

Potential new hemipteran vectors of stolbur phytoplasma in Serbian vineyards

Tatjana CVRKOVIĆ¹, Jelena JOVIĆ¹, Milana MITROVIĆ¹, Oliver KRSTIĆ¹, Slobodan KRNJAJIĆ¹, Ivo TOŠEVSKI^{1,2}

¹Institute for Plant Protection and Environment, Department of Plant Pests, Zemun, Serbia

²CABI Europe - Switzerland, 1 Rue des Grillons, 2800 Delémont, Switzerland

Abstract

The diversity of Auchenorrhyncha species was studied in vineyards affected by 'bois noir' (BN) and their surroundings in Serbia. Auchenorrhyncha specimens were collected for identification, statistic analysis and for stolbur phytoplasma presence detection. A total number of collected species was 49, belonging to 8 families. The most numerous was family Cicadellidae with 30 species, followed by Cixiidae (7), Delphacidae (4), Aphrophoridae (3). Families Dictyopharidae, Issidae, Cercopidae and Membracidae were present with only one species. The most abundant species in all inspected sites was *Psammotettix alienus* (Dahlbom). *Dictyophara europaea* (L.), *Hyalesthes obsoletus* Signoret, *Euscelis incisus* (Kirschbaum) and *Reptalus quinquecostatus* (Dufour) were numerous, as well. The presence of BN was detected in 4 Auchenorrhyncha species: *H. obsoletus*, *R. quinquecostatus*, *Reptalus panzeri* (Low) and *D. europaea*. This is the first record of BN phytoplasmas in *D. europaea*.

Key words: 'bois noir', stolbur, *Dictyophara europaea*, vector, grapevine.

Introduction

'Bois noir' (BN) is the most widespread grapevine yellows in Europe and Mediterranean area. BN is a disease associated with stolbur phytoplasma presence (16SrXII) and for the past two decades was registered as one of the economically most important grapevine diseases in Europe.

Stolbur phytoplasma infects a wide range of wild and cultivated herbaceous plants and can be transmitted by the cixiid planthoppers *Hyalesthes obsoletus* Signoret, *Pentastiridius leporinus* (L.) and *Reptalus panzeri* (Low) (Maixner *et al.*, 1995; Gatineau *et al.*, 2001; Jović *et al.*, 2007) and the leafhoppers *Macrostelus quadripunctulatus* (Kirschbaum) and *Anaceratagallia ribauti* (Ossiannilsson) (Battle *et al.*, 2008, Riedle-Bauer *et al.*, 2008). Stolbur phytoplasma has also been detected in several other cixiid and leafhopper species (Trivellone *et al.*, 2005), but their vector role has not yet been confirmed. The aims of this study were to determine the diversity of Auchenorrhyncha species present in vineyards and to identify new potential insect vectors of stolbur phytoplasma.

Materials and methods

The diversity of Auchenorrhyncha species was studied in three vineyards: Topola (central Serbia), Vršac (northern Serbia) and Rajac (eastern Serbia), where a high percentage of BN infected plants were present.

Insect sampling was carried out in 2006 and 2007, from mid-May to the end of September. Potential hemipteran vectors including leafhoppers and planthoppers were collected using sweep nets and mouth-aspirators from grapevines and weeds along the borders of vineyards, and stored in 96% ethanol for later species and phytoplasma identification.

Total nucleic acids were extracted from individual insects using a modified CTAB method (Gatineau *et al.*, 2001). Phytoplasma was identified in insects using a modification of the stolbur phytoplasma-specific nested PCR protocol, with primers Stol11f/r, R16F2/R1, and R16F3/R2, according to Radonjić *et al.* (2009).

Results and discussion

In total, 4,971 Auchenorrhyncha specimens were collected in and around vineyards, belonging to eight families and 49 species (table 1). The most abundant was the Cicadellidae with 30 species, followed by Cixiidae 7 species, Delphacidae 4 species, Aphrophoridae 3 species, and Dictyopharidae, Issidae, Cercopidae and Membracidae with only one species recorded. The predominant species were *Psammotettix alienus* (Dahlbom) (29.4%). *Dictyophara europaea* (L.) (10%), *H. obsoletus* (9.2%), *Euscelis incisus* (Kirschbaum) (6.4%), *Reptalus quinquecostatus* (Dufour) (5.8%), *Neoliturus fenestratus* (Herrich-Schaffer) and *Errastunus ocellaris* (Fallen) (about 4.2%), were numerous as well, while presence of *Laodelphax striatella* (Fallen), *Philaenus spumarius* (L.), *Doratura impudica* Horvath and *Zyginidia pullula* (Boheman) was between 2.5 and 3.3%.

PCR analyses using DNA from collected adult insects indicated that 4 out of 49 collected species harbored the stolbur phytoplasma: 38% of *H. obsoletus* (91/240), 15% of *R. quinquecostatus* (44/289), 8% of *R. panzeri* (4/49) and 12% of *D. europaea* (41/341) specimens.

In this survey, relatively dense populations of the *D. europaea* were recorded in vineyards and their surroundings, with 12% of captured specimens positive for BN phytoplasma presence.

Table 1. Auchenorrhyncha species collected in and around vineyards affected by BN.

Species
Cixiidae/ Cixinae
<i>Cixius wagneri</i> (China 1942)
<i>Cixius similis</i> (Kirschbaum 1868)
<i>Hyalesthes obsoletus</i> (Signoret 1865)
<i>Reptalus cuspidatus</i> (Fieber 1876)
<i>Reptalus panzeri</i> (Low 1883)
<i>Reptalus quinquecostatus</i> (Dufour 1833)
<i>Tachycixius desertorum</i> (Fieber 1876)
Delphacidae/ Delphacinae
<i>Asiraca clavicornis</i> (F. 1794)
<i>Dicranotropis hamata</i> (Boheman 1847)
<i>Laodelphax striatella</i> (Fallen 1826)
<i>Stenocranus major</i> (Kirschbaum 1868)
Dictyopharidae/Dictyopharinae
<i>Dictyophara europaea</i> (L. 1767)
Membracidae/Smiliinae
<i>Stictocephala bisonia</i> (Kopp et Yonke 1977)
Issidae/Issinae
<i>Issus coleoptratus</i> (F. 1781)
Cercopidae/Haematoloma
<i>Lepyronia coleoptrata</i> (L. 1758)
Aphrophoridae/Aphrophorinae
<i>Philaenus spumarius</i> (L. 1758)
<i>Neophilaenus campestris</i> (Fallen 1805)
<i>Aphrophora alni</i> (Fallen 1805)
Cicadellidae/ Typhlocybinae
<i>Empoasca vitis</i> (Gothé 1875)
<i>Eupterix</i> sp.
<i>Zyginidia pullula</i> (Boheman 1845)
Cicadellidae/ Deltocephalinae
<i>Scaphoideus titanus</i> (Ball 1932)
<i>Fieberiella septentrionalis</i> Wagner 1963
<i>Jassargus obtusivalis</i> (Kirschbaum 1868)
<i>Errastunus ocellaris</i> (Fallen 1806)
<i>Neotalitrus fenestratus</i> (Herrich-Schaffer 1834)
<i>Mocydia crocea</i> (Herrich-Schaffer 1837)
<i>Psammotettix alienus</i> (Dahlbom 1850)
<i>Psammotettix confinis</i> (Dahlbom 1850)
<i>Euscelis incisus</i> (Kirschbaum 1858)
<i>Goniagnathus brevis</i> (Herrich-Schaffer 1835)
<i>Macrostelus</i> sp.
<i>Platymetopius major</i> (Kirschbaum 1868)
<i>Recilia schmidgeni</i> (Wagner 1939)
<i>Doratura impudica</i> Horvath 1897
<i>Allygidius commutatus</i> (Fieber 1872)
<i>Graphocraerus ventralis</i> (Fallen 1806)
<i>Ophiola decumana</i> (Kontkanen 1949)
<i>Streptanus confinis</i> (Reuter 1880)
<i>Mocuellus collinus</i> (Boheman 1850)
<i>Allygus</i> sp.
Cicadellidae/Cicadellinae
<i>Cicadella viridis</i> (L. 1758)
<i>Macropsis fuscula</i> (Zetterstedt 1828)
<i>Macropsis</i> sp.
Cicadellidae/ Aphrodinae
<i>Aphrodes</i> sp.
Cicadellidae/ Idiocerinae
<i>Idiocerus</i> sp.
Cicadellidae/Dorycephalinae
<i>Eupelix cuspidata</i> (Fabricius 1775)
Cicadellidae/Agalliinae
<i>Anaceratagallia ribauti</i> (Ossiannilsson 1938)
<i>Dryodurgades reticulatus</i> (Herrich-Schaffer 1834)

This is the first record of stolbur phytoplasma presence in *D. europaea*. Until now, it was reported that *D. europaea* is infected with ‘flavescence dorée’ phytoplasma and preliminary transmission experiments showed that this species is able to transmit this phytoplasma from clematis to grapevine (Filippin *et al.*, 2009). *D. europaea* is polyphagous insect present in vineyards and herbaceous plants along the borders of vineyards from the middle of May to the end of September. Its ability to carry stolbur phytoplasma gives a new light in the epidemiology of BN disease in vineyards, but further studies are required to assess its vector role.

Acknowledgements

We thank the Ministry of Education and Science of Republic of Serbia for financial support during this study through grant III43001.

References

- BATTLE A., ALTABELLA N., SABATE J., LAVIÑA A., 2008.- Study of the transmission of stolbur phytoplasma to different crop species, by *Macrostelus quadripunctulatus*.- *Annals of Applied Biology*, 152: 235-242.
- FILIPPIN L., JOVIĆ J., CVRKOVIĆ T., FORTE V., CLAIR D., TOŠEVSKI I., BOUDON-PADIEU E., BORGIO M., ANGELINI E., 2009.- Molecular characteristics of phytoplasmas associated with ‘flavescence dorée’ in clematis and grapevine and preliminary results on the role of *Dictyophara europaea* as a vector.- *Plant Pathology*, 58: 826-837.
- GATINEAU F., LARRUE J., CLAIR D., LORTON F., RICHARDMOLARD M., BOUDON-PADIEU E., 2001.- A new natural planthopper vector of stolbur phytoplasma in the genus *Pentastiridius* (Hemiptera: Cixiidae).- *European Journal of Plant Pathology*, 107: 263-271.
- JOVIĆ J., CVRKOVIĆ T., MITROVIĆ M., KRNJAJIĆ S., REDINBAUGH M. G., PRATT R. C., GINGERY R. E., HOGENHOUT S. A., TOŠEVSKI I., 2007.- Roles of stolbur phytoplasma and *Reptalus panzeri* (Cixiinae, Auchenorrhyncha) in the epidemiology of maize redness in Serbia.- *European Journal of Plant Pathology*, 118:85-89.
- MAIXNER M., AHRENS U., SEEMÜLLER E., 1995.- Detection of the German grapevine yellows (Vergilbungskrankheit) MLO in grapevine, alternative hosts and a vector by a specific PCR procedure.- *European Journal of Plant Pathology*, 101: 241-250.
- RADONJIĆ S., HRNČIĆ S., JOVIĆ J., CVRKOVIĆ T., KRSTIĆ O., KRNJAJIĆ S., TOŠEVSKI I., 2009.- Occurrence and distribution of grapevine yellows caused by stolbur phytoplasma in Montenegro.- *Journal of Phytopathology*, 157: 682-685.
- RIEDLE-BAUER M., SARA A., REGNER F., 2008.- Transmission of a stolbur phytoplasma by the Agalliinae leafhopper *Anaceratagallia ribauti* (Hemiptera, Auchenorrhyncha, Cicadellidae).- *Journal of Phytopathology*, 156: 687-690.
- TRIVELLONE V., PINZAUTI F., BAGNOLI B., 2005.- *Reptalus quinquecostatus* (Dufour) (Auchenorrhyncha Cixiidae) as a possible vector of stolbur-phytoplasma in a vineyard in Tuscany.- *Redia*, 88:103-108.

Corresponding author: Tatjana CVRKOVIĆ (e-mail: tanjacvrkovic@yahoo.com), Institute for Plant Protection and Environment, Banatska 33, 11080 Zemun, Serbia.