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Insight into Epidemiological Importance of Phytoplasma Vectors in Vineyards in South Moravia, Czech Republic

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Abstract

Šafářová D., Lauterer P., Starý M., Válová P., Navrátil M.: Insight into epidemiological importance of phytoplasma vectors in vineyards in South Moravia, Czech Republic. Plant Protect. Sci.

Bois noir (BN), caused by *'Candidatus* Phytoplasma solani', is a serious disease of grapevines in Europe. During the 2010–2012 survey in Perná vineyard (South Moravia, Czech Republic) a total of 4854 insect individuals were collected and among these, 95 insect species belonging to Auchenorrhyncha (77 species), Heteroptera (12), and Psylloidea (62) were indentified. The nested polymerase chain reaction-restriction fragment length polymorphism analyses confirmed *Hyalesthes obsoletus* as the main BN vector with 43.8% of phytoplasma positive individuals on average. A significant role of *Anaceratagallia ribauti* (22.6% of phytoplasma positive specimens) should be taken into account based on its occurrence and incidence of infected individuals. Eleven insect species were identified as new carriers of *'Ca.* P. solani' or suggested as potential BN vectors in this work.

Key words: *'Candidatus* Phytoplasma solani'; *'Candidatus* Phytoplasma asteris'; bois noir; vector; *Hyalesthes obsoletus*

Phytoplasmas are wall-less, non-culturable pathogenic plant bacteria classified within the class Mollicutes. These obligate parasites colonise phloem sieve cells of plants and various tissues of the insect vectors. They are transmitted by insects of the order Hemiptera, however, vector species are only restricted to three suborders: Auchenorrhyncha, Heteroptera, and Sternorrhyncha. Among them, about 100 insect species have been confirmed to be phytoplasma vectors (Weintraub & Beanland 2006; Weintraub 2007).

Currently, the wide spread of an economically important phytoplasma, *'Candidatus* Phytoplasma solani' and occurrence of Bois noir (BN) local outbreaks have been reported in grapevines in several European countries (Battle *et al.* 2000; Riedle-Bauer *et al.* 2006; Kuntzmann *et al.* 2008; Kostadinovska *et al.* 2014; Mori *et al.* 2015) as well as in the Czech Republic (Starý *et al.* 2013). Until now, *'Ca.* P. solani' has been detected in more than 50 insect species and at least 7 of them (*Hyalesthes obsoletus* as the most important, along with *Aphrodes bicincta*, *Euscelidius variegatus*, *Euscelis obsoletus*, *Macrosteles quadripunctulatus*, *Reptalus panzeri*, and *Issus* sp.) were confirmed as its vector involved in transmission of BN disease (Maixner 1994; Chuche *et al.* 2016).

The present study deals with the occurrence of Auchenorrhyncha, Heteroptera, and Psylloidea in Perná vineyard in South Moravia, Czech Republic and evaluates their impact as vectors in BN epidemiology.

MATERIAL AND METHODS

The survey was conducted from 2010 to 2012 in a vineyard in Perná (48°51'8''N, 16.37'28''E; 230 m

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a.s.l.), South Moravia, Czech Republic. The locality represents a grapevine agro-ecosystem, with a frequent occurrence of BN (Starý *et al.* 2013). The elimination of competing vegetation from the vineyard by herbicides application under the rows, and by sporadic mowing between rows was the only management practice applied in the green cover. The surrounding ruderal vegetation, with predominating nettles (*Urtica dioica*), was mowed once a year at the beginning of July.

Insects were sampled from the undamaged vegetation (up to 100 cm high) in vineyard in two-week intervals from June to August. Individuals were trapped semi-quantitatively using permanent sweep-netting (net 50 cm in diameter) for 1 hour. Captured individuals were stored in absolute ethanol at –20°C until determination and testing for the presence of phytoplasma was done.

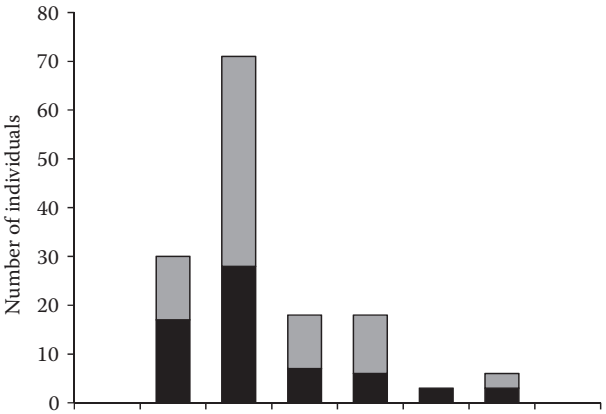
Total DNA from individual insects was obtained using a commercial Wizard Genomic DNA Purification Kit (Promega, Madison, USA). The phytoplasma detection and identification was performed using standard nested polymerase chain reaction (PCR) with P1/P7 primers followed by R16F2/R2, and subsequent restriction fragment length polymorphism (RFLP) analysis using *Alu*I, *Bfm*I, *Mse*I, and *Rsa*I (Fermentas, Vilnius, Lithuania) restriction endonucleases (Starý *et al.* 2013). Numbers of tested insects are presented in Supplementary Table S2 in Electronic Supplementary Material (ESM).

RESULTS

Survey of planthoppers, leafhoppers, and other phytoplasma vectors. During the systematic survey in 2010–2012 a total of 4854 insect individuals were collected and among these, 95 insect species belonging to Auchenorrhyncha (77 species), Heteroptera (12), and Psylloidea (6) were identified. The most abundant species was *Trioza urticae*, with 1832 individuals trapped; the second was *Aphalara avicularis* (449 individuals). Besides them populations of *Emelyanoviana mollicula, Empoasca pteridis, Psammotettix confinis, Dicranotropis hamata,* and the main vector of *'Ca.* P. solani' *Hyalesthes obsoletus* were also abundant. Detailed characterisation of entomofauna, list of species trapped, and their relative abundance are summarised in Supplementary Tables S1 and S2.

Phytoplasma detection in insects. In total, 1138 adults representing 45 species were tested for phytoplasma presences, 28 insect species were bearing *'Ca.* P. solani' (16SrXII-A subgroup) and/or *'Ca.* P. asteris' (16SrI-B, -C, and prevalent -F subgroups). For details see Supplementary Table S2. Phytoplasma *'Ca.* P. solani' was detected in 18 Auchenorryncha species, 3 Heteroptera species, and 2 Psylloidea species. Among them, 9 species known as BN and/or stolbur confirmed vectors: *Anaceratagallia ribauti* (relative abundance 102 individuals), *Aphrodes bicincta* (3), *Euscelidius variegatus* (64), *Euscelis incisus* (5), *Hardya tenuis* (1), *Hyalesthes obsoletus* (146), *Macrosteles quadripunctulatus* (64), *Macrosteles laevis* (25), and *Reptalus panzeri* (4) occurred at this locality, but only two of them showed a higher abundance together with the significant percentage of *'Ca.* P. solani' positive individuals: *Hyalesthes obsoletus* (43.8%) and *Anaceratagallia ribauti* (22.6%).

Based on the high abundance and the highest number of phytoplasma positive individuals, the *Hyalesthes obsoletus* should be expectedly considered the most important vector species. The individuals were sampled from the end of June, with the population density culminating at the beginning of July (Figure 1), to the middle of August, when the last individuals were sporadically caught in the season. The adults were mainly present on *Urtica dioica* (94% of the collected individuals), and to a lesser extent



	Jun II	Jun III	Jul I	Jul II	Jul III	Aug I	Aug II	Aug III
♂	0	13	43	11	12	0	3	0
♀	0	17	28	7	6	3	3	0

Figure 1. Total seasonal abundance and phytoplasma presence in *Hyalesthes obsoletus* captured in vineyard from 2010 to 2012

Data summarised according the month decades (I–III), phytoplasma positive (black column) and phytoplasma negative (grey column) individuals

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on *Convolvulus arvensis* (6%) plants. The second important prevalent vector, *Anaceratagallia ribauti*, was sampled from the second half of June to the end of August, with the population culmination during July. The epidemiological situation of BN might have been additionally influenced by vector *Macrosteles quadripunctulatus* (relative abundance 64 individuals, 4.2% positive individuals) that was sampled from the beginning of June to the end of August with population density culmination noted during July.

Surprisingly, *'Ca.* P. solani' was detected in the confirmed *'Ca.* P. asteris' vector *Neoaliturus fenestratus* (relative abundance 13 individuals; 61.5% positive individuals). A high incidence of *'Ca.* P. solani' was also noted in the abundant *Mocuellus collinus* (51; 36.1%), *Lygus rugulipennis* (69; 46.2%), and its presence was for the first time detected in *Doratura homophyla* (65; 16.1%), *Empoasca pteridis* (165; 35.0%), *Psammotettix confinis* (139; 19.4%), and *Aphalara avicularis* (449; 11.6%).

The occurrence of other eight *'Ca.* P. asteris' vector species was noted at the same locality (*Aphrodes bicincta, Empoasca decipiens, Euscelidius variegatus, Euscelis incisus, Macrosteles laevis, Macrosteles qudripunctulatus, Macrosteles viridigriseus,* and *Neoliturus fenestratus*), but only *M. quadripunctulatus* showed higher abundance and AY phytoplasma infestance (relative abundance 64 individuals, 16.7% positive individuals). A considerable percentage of phytoplasma positive individuals were found in potential vectors *P. alienus* (54; 12.5%) and *Javesella pellucida* (98; 9.28%). Totally 9 species were bearing both mentioned phytoplasmas, either *'Ca.* P. solani' or *'Ca.* P. asteris' (for details see Supplementary Table S2).

In summary, the new potential vectors of *'Ca.* P. solani' (STOL) and/or *'Ca.* P. asteris' (AY) detected in our study were: *Doratura homophyla* (AY, STOL), *Empoasca pteridis* (STOL), *Ophiola decumana* (STOL), *Psammotettix confinis* (STOL), *Psammotettix kolosvarensis* (AY), *Streptanus aemulans* (AY, STOL), *Dicranotropis hamata* (AY, STOL), *Javesella pellucida* (STOL), *Adelphocoris lineolatus* (STOL), *Liocoris tripustulatus* (STOL), *Aphalara avicularis* (STOL), and *Trioza urticae* (STOL).

DISCUSSION

The research into *'Ca.* P. solani' and its vectors carried out so far within different European vineyard agroecosystems has been limited, data have still been only partial, and in some cases contradictory (Fos *et al.* 1992; Battle *et al.* 2000; Orenstein *et al.* 2003; Riedle-Bauer *et al.* 2006; Cvrković *et al.* 2011; Mehle *et al.* 2011; Mitrović *et al.* 2012; Atanasova *et al.* 2015). BN epidemiology is connected with two main epidemiological cycles, nettle cycle characterised by *'Ca.* P. solani' tuf-a type and *Urtica dioica* as a reservoir plant, and bindweed cycle with *'Ca.* P. solani' tuf-b type and *Convolvulus arvensis* present (Langer & Maixner 2004). The only *'Ca.* P. solani' tuf-b type and *U. dioica* as dominant reservoir plant have been reported until now in South Bohemia (Fialová *et al.* 2009; Starý *et al.* 2013), but it could be similar to the situation recently observed at some localities in Austria characterised by the presence of specific tuf-b2 genotype (Aryan *et al.* 2014).

Studies on the vineyard community of Auchenorrhyncha, Heteroptera, and Psylloidea species (BN vector and/or potential vector) have been conducted in different European countries, however only few of them under Central European climatic conditions (Safarova *et al.* 2011; Aryan *et al.* 2014; Tancik & Seljak 2017).

In the present study, the expected key role of *Hyalesthes obsoletus* in the epidemiology of bois noir disease in Perná vineyard (Czech Republic) was demonstrated. The detected infection rate (34–62%) is similar to the observations previously made in other European vineyards (Fos *et al.* 1992; Sforza *et al.* 1998; Ge & Maixner 2003; Riolo *et al.* 2007; Sabaté *et al.* 2007; Cvrković *et al.* 2011; Mitrović *et al.* 2012; Aryan *et al.* 2014).

Macrosteles quadripunctulatus and *M. laevis* are frequent polyphagous species and both of them were experimentally confirmed as *'Ca.* P. solani' vectors, with *M. quadripunctulatus* as a vector to grapevines and vegetables and *M. laevis* only to vegetables (Batlle *et al.* 2008). During our survey and under the conditions at the studied vineyard, the both *Macrosteles* species were evaluated as not so important in phytoplasma spread into grapevines, as only 4.2% of the phytoplasma infected *M. quadripunctulatus* individuals were caught; even though the species abundance was higher compared to other *'Ca.* P. solani' vectors detected there, and no infected *M. laevis* individuals were found.

Anaceratagalia ribauti is another insect species that is able to transmit *'Ca.* P. solani' among the host plants. Its vector status was demonstrated by proving its ability of phytoplasma acquisition from

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bindweed and subsequent transmission to broad bean and periwinkle (Riedle-Bauer *et al.* 2006, 2008; Aryan *et al*. 2014). The frequent occurrence of *A. ribauti* observed in the studied vineyard was reported from various European countries, too. But under the predominant presence of confirmed vector *H. obsoletus* it was evaluated as of less or none epidemiological impact (Riedle-Bauer *et al.* 2006; Cvrković *et al.* 2011; Mehle *et al.* 2011; Landi *et al.* 2013; Aryan *et al*. 2014). However, despite the fact that the transmission of BN to grapevine by *A. ribauti* was not observed, its potential importance in BN pathosystem cannot be excluded due to its relatively high abundance, infectivity, and proven ability to transmit BN among reservoir herbaceous plants.

In summary, in this study, 23 insect species bearing *'Ca.* P. solani', including 5 BN confirmed vectors, were found. Ten of the studied insect species had been tested earlier in transmission trials with negative results: *Dicranotropis hamata*, *Psammotettix alienus*, *Aphrodes makarovi*, *Errastunus ocellaris*, *Jassargus obtusivalvis*, *Mocuellus collinus*, *Mocydia crocea*, *Neoaliturus fenestratus*, *Psammotettix confinis*, and *Streptanus aemulans* (Riedle-Bauer *et al.* 2008). These findings cannot not be taken as a proof of their non-vector status, as success often depends on the test model chosen, the number of trapped as well as individual insects used, and on the number of repetitions. For example, *Euscelis incisus* was accepted as a stolbur vector (Valenta *et al*. 1961), but negative results in a transmission test under specific conditions were obtained, too (Riedle-Bauer *et al.* 2008).

On the other hand, the high number of other phytoplasma positive insect species does not directly indicate their importance in phytoplasma spread; their live history should be taken into account prior to their assignment as a potential vector. In this regard, polyphagous species developing and feeding on dicotyledonous weeds, such as *Philaenus spumarius*, earlier confirmed as a vector of *'Ca.* P. mali' (Hegab & El-Zohairy 1986); *Aphrodes macarovi*, a new species close to *'Ca.* P. solani' vector *Aphrodes bicincta* (Tishechkin 1998); *Empoasca* spp. including the vector of *'Ca.* P. asteris' *Empoasca decipiens* (Galetto *et al.* 2011); *Psammotettix* spp. including the vector of *'Ca.* P. asteris' *Psammotettix alienus* (Landi *et al.* 2013), could be evaluated as putative vectors but their vector status should be confirmed in transmission trials. The biology of these species and their ability to develop on bindweed or nettle should be elucidated, too.

The insect species feeding on monocotyledonous plants (mainly *Poaceae*) such as *Dicranotropis hamata*, *Doratura* spp., *Errastunus ocellaris*, *Mocuellus collinus*, and *Mocydia crocea* cannot be considered significant vectors of *'Ca.* P. solani' due to their feeding plant preference, although they are capable of phytoplasma acquisition during accidental feeding on dicotyledonous plants.

Besides *'Ca.* P. solani', the second phytoplasma *'Ca.* P. asteris' was detected in many insect species even though this phytoplasma was not detected in plants at the studied locality. Contrary to reports from other European countries (Duduk *et al.* 2007; Landi *et al.* 2013) about the prevalence of the 16SrI-B and 16SrI-C subgroups strains, in our study the 16SrI-F strain predominanted, in line with earlier preliminary results from a similar locality (Orságová *et al.* 2011).

Although *Hyalesthes obsoletus* is the most important vector in the studied agro-ecosystems, it cannot be excluded that '*Ca.* P. solani' could be transmitted among reservoir hosts and/or target crop by another insect species. Based on our observation, attention should be focused to *Anaceratagalia ribauti* as the potentially important vector and effective evaluation of its transmission ability to grapevine should follow.

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Electronic supplementary material (ESM)

Table S1. List of insect species captured in Perná vineyard and their phytoplasma vector status according to literature

Classification	Species	Confirmed vector	Insect bearing phytoplasma
Auchenorrhyncha			
Aphrophoridae	*Aphrophora alni* (Fallén, 1805)		
	Lepyronia coleoptrata (Linnaeus, 1758)		
	Philaenus spumarius (Linnaeus, 1758)		STOL[1]; AY[2]
Cicadellidae	*Agallia consobrina* Curtis, 1833		
	Allygidius abbreviatus (Lethierry, 1878)		
	***Anaceratagallia ribauti* (Ossiannilsson, 1938)**	STOL[3]	AY[4]
	***Aphrodes bicincta* (Schrank, 1776)**	STOL[5*, 6*, 13#]; AY[25*]	AY[2, 6]
	Aphrodes makarovi Zachvatkin, 1948		STOL[23]
	Arocephalus languidus (Flor, 1861)		
	Arthaldeus striifrons (Kirschbaum, 1868)		
	Artianus interstitialis (Germar, 1821)		
	Balcanocerus larvatus (Herrich-Schäffer, 1837)		
	Balclutha rhenana Wagner, 1939		

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Classification	Species	Confirmed vector	Insect bearing phytoplasma
Cicadellidae	*Chlorita paolii* (Ossiannilsson,1939)		
(continuation)	*Chlorita viridula* (Fallén, 1806)		
	Cicadella viridis (Linnaeus, 1758)		STOL[9]
	Cicadula persimilis (Edwards, 1920)		
	Deltocephalus pulicaris (Fallén, 1806)		
	Doratura homophyla (Flor, 1861)		
	Doratura impudica Horváth, 1897		
	Elymana sulphurella (Zetterstedt, 1828)		
	Emelyanoviana mollicula (Boheman, 1845)		STOL[7]
	Empoasca decipiens Paoli, 1930	AY[10]	STOL[24]
	Empoasca pteridis (Dahlbom, 1850)		
	Empoasca vitis (Goethe, 1875)		
	Enantiocephalus cornutus (Herrich-Schäffer, 1838)		
	Errastunus ocellaris (Fallén, 1806)		STOL[7]; AY[4]
	Eupteryx atropunctata (Goeze, 1778)		STOL[7]
	Eupteryx aurata (Linnaeus, 1758)		
	Eupteryx calcarata Ossiannillsson, 1936		
	Eupteryx cyclops Matsumura, 1906		
	Eupteryx florida Ribaut, 1936		
	Eupteryx notata Curtis, 1837		
	Eupteryx tenella (Fallén, 1806)		
	Eupteryx urticae (Fabricius, 1803)		
	***Euscelidius variegatus* (Kirschbaum, 1858)**	STOL[13#]; AY[11, 12]	
	Euscelidius schenckii (Kirschbaum, 1868)		
	***Euscelis incisus* (Kirschbaum, 1858)**	STOL[5*]; AY[11, 26]	AY[2]
	Evacanthus acuminatus (Fabricius, 1794)		STOL[23]
	Graphocraerus ventralis (Fallén, 1806)		
	***Hardya tenuis* (Germar, 1821)**	STOL[14]	

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Classification	Species	Confirmed vector	Insect bearing phytoplasma
Cicadellidae	*Jassargus obtusivalvis* (Kirschbaum, 1868)		AY[2, 4]
(continuation)	*Macropsis scutellata* (Boheman, 1845)		
	***Macrosteles laevis* (Ribaut, 1927)**	STOL[15]; AY[6*]	AY[2]
	Macrosteles ossiannilssoni Lindberg, 1954		
	***Macrosteles quadripunctulatus* (Kirschbaum, 1868)**	STOL[16], AY [6*, 11, 12]	
	Macrosteles sardus Ribaut, 1948		STOL[7]
	Macrosteles viridigriseus (Edwards, 1922)	AY[27*]	
	Mocuellus collinus (Boheman, 1850)		STOL[7], AY[4]
	Mocydia crocea (Herrich-Schäffer, 1837)		STOL[8], AY[4]
	Neoaliturus fenestratus (Herrich-Schäffer, 1834)	AY[17*]	STOL[7, 18]
	Ophiola decumana (Kontkanen, 1949)		AY[19]
	Platymetopius major (Kirschbaum, 1868)		
	Platymetopius rostratus (Herrich-Schäffer, 1834)		
	Psammotettix alienus (Dahlbom, 1850)		STOL[1]; AY[1, 7]
	Psammotettix confinis (Dahlbom, 1850)		AY[19]
	Psammotettix kolosvarensis (Matsumura, 1908)		STOL[7]
	Rhoananus hypochlorus (Fieber, 1869)		
	Stictocoris picturatus (C. Sahlberg, 1871)		
	Streptanus aemulans (Kirschbaum, 1868)		
	Streptanus sordidus (Zetterstedt, 1828)		
	Zyginidia pullula (Boheman, 1845)		
Cixiidae	*Cixius nervosus* (Linnaeus, 1758)		
	***Hyalesthes obsoletus* Signoret, 1865**	STOL[6*, 20]	
	***Reptalus panzeri* (P. Löw, 1883)**	STOL[1, 21]	
Cercopidae	*Cercopis sanguinolenta* (Scopoli, 1763)		
Delphacidae	*Asiraca clavicornis* (Fabricius, 1794)		
	Chloriona unicolor (Herrich-Schäffer, 1835)		
	Dicranotropis hamata (Boheman, 1847)		

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Classification	Species	Confirmed vector	Insect bearing phytoplasma
Delphacidae	*Javesella pellucida* (Fabricius, 1794)		AY[2]
(continuation)	*Laodelphax striatella* (Fallén, 1826)		STOL[8, 14], AY[2, 14]
	Megadelphax sordidula (Stål, 1853)		
	Ribautodelphax albostriata (Fieber, 1866)		
	Stenocranus minutus (Fabricius, 1787)		
Dictyopharidae	*Dictyophara europaea* (Linnaeus, 1767)		STOL[1]
	Stictocephala bisonia Kopp et Yonke, 1977		STOL[8]
Heteroptera			
Heterogastridae	*Heterogaster urticae* (Fabricius, 1775)		
Miridae	*Adelphocoris lineolatus* (Goeze, 1778)		
	Apolygus lucorum (Meyer-Dür, 1843)		
	Brachycoleus decolor Reuter, 1887		
	Chlamydatus pullus (Reuter, 1871)		
	Halticus apterus (Linnaeus, 1761)		
	Liocoris tripustulatus (Fabricius, 1781)		
	Lygus pratensis (Linnaeus, 1758)		STOL[22]
	Lygus rugulipennis Poppius, 1911		STOL[2, 22]
	Orthops basalis (Costa, 1852)		
	Trigonotylus caelestialium (Kirkaldy, 1902)		
Rhyparochromidae	*Megalonotus sabulicola* (Thomson, 1870)		
Psylloidea			
Aphalaridae	*Aphalara avicularis* Ossiannilsson, 1981		
	Aphalara freji Burckhardt & Lauterer, 1997		
	Aphalara maculipennis Löw, 1886		
Triozidae	*Bactericera nigricornis* (Förster, 1848)		
	Heterotrioza chenopodii (Reuter, 1876)		
	Trioza urticae (Linnaeus, 1758)		

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STOL represents '*Ca*. P. solani'; AY represents '*Ca*. P. asteris'; confirmed '*Ca*. P. solani' vector marked in bold; # transmission assays to *in vitro* grapevine plants; *evaluation based on symptomatology without molecular pathogen characterisation

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Table S2. Phytoplasma detection in insects captured in Perná vineyard

Clasification	Species	Number of collected adults	Number of PCR positive/ analysed adults	Phytoplasma identified	Number of collected larvae	Number of PCR positive/ analysed larvae	Phytoplasma identified
Auchenorrhyncha							
Aphrophoridae	*Philaenus spumarius* (Linnaeus, 1758)	1	0/1		0		
Cicadellidae	*Agallia consobrina* Curtis, 1833	2	0/2		0		
	***Anaceratagallia ribauti* (Ossiannilsson, 1938)**	102	26/102	3× AY-F 23× STOL	8	0/4	
	***Aphrodes bicincta* (Schrank, 1776)**	3	nt		7	2/7	2× AY-F
	Aphrodes makarovi Zachvatkin, 1948	6	1/2	1× STOL	0		
	Chlorita paolii (Ossiannilsson,1939)	123	0/24		8	nt	
	Doratura homophyla (Flor, 1861)	65	8/31	3× AY-F* 5× STOL*	71	nt	
	Emelyanoviana mollicula (Boheman, 1845)	213	0/24		12	nt	

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Clasification	Species	Number of collected adults	Number of PCR positive/ analysed adults	Phytoplasma identified	Number of collected larvae	Number of PCR positive/ analysed larvae	Phytoplasma identified
Cicadellidae	***Empoasca decipiens* Paoli, 1930**	3	1/2	1× STOL			
(continuation)	*Empoasca pteridis* (Dahlbom, 1850)	165	7/20	7×STOL*	7	nt	
	Empoasca vitis (Goethe, 1875)	3	0/3		1	nt	
	Errastunus ocellaris (Fallén, 1806)	42	1/17	1× AY-F	6	nt	
	Eupteryx atropunctata (Goeze, 1778)	41	0/24		0		
	Eupteryx aurata (Linnaeus, 1758)	52	0/24		0		
	Eupteryx calcarata Ossiannillsson, 1936	95	0/21		0		
	Eupteryx urticae (Fabricius, 1803)	69	0/24		34	nt	
	***Euscelidius variegatus* (Kirschbaum, 1858)**	10	2/10	1× AY-F	14	nt	
				1× STOL			
	***Euscelis incisus* (Kirschbaum, 1858)**	5	2/4	2× STOL	2	nt	
	***Hardya tenuis* (Germar, 1821)**	1	nt				
	Jassargus obtusivalvis (Kirschbaum, 1868)	26	2/14	2× AY-F	18	0/6	
	***Macrosteles laevis* (Ribaut, 1927)**	25	0/21		1	nt	
	***Macrosteles quadripunctulatus* (Kirschbaum, 1868)**	64	5/24	4× AY-B	4	nt	
				1× STOL			
	Macrosteles sardus Ribaut, 1948	2	0/1		0		
	Mocuellus collinus (Boheman, 1850)	51	17/36	4× AY-F			
				13× STOL			
	Mocydia crocea (Herrich-Schäffer, 1837)	5	1/5	1× STOL	2	0/2	
	Neoaliturus fenestratus (Herrich-Schäffer, 1834)	13	8/13	8× STOL	0		
	Ophiola decumana (Kontkanen, 1949)	37	2/24	2× STOL*	17	nt	
	Psammotettix alienus (Dahlbom, 1850)	54	3/24	3× AY-F	15	0/2	
	Psammotettix confinis (Dahlbom, 1850)	139	10/31	4× AY-F	6	nt	
				6× STOL*			
	Psammotettix kolosvarensis (Matsumura, 1908)	35	5/20	2× AY-B*	1	nt	
				3× AY-F			

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Clasification	Species	Number of collected adults	Number of PCR positive/ analysed adults	Phytoplasma identified	Number of collected larvae	Number of PCR positive/ analysed larvae	Phytoplasma identified
Cicadellidae	*Stictocoris picturatus* (C. Sahlberg, 1871)	9	0/7		3	nt	
(continuation)	*Streptanus aemulans* (Kirschbaum, 1868)	8	5/8	1× AY-B* 1× AY-F* 3× STOL*	25	8/11	8× AY
Cixiidae	***Hyalesthes obsoletus* (Signoret, 1865)**	146	64/146	64× STOL			
	***Reptalus panzeri* (P. Löw, 1883)**	4	0/1		0		
Delphacidae	*Dicranotropis hamata* (Boheman, 1847)	139	12/77	1× AY-C* 3× AY-F* 8× STOL*	57	nt	
	Javesella pellucida (Fabricius, 1794)	98	10/97	1× AY-C 8× AY-F 1× STOL*	0		
	Laodelphax striatella (Fallén, 1826)	51	1/40	1× AY-F	4	nt	
Dictyopharidae	*Dictyophara europaea* (Linnaeus, 1767)	8	0/2		4	1/3	1× STOL
Membracidae	*Stictocephala bisonia* Kopp et Yonke, 1977	18	2/13	2× STOL			
Heteroptera							
Miridae	*Adelphocoris lineolatus* (Goeze, 1778)	22	2/19	2× STOL*			
	Liocoris tripustulatus (Fabricius, 1781)	19	1/8	1× STOL*			
	Lygus pratensis (Linnaeus, 1758)	9	0/9				
	Lygus rugulipennis Poppius, 1911	69	12/26	12× STOL	7	nt	
Psylloidea							
Aphalaridae	*Aphalara avicularis* Ossiannilsson, 1981	449	5/43	5× STOL*	0		
	Aphalara maculipennis Löw, 1886	9	0/4		1	nt	
Triozidae	*Trioza urticae* (Linnaeus, 1758)	1832	1/82	1× STOL*	0		

nt – not tested; STOL – '*Candidatus* Phytoplasma solani'; AY – '*Canadidatus* Phytoplasma asteris', subgroubs marked by letters; *newly detected phytoplasma host in this work; confirmed '*Ca*. P. solani' vector in bold –confirmed '*Ca*. P. solani' vector to grapevine marked by grey background