

Molecular tests to determine 'Candidatus Phytoplasma pyri' presence in psyllid vectors from a pear tree orchard in the Czech Republic – a preliminary report

Jana KUČEROVÁ¹, Luboš TALÁCKO¹, Pavel LAUTERER², Milan NAVRÁTIL³, Renata FIALOVÁ³

¹Research and Breeding Institute of Pomology Holovousy, Hořice, Czech Republic

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

³Department of Cell Biology and Genetics, Faculty of Science, Palacky University in Olomouc, Olomouc-Holice, Czech Republic

Abstract

The frequency of individuals of the psyllid species *Cacopsylla pyri*, *Cacopsylla pyrisuga* and *Cacopsylla pyricola* infected with 'Candidatus Phytoplasma pyri' has been monitored in a pear tree orchard managed according to integrated pear production in eastern Bohemia (Czech Republic). The insects were captured on pear trees in two to four weeks intervals during the vegetative season in 2006. Phytoplasmas in psyllid samples were detected by PCR/RFLP. *C. pyri*, *C. pyrisuga* and *C. pyricola* were identified in the surveyed orchard, but only a low portion of these psyllid species was infected with the phytoplasma.

Key words: Pear decline, *Cacopsylla pyri*, *Cacopsylla pyrisuga*, *Cacopsylla pyricola*, PCR.

Introduction

Pear decline phytoplasma, newly denominated as 'Candidatus Phytoplasma pyri' (Seemüller and Schneider, 2004), belongs to the apple proliferation group and causes serious diseases in pear crops. Psyllids seem to play a crucial role in the transmission of the agent of this disease. Recent studies demonstrated the important role of *Cacopsylla* species in the epidemiology of pear decline disease. The disease is spread by *Cacopsylla pyri* (L.) (Garcia-Chapa *et al.*, 2005) and *Cacopsylla pyricola* (Foerster) (Davies *et al.*, 1992). The frequency of psyllids infected with the phytoplasma has been studied in a pear tree orchard in the Czech Republic. Phytoplasmas in psyllid samples were detected by PCR/RFLP. This is a preliminary report because the survey will continue.

Materials and methods

Studies on the occurrence of potential psyllid vectors of 'Ca. P. pyri' have been conducted in a pear tree orchard managed according to the rules of integrated pear production in eastern Bohemia. The 6% of the trees in this orchard were infected with 'Ca. P. pyri'. The insects were captured by sweep-netting on pear trees in two or four-weeks intervals during the vegetative season in 2006. Insects were identified, numbered and then stored at -20 °C in absolute ethanol until DNA extraction. All individuals of *C. pyri*, *Cacopsylla pyrisuga* (Foerster) and *C. pyricola* were subjected to molecular assays to determine the presence of phytoplasmas.

The phytoplasma detection was done by PCR. The extracts of total DNA from single specimens of *C. pyri*, *C. pyricola*, and *C. pyrisuga* were obtained using a commercial kit (Wizard Genomic DNA Purification Kit, Promega, USA). Products were diluted (1: 10) with ster-

ile deionised water. The DNA was amplified by 35 cycles in a thermocycler (Techne). PCR products were diluted with sterile distilled water (1: 39) prior to amplification by nested-PCR using R16F2/R2 (Gundersen and Lee, 1996) and fU5/rU3 (Lorenz *et al.*, 1995) primer pairs. Final R16F2/R2 amplicons (10 µl) were digested with *RsaI* and *BfmI* (Fermentas, Lithuania) 16 hours at 37 °C. The digests were mixed with SYBRGreen I before electrophoresis for visualization under UV light and were run on 3% agarose gels in TBE buffer.

Results

C. pyri, *C. pyrisuga* and *C. pyricola* were identified in the surveyed orchard (table 1). The overwintering generation of all monitored psyllid species was present in the orchard in April. In contrast to *C. pyrisuga* and *C. pyricola*, new generation of *C. pyri* was observed from the end of May until beginning of August. It seems that the new generation of *C. pyrisuga* and *C. pyricola* was more affected and decimated by insecticide treatments than the new adults of *C. pyri*.

Table 1. Number of captured psyllids in surveyed pear tree orchard during the vegetative season of 2006.

Date	<i>C. pyri</i>	<i>C. pyricola</i>	<i>C. pyrisuga</i>
07.04	28	3	61
19.04	5	3	176
16.05	0	0	2
29.05	18	0	0
20.06	18	2	0
04.07	7	0	0
18.07	80	0	0
01.08	41	1	0

Table 2. Portion of psyllids infected with ‘*Ca. P. pyri*’ captured in the surveyed pear tree orchard during the vegetative season of 2006. (nt = not tested).

Species	Overwintering generation tested/positive	New generation tested/positive
<i>C. pyrisuga</i>	132 / 5 (3.8 %)	nt / nt
<i>C. pyri</i>	nt / nt	76 / 1 (1.3 %)

Results of PCR/RFLP tests of randomly selected individuals of putative pear psyllid vectors from the surveyed orchard are summarised in table 2. Infection with ‘*Ca. P. pyri*’ was found in the overwintering generation of *C. pyrisuga* and in the new generation *C. pyri*, respectively.

Discussion

C. pyri, *C. pyrisuga* and *C. pyricola* were identified in the surveyed orchard, but with a low infection rate with ‘*Ca. P. pyri*’. *C. pyri* is the most common psyllid in Spanish pear-tree orchards as reported by Avinent *et al.* (1997). Phytoplasmas were detected by PCR in eight out of 33 psyllid samples (24%).

The results of first years of our survey do not confirm results by Avinent *et al.* (1997).

Davies *et al.* (1995) identified *C. pyricola* as main vector for ‘*Ca. P. pyri*’. Because of the low population of the monitored psyllid species during the first year of our survey, individuals were not tested by PCR.

The highest number of individuals was found in the overwintering generation of *C. pyrisuga*. By literature it has not been clearly demonstrated that the disease is transmitted by *C. pyrisuga*. ‘*Ca. P. pyri*’ was detected by PCR in five out of 132 psyllid samples in our samples of this species.

In the matter of phytoplasma transmission, *C. pyrisuga* seems to be unimportant in pear tree orchards managed according to integrated pear production in the Czech conditions on the basis of results so far obtained.

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Corresponding author: Jana KUČEROVÁ (e-mail: kucerova@vsuo.cz), Research and Breeding Institute od Pomology Holovously, 508 01 Hořice, Czech Republic.