

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

DANA ŠAFÁŘOVÁ¹, PAVEL LAUTERER², MARTIN STARÝ¹, PAVLA VÁLOVÁ¹ and MILAN NAVRÁTIL^{1*}

¹Department of Cell Biology and Genetics, Faculty of Science, Palacký University, Olomouc, Czech Republic;

²Department of Entomology, The Moravian Museum, Brno, Czech Republic

*Corresponding author: milan.navratil@upol.cz

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

<https://doi.org/10.17221/8/2018-PPS>

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

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

<https://doi.org/10.17221/8/2018-PPS>

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

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

1. Cvrković A., Jović J., Mitrović M., Krstić O., Krnjalić S., Toševski I. (2011): Potential new hemipteran vectors of stolbur phytoplasma in Serbian vineyards. *Bulletin of Insectology*, 64 (supplement): S129–S130.
2. Orságová H., Březíková M., Schlesingerová G. (2011): Presence of phytoplasmas in hemipterans in Czech vineyards. *Bulletin of Insectology*, 64 (supplement): S119–S120.
3. 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.
4. Mitrović M., Jović J., Cvrković T., Krstić O., Trkulja N., Toševski I. (2012): Characterisation of a 16SrII phytoplasma strain associated with bushy stunt of hawkweed oxtongue (*Picris hieracioides*) in south-eastern Serbia and the role of the leafhopper *Neolaiturus fenestratus* (Deltocephalinae) as a natural vector. *European Journal of Plant Pathology*, 134: 647–660.
5. Valenta V., Musil M., Mišiga S. (1961): Investigation on European yellows-type viruses. I. The stolbur virus. *Phytopathologische Zeitschrift*, 42: 1–38.
6. Brčak J. (1979): Leafhopper and planthopper vectors of plant disease agents in central and southern Europe. In: Maramorosch K, Harris K F, eds., *Leafhopper vectors and plant disease agents*. London, Academic Press: 97–146.
7. Riedle-Bauer M., Tiefenbrunner W., Otreba J., Hanak K., Schildberger B., Regner F. (2006): Epidemiological observations on Bois Noir in Austrian vineyards. *Mitteilungen Klosterneuburg*, 56: 177–181.
8. Fos A., Danet J.-L., Zreik L., Garnier M., Bove J.M. (1992): Use of a monoclonal antibody to detect the Stolbur mycoplasma-like organism in plants and insects and to identify a vector in France. *Plant Disease*, 76: 1092–1096.
9. Mikec I., Križanac I., Budinščak Ž., Musić M.Š., Krajačić M., Škorić D. (2006): Phytoplasmas and their potential vectors in vineyards of indigenous Croatian varieties. In: *Extended Abstracts 15th Meeting ICVG*. Stellenbosch, South Africa: 218–220. Available from <http://icvg.org/data/extabstr2006part1.pdf> (accessed Dec 7, 2017).
10. Galetto L., Marzachi C., Demichelis S., Bosco D. (2011): Host plant determines the phytoplasma transmission competence of *Empoasca decipiens* (Hemiptera: Cicadellidae). *Journal of Economic Entomology*, 104: 360–366.
11. Bosco D., Minucci C., Boccardo G., Conti M. (1997): Differential acquisition of chrysanthemum yellows phytoplasma by three leafhopper species. *Entomologia Experimentalis et Applicata*, 83: 219–224.
12. Palermo S., Arzone A., Bosco D. (2001): Vector-pathogen-host plant relationships of chrysanthemum yellows (CY) phytoplasma and the vector leafhoppers *Macrosteles quadripunctulatus* and *Euscelidius variegatus*. *Entomologia Experimentalis et Applicata*, 99: 347–354.
13. Laviña A., Sabaté J., Batlle A. (2006): Spread and transmission of Bois noir phytoplasma in two regions of Spain. In: *Extended Abstracts 15th Meeting ICVG*. Stellenbosch, South Africa. pp. 218–220. Available at <http://icvg.org/data/extabstr2006part2.pdf> (accessed Dec 7, 2017).
14. Sabaté J., Laviña A., Batlle A. (2003): Potential vectors of grapevine bois noir phytoplasma in Spain and evaluation of their transmission capacity. In: *Extended Abstracts 14th Meeting ICGV*. Locorotondo, Italy: 113. Available at <http://icvg.org/data/2003Session3.pdf> (accessed Dec 7, 2017).
15. Güclü S., Ozbek H. (1991): Studies on the vectors of MLOs caused stolbur disease of potatoes in Erzurum. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, 22: 35–42.
16. Batlle A., Altabella N., Sabate J., Lavina A. (2008): Study of the transmission of stolbur phytoplasma to different crop species, by *Macrosteles quadripunctulatus*. *Annals of Applied Biology*, 152: 235–242.

<https://doi.org/10.17221/8/2018-PPS>

17. Raccah B., Klein M. (1982): Transmission of safflower phyllody mollicute by *Neoliturus fenestratus*. *Phytopathology*, 72: 230–232.
18. Batlle A., Martinez M.A., Lavina A. (2000): Occurrence, distribution and epidemiology of grapevine yellows in Spain. *European Journal of Plant Pathology*, 106: 811–816.
19. Drobnjaković T., Perić P., Marčić D., Picciau L., Alma A., Mitrović J., Duduk B., Bertaccini A. (2010): Leafhoppers and Cixiids in phytoplasma-infected carrot fields: Species Composition and Potential. *Phytoplasma Vectors. Pesticides and Phytomedicine (Belgrade)*, 25: 311–318.
20. Maixner M. (1994): Transmission of German grapevine yellows (Vergilbungskrankheit) by the planthopper *Hyalesthes obsoletus* (Auchenorrhyncha: Cixiidae). *Vitis*, 33: 103–104.
21. 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.
22. Březíková M., Linhartová S. (2007): First report of potato stolbur phytoplasma in hemipterans in southern Moravia. *Plant Protection Science*, 43: 73–76.
23. Sanna F., Quaglino F., Filisetti S., Casati P., Faccincani M., Bianco P.A., Mori N. (2016): Preliminary results on putative vectors of ‘*Candidatus* Phytoplasma solani’ in bois noir-affected vineyards in Franciacorta (Lombardy region, North Italy). *Mitteilungen Klosterneuburg*, 66: 28–31.
24. Carraro L., Ferrini F., Martini M., Ermacora P., Loi N. (2008): A serious epidemic of stolbur on celery. *Journal of Plant Pathology*, 90: 131–135.
25. Posnette A.F., Ellenberger C.E. (1963): Further studies of green petal and other leafhopper-transmitted viruses infecting strawberry and clover. *Annals of Applied Biology*, 51: 69–83.
26. Alma A., Palermo S., Boccardo G., Conti M. (2001): Transmission of chrysanthemum yellows, a subgroup 16SrI-B phytoplasma, to grapevine by four leafhopper species. *Journal of Plant Pathology*, 83: 181–187.
27. Frazier N.W., Posnette A.F. (1956): Leafhopper transmission of clover virus causing green petal disease strawberry. *Nature*, 177: 1040–1041.

Table S2. Phytoplasma detection in insects captured in Perná vineyard

Classification	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							
<i>Aphrophoridae</i>	<i>Philaenus spumarius</i> (Linnaeus, 1758)	1	0/1		0		
<i>Cicadellidae</i>	<i>Agallia consobrina</i> Curtis, 1833	2	0/2		0		
	<i>Anaceratagallia ribauti</i> (Ossiannilsson, 1938)	102	26/102	3× AY-F 23× STOL	8	0/4	
	<i>Aphrodes bicincta</i> (Schrank, 1776)	3	nt		7	2/7	2× AY-F
	<i>Aphrodes makarovi</i> Zachvatkin, 1948	6	1/2	1× STOL	0		
	<i>Chlorita paolii</i> (Ossiannilsson, 1939)	123	0/24		8	nt	
	<i>Doratura homophyla</i> (Flor, 1861)	65	8/31	3× AY-F* 5× STOL*	71	nt	
	<i>Emelyanoviana mollicula</i> (Boheman, 1845)	213	0/24		12	nt	

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

<https://doi.org/10.17221/8/2018-PPS>

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

nt – not tested; STOL – ‘*Candidatus* Phytoplasma solani’; AY – ‘*Canadidatus* Phytoplasma asteris’, subgroups 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