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Monitoring of psyllid species (Hemiptera, Psylloidea) in apple and pear orchards in East Bohemia

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Abstract

During the years 2009–2010, a monitoring of the psyllid species occurring in apple and pear orchards with different types of plantation management was carried out in orchards in East Bohemia. Species known as vectors of quarantine phytoplasmas '*Candidatus* Phytoplasma mali' and '*Candidatus* Phytoplasma pyri', were studied in priority. The infection with phytoplasmas was determined by amplification of DNA using polymerase chain reaction with subsequent restriction fragment length polymorphism analysis in selected individuals. Specimens of *Cacopsylla picta, C. pyricola, C. pyri, C. pyrisuga* were found to be phytoplasma infected with in the studied territories.

Key words: psyllid species, vectors of phytoplasmas, '*Candidatus* Phytoplasma mali', '*Candidatus* Phytoplasma pyri', PCR/RFLP.

Introduction

'*Candidatus* Phytoplasma mali' and '*Candidatus* Phytoplasma pyri' are associated with serious diseases in apple and pear growing areas. '*Ca.* P. mali' is the agent associated with apple proliferation (AP) and '*Ca.* P. pyri' is the agent associated with pear decline (PD) (Seemüller and Schneider, 2004). Psyllids of *Cacopsylla* genus (Hemiptera, Psylloidea) are vectors of these phytoplasmas. In Central and Southern Europe *Cacopsylla picta* (Foerster 1848) has been determined as a vector of AP, (Frisinghelli *et al*., 2000; Jarausch *et al*., 2003) whereas *C. melanoneura* (Foerster 1848) was only confirmed as vector of AP in northwestern Italy (Tedeschi *et al.*, 2002). PD is transmitted in Europe by *C. pyri* (Garcia-Chapa *et al.,* 2005), *C. pyrisuga* (Križanac *et al.*, 2008) and *C. pyricola* (Jensen *et al*., 1964).

The aim of this study was to monitor the occurrence of these known phytoplasma vector in apple and pear orchards with different types of plantation management: conventional, organic, integrated production and old deserted orchards under the climatic conditions in Czech Republic.

Materials and methods

The observation of occurrence of known AP and PD vector species was carried out in 4 apple plantations and 4 pear plantations with conventional, organic, integrated production and old deserted orchards in East Bohemia. The insects were collected with sweep-netting from March to September at every 2 weeks or more often. Insects were determined, numbered and then stored at -20°C in absolute ethanol for later identification. The infection of psyllid species with phytoplasmas was determined by PCR. Total DNA was extracted from two individuals of the same *Cacopsylla* species, using a commercial kit (Wizard Genomic DNA Purification Kit, Promega, USA). DNA products, diluted with sterile water in proportion 1: 10, was amplified by 35 cycles in a thermocycler (Techne). Nested PCR was carried out with the primers R16F2n/R2 (Gundersen and Lee, 1996) and fU5/rU3 (Lorenz *et al.*, 1995). Final products were submitted to RFLP analyses using *Rsa*I and *Bfm*I (Fermentas, Vilnius, Lithuania). The PCR and RFLP products were analyzed on 1.5% agarose gels in TBE buffer and stained with SYBR Green.

Results

In table 1 there are mentioned species of *Cacopsylla*, tested for the presence of phytoplasmas by PCR. Individuals of *C. mali*, *C. melanoneura* and *C. pyri* were tested in the year 2009. The presence of phytoplasma was found in the samples of *C. pyri*. Abundance of *C. pyri* (mainly in commercial and integrated orchards), *C. pyricola*, *C. pyrisuga*, *C. picta* and *C. mali* (mainly in organic and old deserted orchards) was observed, but only several individuals of *C. melanoneura* were collected during the growing season 2010. Owing to the low quantity of collected individuals, *C. melanoneura* has not been tested by PCR and testing of *C. mali* samples is in progress.

The comparison of abundance of psyllid species in apple and pear orchards with different types of plantation management during two growing seasons showed disparities within a single years as well as with regard to plantation management. In 2010, a high incidence of *C. pyri* in pear orchards, especially in integrated and commercial orchards, and of *C. mali* in apple orchards, mainly in organic and old deserted orchards was observed (figure 1).

Table 1. Results of phytoplasmas detection in *Cacopsylla* species by PCR in years 2009 and 2010.

Species/year of observation	Number of tested samples and results of PCR					
	2009			2010		
	total	positive	%	total	positive	%
Cacopsylla mali	45	0	0	0	0	0
Cacopsylla melanoneura	7	0	0	0	0	0
Cacopsylla picta	0	0	0	90	4	4.4
Cacopsylla pyri	454	1	0.2	512	10	2
Cacopsylla pyrisuga	0	0	0	47	1	2.1
Cacopsylla pyricola	0	0	0	17	1	5.9

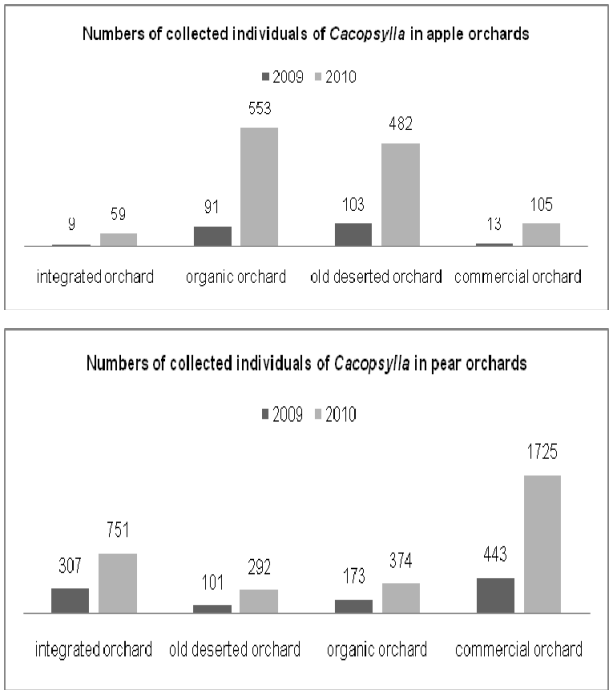

Figure 1. Numbers of collected individuals of *Cacopsylla* spp. in apple and pear orchards with different plantation management in years 2009 and 2010.

Discussion

C. pyri represents the big problem for the quality production in pear plantations with commercial and integrated plantation management. A huge amount of psyllid species, especially *C. mali*, occur in the organic and old deserted orchards. According to the PCR/RFLP examination of phytoplasma presence, *C. pyri, C. pyricola* and *C. pyrisuga* can be vectors of '*Ca.* P. pyri' in Czech Republic. Samples of *C. mali* collected in the year 2010 have not been tested yet, however, the presence of the AP in this vector was not described in other countries to this date (Jarausch *et al.*, 2003). Further work to verify the situation toward better disease management in orchards is in progress.

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