity of Topola; (6) Mt. Radalj (near Loznica); (7) Zavlaka (near Osečina); (8) Okletac (near Bajina Bašta); (9) Potpeći (near Užice); (10) Aleksandrovac; (11) Kraljevo; (12) Prijepolje.

Slika 1 – Mapa Srbije sa lokalitetima sakupljanja jova i Alder yellows fitoplazme između 2006. i 2010. godine: (1) Šuvajić (u blizini Velikog Gradišta); (2) Debeli Lug (u blizini Majdanpeka); (3) Jabukovac (u blizini Negotina); (4) Vodopad Bigar (Manastir Sveti Onufrije u blizini sela Temska); (5) okolina Topole; (6) Planina Radalj (u blizini Loznice); (7) Zavlaka (u blizini Osečine); (8) Okletac (u blizini Bajine Bašte); (9) Potpeći (u blizini Užica); (10) Aleksandrovac; (11) Kraljevo; (12) Prijepolje.

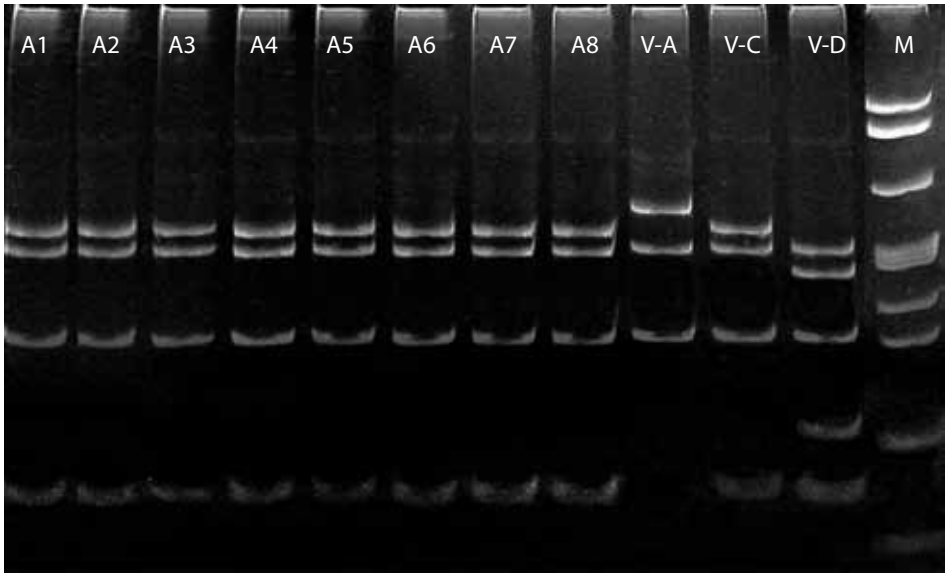
Table 1 – Occurrence of AldY phytoplasmas in symptomatic and asymptomatic alders collected on 12 localities in Serbia between 2006 and 2010.

Tabela 1 – Prisustvo AldY fitoplazme u simptomatskim i asimptomatskim jovama sakupljenim na 12 lokaliteta u Srbiji između 2006. i 2010. godine.

Locality Lokalitet	Alder species Alder vrsta	Symptoms observed Registrovani simptomi	PCR positive/ analyzed samples[a] PCR pozitivni/ analizirani uzorci[a]
Šuvajić	Alnus glutinosa	leaf yellowing	5/6
Debeli Lug	*Alnus glutinosa*	leaf yellowing, asymptomatic	6/6
Jabukovac	*Alnus incana*	asymptomatic	4/6
Monastery Sveti Onufrije	*Alnus glutinosa*	leaf yellowing, asymptomatic	5/6
Topola	*Alnus glutinosa*	leaf yellowing, asymptomatic	6/6
Mt. Radalj	Alnus glutinosa	leaf yellowing,	4/6
Zavlaka	*Alnus glutinosa*	leaf yellowing, asymptomatic	4/6
Okletac	*Alnus glutinosa*	leaf yellowing, asymptomatic	5/6
Potpeći	*Alnus glutinosa*	asymptomatic	4/6
Aleksandrovac	*Alnus glutinosa*	asymptomatic,	4/6
Kraljevo	*Alnus glutinosa*	asymptomatic	3/6
Prijepolje	*Alnus glutinosa*	leaf yellowing, asymptomatic	4/6
In total Ukupno			54/72

(a) identification of AldY phytoplasmas was performed using nested PCR with primer pairs P1/P7 and 16r758f/M23Sr followed by restriction analysis with *Taq*I endonuclease.
(a) identifikacija AldY fitoplazme je izvršena pomoću nested PCR analize sa P1/P7 i 16r758f/M23Sr i restrikcionom analizom sa *Taq*I endonukleazom.

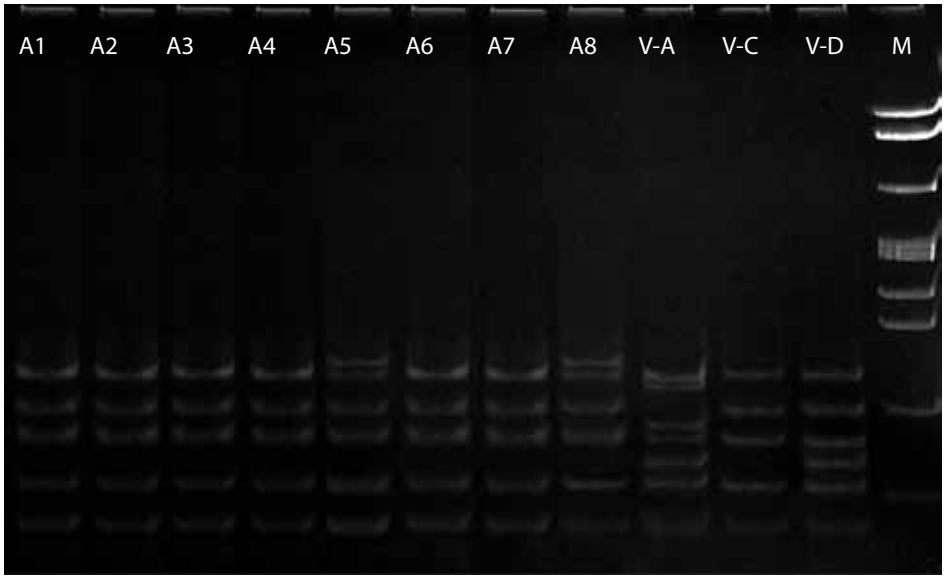
Molecular analyses. A total of 72 alder samples were subjected to nested PCR analyses with P1/P7 and 16r758f/M23Sr primers, of which 54 tested positive for the presence of alder yellows phytoplasma (Table 1). RFLP profiles of 16S rRNA gene showed the presence of phytoplasmas of the 16SrV-C subgroup (Picture 2) in all positive samples. Further characterization of the ribosomal protein gene operon showed two different *Mse*I RFLP profiles, one similar to FD-C



Picture 2 – RFLP analyses of the 1050 bp 16SrRNA gene amplified by nested PCR with primer pair P1/P7 followed with 16r758f/M23Sr primers, digested with *Taq*I and separated by electrophoresis through 13% polyacrylamide gels. A1-4: alder samples from Central Serbia; A5-8: alder samples from East Serbia; V-A: elm yellows (EY) phytoplasma of the 16SrV-A ribosomal subgroup maintained in periwinkle (provided by W.A. Sinclair, New York); V-C: *Flavescence dorée* phytoplasma of the 16SrV-C subgroup (FD-C) isolated from naturally infected grapevine from Central Serbia; V-D: *Flavescence dorée* phytoplasma of the 16SrV-D subgroup (FD-D) isolated from naturally infected grapevine from Veneto region, Italy (provided by E. Angelini, Conegliano); M: marker, ΦX174 DNA/HaeIII digested, Fermentas.

Slika 2 – RFLP analiza 1050 bp nested PCR produkata 16S rRNK gena umnoženih pomoću P1/P7 i 16r758f/M23Sr para prajmera digestovanih sa *Taq*I endonukleazom i elektroforetski razdvojenih kroz 13% poliakrilamidni gel. A1-4: uzorci jova iz centralne Srbije; A5-8: uzorci jova iz istočne Srbije; V-A: elm yellows (EY) fitoplazma ribozomalne podgrupe 16SrV-A održavana u perivinki (W.A. Sinclair, New York); V-C: *Flavescence dorée* (FD-C) fitoplazma ribozomalne podgrupe 16SrV-C izolovana iz prirodno inficirane vinove loze iz centralne Srbije; V-D: *Flavescence dorée* (FD-D) fitoplazma ribozomalne podgrupe 16SrV-D izolovana iz prirodno inficirane vinove loze iz Veneto regiona u Italiji (E. Angelini, Conegliano); M: marker, ΦX174 DNA/HaeIII digested, Fermentas.

and one (only in samples from eastern Serbia) similar to the AldY strain previously described by Lee *et al.*, 2004 (Picture 3). RFLP profiles showed the presence of phytoplasmas of the 16SrV-C subgroup in all samples collected from alder trees with characteristic symptoms of yellowing, but also in certain number of trees which were symptomless.



Picture 3 – RFLP analyses of the 1200 bp ribosomal protein operon sequence comprising *rpl22-rps3* genesamplified by nested PCR with primer pair rp(V)F1/rpR1 followed by rp(V)F1A/rp(V)R1A primers, digested with *Mse*I, and separated by electrophoresis through 13% polyacrylamide gels. Abbreviations of the isolates and reference strains are the same as described on Picture 2.; M: marker, ΦX174 DNA/HaeIII digested, Fermentas.

Slika 3 – RFLP analiza 1200 bp nested PCR produkata operona ribozomalnih proteina *rpl22-rps3* umnoženih pomoću rp(V)F1/rpR1 i rp(V)F1A/rp(V)R1A para prajmera digestovanih sa *Mse*I endonukleazom i elektroforetski razdvojenih kroz 13% poliakrilamidni gel. Oznake izolata i referentnih sojeva su iste kao na Slici 2.; M: marker, ΦX174 DNA/HaeIII digested, Fermentas.

According to recently published data, there is some extent of confirmation about phytoplasma exchange between wild alders and grapevine in west Europe, particularly in France and West Germany, as it is shown by Maixner et al. (2000) and Arnaud et al. (2007). These findings objectively launched hypothesis that first FD outbrakes observed in France in the mid 50's, after accidental introduction of the leafhopper *S. titanus,* could originate from alder trees driven by intermediary transmission of alder yellows phytoplasma by the leafhopper *Oncopsis alni* to grapevine (Arnaud et al*.,* 2007). It is worth noting that alder trees and vineyards are often in close contacts in some regions of France or in Palatinate region in West Germany.

DISCUSSION

Phytoplasma exchange from the wild flora and agricultural crops is not a rare phenomenon, and is usually correlated with changing in behavior or ecological demands of particular hemipteran vectors. Host shift of *Reptalus panzeri* from shrubs to maize, for example, lead to severe outbreaks of Stolbur phytoplasma on maize (Jović et al., 2009). Similar situation has been reported for *Hyalesthes obsoletus* involvement in rapid and severe outbreaks of stolbur phytoplasma on lavender (*Lavandula angustifolia*) (Sforza et al., 1999; Gaudin et al., 2011) in France or on potato crop in Serbia (Jović et al., 2011). In last decade, increasing number of reports about accidental or sometimes permanent, but low rate presence of phytoplasmas in different crop systems became relatively common event. This is obviously in correlation with substantial changes in assemblages of Hemipteran vectors inside or around particular agro-ecosystems. One of the possible reasons should be an intensive fertilization practices used in agriculture in past 50 years which lead to an increase of concentrations of total nitrogen, amino acid and organic compounds in both the crops and weeds (Jović et al., 2009), resulting in a nutritional balance that facilitates leafhopper fecundity and better survival of the offspring (Brodbeck et al., 1999; Olmstead et al., 1997).

Following the experience of the researchers involved in the study of epidemiology of grapevine yellows disease in west Europe, there is a strong indication that alder yellows phytoplasma should be considered as a potential threat. Our study clearly indicates that alder yellows phytoplasmas are widely distributed in Serbia with high incidence in alders. On the other hand, there are substantial differences in ecology of alder trees in Serbia and west Europe, i.e. alders are obviously related with mesic and cold habitats which are obviously not suitable habitats attractive for Serbian vine growers. Thus, there is a big spatial gap in topology which strictly separates alder's compartments from vineyards, giving less possibilities of accidental transmission of alder yellows phytoplasma by its natural vector *Oncopsis alni*.

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REFERENCES

Angelini, E., Clair, D., Borgo, M., Bertaccini, A., Boudon-Padieu, E. (2001): Flavescence dorée in France and Italy – occurrence of closely related phytoplasma isolates and their near relationships to Palatinate grapevine yellows and an alder phytoplasma. Vitis 40, 79–86.

Angelini, E., Negrisolo, E., Clair, D., Borgo, M., Boudon-Paddieu, E. (2003): Phylogenetic relationships among "flavescence dorée" strains and related phytoplasmas determined by heteroduplex mobility assay and sequence of ribosomal and non-ribosomal DNA. Plant Pathology 52, 663-672.

Arnaud, G., Malembic-Maher, S., Salar, P., Bonnet, P., Maixner, M., Marcone, M., Boudon-Padieu, E., Foissac, X. (2007): Multilocus sequence typing confirms the close genetic interreladedness of three distinct Flavescence dorée phytoplasma strain clusters and group 16SrV phytoplasmas infecting grapevine and alder in Europe. Applied and environmental microbiology 73, 4001-4010.

Brodbeck, B.V., Andersen, P.C., Mizell, R.F. (1999): Effects of total dietary nitrogen and nitrogen form on the development of xylophagous leafhoppers. Archives of Insect Biochemistry and Physiology 42, 37-50.

Cvrković, T., Jović J., Mitrović, M., Petrović, A., Krnjajić, S., Malembic-Maher, S., Toševski, I. (2008): First report of alder yellows phytoplasma on common alder (*Alnus glutinosa*) in Serbia. Plant Pathology 57, 773.

Daire, X., Clair, D., Reinert, W., Boudon-Padieu, E. (1997): Detection and differentiation of grapevine yellows phytoplasmas belonging to the elm yellows group and to the stolbur subgroup by PCR amplification of non-ribosomal DNA. European Journal of Plant Pathology 103, 507–14.

De Sousa, E., Casati, P., Cardoso, F., Baltazar, C., Durante, G., Quaglino, F., Bianco, P.A. (2009): Flavescence dorée phytoplasma affecting grapevine(Vitis vinifera) newly reported in Portugal. New Disease Reports 19, 33.

Deng, S., Hiruki, C. (1991): Amplification of 16S rRNA genes from culturable and non-culturable molicutes. Journal of Microbiological Methods 14, 53-61.

Filippin, L., Jović, J., Cvrković, T., Forte, V., Clair, D., Toševski, I.., Boudon-Padieu, E., Borgo, M., Angelini E. (2009): Molecular Peculiarities of Phytoplasmas Associated with *Flavescence dorée* in Clematis and Grapevine and Preliminary Results on the Role of *Dictyophara europaea* (L.) as a Vector. Plant Pathology 58(5), 826–837.

Gaudin, J., Semetey, O., Foissac, X., Eveillardi, S. (2011): Phytoplasma titer in diseased lavender is not correlated to lavender tolerance to stolbur phytoplasma. Bulletin of Insectology 64 (Supplement), S179-S180.

Gibb, K.S., Padovan, A.C., Mogen, B.D. (1995): Studies on sweet potato little-leaf phytoplasma detected in sweet potato and other plant species growing in Northern Australia. Phytopathology 85(2), 169-174.

Jović, J., Cvrković, T., Mitrović, M., Krnjajić, S., Petrović, A., Redinbaugh, M.G., Pratt R.C., Hogenhout S.A., Toševski I. (2009): Stolbur phytoplasma transmission to maize by *Reptalus panzeri* and the disease cycle of maize redness in Serbia. Phytopathology 99(9), 1053-1061.

Jović, J., Ember, I., Mitrović, M., Cvrković, T., Krstić, O., Krnjajić, S., Acs, Z., Kolber, M., Toševski, I. (2011): Molecular detection of potato stolbur phytoplasma in Serbia. Bulletin of Insectology 64, S83-S84.

Krnjajić, S., Mitrović, M., Cvrković, T., Jović, J., Petrović, A., Forte, V., Angelini, E., Toševski, I. (2007): Occurrence and distribution of Scaphoideus titanus in multiple outbreaks of "flavescence dorée" in Serbia. Bulletin of Insectology 60 (2), 197-198.

Lederer, W., Seemüller, E. (1991): Occurrence of mycoplasma-like organisms in diseased and non-symptomatic alder trees (*Alnus* spp.). European Journal of Forest Pathology 21, 90-96.

Lee, I.M., Gundersen-Rindal, D.E., Davis, R.E., Bartoszyk, I.M. (1998): Revised classification scheme of phytoplasmas based an RFLP analyses of 16S rRNA and ribosomal protein gene sequences. International Journal of Systematic Bacteriology 48, 1153-1169.

Lee, I.M., Martini, M., Marcone, C., Zhu, S.F. (2004): Classification of phytoplasma strains in the elm yellows group (16SrV) and proposal of 'Candidatus Phytoplasma ulmi' for the phytoplasma associated with elm yellows. International Journal of Systematic and Evolutionary Microbiology 54, 337–47.

Maixner, M., Reinert, W. (1999): *Oncopsis alni* (Schrank) (*Auchenorrhyncha : Cicadellidae*) as a vector of the alder yellows phytoplasma of *Alnus glutinosa* (L.) Gaertn. European Journal of Plant Pathology 105, 87-94.

Maixner, M., Reinert, W., Darimont, H. (2000): Transmission of grapevine yellows by *Oncopsis alni* (Schrank) (*Auchenorrhyncha : Macropsinae*). Vitis 39, 83-84.

Malembic-Maher, S., Salar, P., Vergnes, D., Foissac, X. (2007): Detection and diversity of "flavescence dorée" - related phytoplasmas in alders surrounding infected vineyards in Aquitaine (France). Bulletin of Insectology 60(2), 329-330.

Martini M., Murari E., Mori N., Bertaccini A., (1999): Identification and epidemic distribution of two flavescence dore´e-related phytoplasmas in Veneto (Italy). Plant Disease 83, 925–30.

Maurer, R., Seemüller, E., Sinclair, W. A. (1993): Genetic relatedness of mycoplasmalike organisms affecting elm, alder, and ash in Europe and North America. Phytopathology 83, 971-976.

Olmstead, K.L., Denno, R.F., Morton, T.C., Romeo, J.T. (1997): Influence of Prokelisia planthoppers on amino acid composition and growth of *Spartina alterniflora*. Journal of Chemical. Ecology 23, 303-321.

Padovan, A.C., Gibb, K.S., Bertaccini, A., Vibio, M., Bonfiglioli, R.E., Magarey, P.A., Sears, B.B. (1995): Molecular detection of the Australian grapevine yellows phytoplasmas and comparison with grapevine yellows phytoplasmas from Italy. Australian Journal of Grape Wine Research, 1: 25-31.

Reisenzein, H, Steffek, R. (2011): First outbreaks of grapevine 'flavescence dorée' in Austrian viticulture. Bulletin of Insectology 64, S223-S224.

Schaerer, S., Johnston, H., Gugerli, P., Linder, C., Shaub, L., Colombi, L. (2007): "Flavescence dorée" in Switzerland: spread of the disease in canton of Ticino and of its insect vector, now also in cantons of Vaud and Geneva. Bulletin of Insectology 60(2), 375-376.

Sforza, R., Bourgoin, T., Wilson, S.W., Boudon-Padieu, E. (1999): Field observations, laboratory rearing and descriptions of immatures of the planthopper Hyalesthes obsoletus (Hemiptera: Cixiidae). European Journal of Entomology 96, 409–418.

Smart, C.D., Schneider, B., Blomquist, C.L., Guerra, L.J., Harrison, N.A., Ahrens, U., Lorenz, K.H., Seemüller, E., Kirckpatrick, B.C. (1996): Phytoplasma-specific PCR primers based on sequences of the 16S-23S rRNA spacer region. *Applied* and Environmental *Microbiology* 62, 1988-1993.

Škorić, D., Šeruga-Musić, M., Plavec, J., Križanac, I. (2011): Geographical distribution of 'flavescence dorée' phytoplasmas in Croatian grapevines. Bulletin of Insectology 64, S243-S244.

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RASPROSTRANJENJE ALDER YELLOWS FITOPLAZME NA CRNOJ I BELOJ JOVI (*ALNUS GLUTINOSA* I *ALNUS INCANA*) U SRBIJI

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IZVOD

Alder yellows (AldY) fitoplazma koja je u asocijaciji sa crnom jovom (*Alnus glutinosa*) i belom jovom (*A. incana*) pripada 16SrV ribozomalnoj grupi fitoplazmi. Ova fitoplazma je srodna fitoplazmi zlatastog žutila vinove loze *Flavescence dorée* (FD), koja je karantinski patogen od ekonomskog značaja u vinogradima južne Evrope uključujući i Srbiju. Do sada je prisustvo alder yellows fitoplazme utvrđeno u mnogim evropskim zemljama uključujući Francusku, Nemačku, Švajcarsku, Austriju, Italiju i Baltički region. Inficirane jove ispoljavaju simptome žutila listova, malih listova, redukcije lisne mase, ili ponekad ne ispoljavaju simptome inficiranosti. U cilju utvrđivanja prisustva i rasprostranjenja ove fitoplazme na široj teritoriji Srbije, sprovedeno je uzorkovanje simptomatskih i asimptomatskih jova. Rezultati istraživanja su potvrdili široku distribuciju alder yellows fitoplazme u Srbiji i prisustvo fitoplazme kako u simptomatskim tako i u asimptomatskim stablima. Od ukupno 72 uzorkovane biljke, 54 su bile inficirane fitoplazmom. Analizom RFLP profila 16S rRNK gena utvrđeno je prisustvo 16SrV-C podgrupe fitoplazmi. Dalja karakterizacija PCR-RFLP analizom operona ribozomalnih proteina svih pozitivnih izolata potvrdila je prisustvo 16SrV-C podgrupe fitoplazmi. U diskusiji je istaknut značaj širokog rasprostranjenja AldY fitoplazme i uticaja na epidemiologiju FD fitoplazme kao i na kontrolu bolesti.

Ključne reči: 16S rRNA, epidemiologija bolesti, *Flavescence dorée*, PCR-RFLP, *rpl22-rps3*, simptomi.

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