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Survey of psyllid vectors of fruit tree phytoplasmas in Bulgaria: a preliminary report

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Abstract

The spread and the frequency of individuals from the psyllid genus *Cacopsylla* has been investigated in spring 2011 in four fruit tree orchards in three different regions of Bulgaria. Insects were captured and identified morphologically for species determination. Phytoplasma infection in psyllid samples was analysed by universal and specific PCR. All psyllid species described as vectors of fruit tree phytoplasmas were present in the investigated areas. Four individuals of *Cacopsylla pruni* from two different regions were carrying '*Candidatus* Phytoplasma prunorum', the agent of European stone fruit yellows (ESFY). This is the first ESFY detection in these regions in Bulgaria.

Key words: '*Candidatus* Phytoplasma prunorum', European stone fruit yellows, psyllid vectors, PCR detection.

Introduction

A few species of the psyllid genus *Cacopsylla* (Hemiptera Psyllidae) have been demonstrated to be vectors of European fruit tree phytoplasmas: '*Candidatus* Phytoplasma mali' associated with apple proliferation (AP), '*Candidatus* Phytoplasma prunorum', the agent of European stone fruit yellows (ESFY) and '*Candidatus* Phytoplasma pyri', the agent of pear decline (PD) (Seemüller and Schneider, 2004). Two psyllids, *Cacopsylla picta* (Foerster) and *Cacopsylla melanoneura* (Foerster) are recognised vectors of '*Ca.* P. mali'. The psyllid *Cacopsylla pruni* Scopoli was described as vector of '*Ca.* P. prunorum' whereas three psyllid species are recognised or presumed vectors of '*Ca.* P. pyri': *Cacopsylla pyri* (L.), *Cacopsylla pyricola* (Foerster) and *Cacopsylla pyrisuga* (Foerster) (reviewed by Jarausch and Jarausch, 2010).

ESFY and PD have been first detected in Bulgaria near Plovdiv (Topchiiska *et al*., 2000). But so far no report exists concerning cases of infected psyllid species in Bulgaria. AP has been reported to occur in Bulgaria since long time (www.eppo.org); however the *Cacopsylla* species vectoring fruit tree phytoplasmas were described in Bulgaria before they have been identified as phytoplasma vectors (Harizanov, 1966a; 1966b; 1982). However almost nothing is known about the incidence and the spread of these quarantine diseases in Bulgaria. Therefore, the aim of the present work was to gain first information on the spread and frequency of psyllid vector species and to determine their natural infection status in selected fruit tree orchards in Bulgaria.

Materials and methods

A survey was conducted in spring 2011 in four fruit tree orchards in three different regions in Bulgaria. Insects were caught using sweep-netting. Captured psyllids were frozen at −20°C and psyllid species identification was done using different determination keys (Hodkinson and White, 1979; Ossiannilsson, 1992; Burckhardt and Jarausch, 2007). DNA was extracted from single psyllid individuals with a CTAB-based protocol as described by Maixner *et al.* (1995).

PCR amplification of phytoplasma DNA was achieved with universal ribosomal primers fU5/P7 (Lorenz *et al.*, 1995; Schneider *et al.*, 1995). For specific PCR of positive *C. pruni*, ESFY-specific non-ribosomal primers ECA1/ECA2 were applied (Jarausch *et al.,* 1998).

Results and discussion

In the surveyed fruit tree orchards all known and putative psyllid vectors of fruit tree phytoplasmas were identified: *C. pruni, C. picta, C. melanoneura, C. pyri, C. pyrisuga* and *C. pyricola.* They were captured in three different region: Dupnica, Sofia (Gorni Lozen, Vrajdebna), and Plovdiv. (figure 1, table 1).

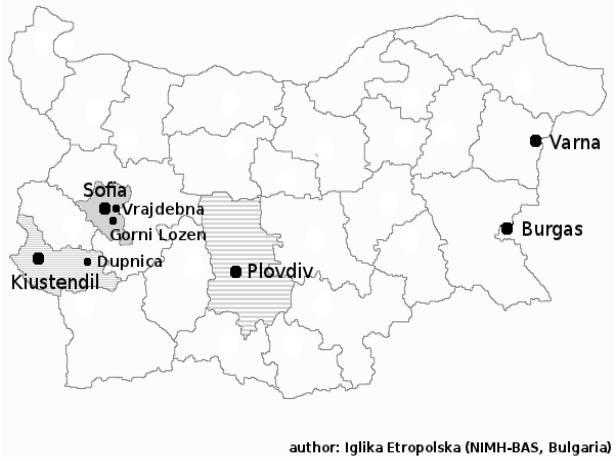

Figure 1. Location of fruit tree orchards surveyed in spring 2011 in Bulgaria.

Table 1. Number of individuals of *Cacopsylla* species captured in fruit tree orchards and results of phytoplasma detection by PCR in individual insects.

Psyllid species	Phytoplasma	Region			
		Dupnica	Gorni Lozen	Vrajdebna	Plovdiv
Cacopsylla pruni	'*Ca.* P. prunorum'	3 / 94 (3.2%)*	1 / 16 (6.3%)	nt	nt
Cacopsylla picta	'*Ca.* P. mali'	0 / 3	0 / 0	nt	0 / 1
Cacopsylla melanoneura	'*Ca.* P. mali'	0 / 23	0 / 1	nt	0 / 11
Cacopsylla pyri	'*Ca.* P. pyri'	0 / 0	0 / 0	0 / 181	nt
Cacopsylla pyricola	'*Ca.* P. pyri'	0 / 2	0 / 0	0 / 1	nt
Cacopsylla pyrisuga	'*Ca.* P. pyri'	0 / 84	0 / 6	0 / 1	nt

* PCR positive versus total number of individuals tested; nt = not tested.

In total, 440 individuals of the six *Cacopsylla* species were collected in the different fruit tree orchards and individually analysed for phytoplasma presence. Among the different known and putative vector species, only 4 individuals of *C. pruni* were found to be infected by phytoplasmas with universal ribosomal primers. The specific PCR revealed the presence of '*Ca.* P. prunorum' in all four phytoplasma-infected *C. pruni* insects. Interestingly, the infected specimens originated from two different collection sites. This is the first report of ESFY detection in these regions.

Discussion

Psyllid species described in Bulgaria by Harizanov (1966a, 1966b, 1982) were from different regions from those investigated in the present study. In all surveyed orchards an important number of psyllid vector species was found; the collected specimens showed variations in presence and abundance at the different sites. In two of the investigated pear orchards (Dupnica and Gorni Lozen, table 1) only *C. pyrisuga* and *C. pyricola* were present while at the location of Vragdebna *C. pyri* was the only pear psyllid species found. Despite abundances of *C. pyri* and *C. pyrisuga*, none of the individuals captured during this preliminary survey was phytoplasma infected. The detection of '*Ca.* P. prunorum' in its vector species in two different regions indicates a broader distribution of ESFY in Bulgaria as known so far. The future investigations will aim to monitor regularly insects and plants in these regions and other fruit tree growing areas in Bulgaria for possible phytoplasma infection.

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References

BURCKHARDT D., JARAUSCH W., 2007.- Pictorial key of central European *Cacopsylla* species associated with Rosaceae. [online] From URL: http://www.psyllidkey.eu.

HARIZANOV A., 1966a.- Chemical experiments for control of the psyllids in the fruit crops.- *Horticultural and Viticultural Science*, 3(2): 143-149.

HARIZANOV A., 1966b.- Biological studies *of Psylla costalis, Psylla pruni*, and *Psylla pyrisuga*.- *Research papers of Agricultural Institute* 'V. *Kolarov'*, 15(2): 1966.

HARIZANOV A., 1982.- The pear psyllas.- *Plant Protection Newsletter*, 4: 23-27.

HODKINSON I. D, WHITE I. M., 1979.- Homoptera: Psylloidea. *Handbook for the Identification of British Insects*, 2(5a): 1-98.

JARAUSCH B., JARAUSCH W., 2010.- Psyllid vectors and their control, pp 250-271. In: *Phytoplasmas: Genomes, Plant Hosts and Vectors* (WEINTRAUB P. G., JONES P., Eds).- CABI, Wallingford, UK..

JARAUSCH W., LANSAC M., SAILLARD C., BROQUAIRE J. M., DOSBA F., 1998.- PCR assay for specific detection of European stone fruit yellows phytoplasmas and its use for epidemiological studies in France.- *European Journal of Plant Pathology*, 104: 17-27.

LORENZ K.-H., SCHNEIDER B., AHRENS U., SEEMÜLLER E., 1995.- Detection of the apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and nonribosomal DNA.- *Phytopathology*, 85: 771-776.

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.

OSSIANNILSSON F., 1992.- The Psylloidea (Homoptera) of Fennoscandia and Denmark.- *Fauna Entomologica Scandinavica*, 29**:** 1-346.

SCHNEIDER B., SEEMÜLLER E., SMART C. D., KIRKPATRICK B. C., 1995.- Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas, pp. 369-380. In: *Molecular and Diagnostic Procedures in Mycoplasmology, Vol. I* (RAZIN S., TULLY, J. G., Eds).- Academic Press, San Diego, CA, USA.

SEEMÜLLER E., SCHNEIDER B., 2004.- '*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma pyri' and '*Candidatus* Phytoplasma prunorum', the causal agents of apple proliferation, pear decline and European stone fruit yellows, respectively.- *International Journal of Systematic and Evolutionary Microbiology*, 54: 1217-1226.

TOPCHIISKA, M., MARCONE, C., SEEMÜLLER, E., 2000.- Detection of pear decline and European stone fruit yellows in Bulgaria.- *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, 107: 658-663.

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