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´*Candidatus* Phytoplasma prunorum´: the phytoplasma infection of *Cacopsylla pruni* from apricot orchards and from overwintering habitats in Moravia (Czech Republic)

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Abstract

The incidence of individuals of *Cacopsylla pruni* infected with ´*Candidatus* Phytoplasma prunorum´ was analysed by PCR/RFLP in single insects captured during a growing season in selected apricot plantations in southern Moravia (Czech Republic). A portion of adults of both overwintering and new generation of the psyllid was determined as positive for the phytoplasma presence. It was found that conifers growing in the investigated hilly sites of middle Moravia harbour *C. pruni* during the winter season. The presence of the phytoplasma positive individuals of the species was determined in 3 of 4 batches of specimens from overwintering sites.

Key words: conifers, psyllid, vector.

Introduction

´*Candidatus* Phytoplasma prunorum´ (formerly European stone fruit yellows phytoplasma) is one of the most important fruit tree phytoplasmas causing serious damages in cultivated *Prunus* species in Europe. In the Czech Republic the pathogen occurs in warmer habitats in southern Moravia (Navrátil *et al.*, 1998) that constitutes a considerable apricot and peach production area within the country.

The psyllid *Cacopsylla pruni* Scopoli was described as vector of ´*Candidatus* Phytoplasma prunorum´ by Carraro *et al.* (1998, 2001). The species is abundant and widely distributed over the Moravian region. *C. pruni* is narrowly oligophagous on *Prunus* species, whereas wild *Prunus* plants, mainly blackthorn (*P. spinosa*), are its preferred host plants (Lauterer, 1999). The psyllid is monovoltine and hibernates at adult stage on shelter plants, mainly conifers.

The aim of the work was to determine the occurrence of *C. pruni* inside selected apricot plantations and the incidence of individuals positive for ´*Ca*. P. prunorum´. A further objective was to assess the presence and the phytoplasma infection of the psyllids from overwintering habitats.

Materials and methods

Insects were caught using sweep-netting during vegetation season of 2006 every two weeks between beginning of April and mid-July in apricot trees in three sites located in the southern Moravia region: in two experimental apricot plantations (designated as L-g and L-m) in Lednice location (altitude 176 m a.s.l.) and in a commercial apricot orchard in Velké Němčice (VN) location (altitude 184 m a.s.l.). The sample collection unit is defined as number of individuals of a species obtained by sweep-netting of 45 trees. After identification, adults of *C. pruni* were stored in absolute ethanol (–20°C) until tested for determining phytoplasma presence. Other captures of hemipteran insects were performed during the winter season monthly from November 2006 to February 2007 in middle Moravia region on conifers (*Picea*) in hilly sites of Drahanská vrchovina Highland (altitude between 480 and 530 m a.s.l.). The adults of *C. pruni* were stored in absolute ethanol (–20°C) until tested.

The extracts of total DNA from single psyllid individuals were obtained using a commercial kit (Wizard Genomic DNA Purification Kit, Promega, USA). The detection of phytoplasmas in single individuals of *C. pruni* was performed using nested-PCR with universal primers R16F1/R0 and R16F2/R2 (Lee *et al*., 1995) derived from 16S rDNA. Amplification products obtained with primers R16F2/R2 were subjected to digestion with *Rsa*I, *Bfm*I, and *AluI* (Fermentas, Lithuania) restriction endonucleases.

Results

The species *C. pruni* occurred in observed apricot orchards from the beginning of April till the end of June 2006. The numbers of psyllids caught on apricots during the growing season 2006 are reported in table 1. The psyllids were more abundant inside both apricot plantations in Lednice location, with the peak in the numbers of captured individuals from mid-April to beginning of May. As regards with the new generation of *C. pruni* inside the location, maximal numbers were collected at the end of June. Very low numbers of *C. pruni* were collected in the apricot orchard in Velké Němčice location. The results of phytoplasma detection in the individuals of *C. pruni* captured in apricot trees during the growing season are shown in table 1.

Table 1. Results of detection of ´*Ca*. P. prunorum´ in *C. pruni* caught on apricots during the growing season 2006 (number of phytoplasma positive individuals/number of captured individuals).

Caught date	Location		
	VN	L-g	L-m
April 3	0/1 (0.0%)	0	4/12 (33.3%)
April 18	0/7 (0.0%)	14/64 (22.9%)	9/22 (26.3%)
May 2	0/4 (0.0%)	10/38 (26.3%)	6/22 (27.3%)
May 16	0	0/4 (0.0%)	0
May 29	0	1/3 (33.3%)	0/3 (0.0%)
June 13	0	0	0/11 (0.0%)
June 26	0/1* (0.0%)	1/13* (7.7%)	20/67* (29.9%)
July 11	0	0	0

* new generation

Table 2. Detection of ´*Ca*. P. prunorum´ in *C. pruni* caught on conifers during the winter season.

Collection date	Phytoplasma positive (%)	Tested
November 25, 2006	4 (10.0)	40
December 26, 2006	4 (13.3)	30
January 7, 2007	0 (0.0)	10
February 10, 2007	1 (1.3)	40

Individuals of *C. pruni* were regularly collected on conifers at the investigated hilly sites from November 2006 to February 2007. The presence of ´*Ca*. P. prunorum´ positive individuals of *C. pruni* was found in 3 out of 4 batches tested (see table 2).

Discussion

In France, Yvon *et al*. (2004) reported detection of two ESFY phytoplasma positive individuals of *C. pruni* from natural populations overwintering on conifers. We found phytoplasma positive individuals of *C. pruni* among adults captured in the winter season on *Picea* trees growing in elevations of the Drahanská vrchovina Highland. In view of the relatively high numbers of *C. pruni* adults and other members of the genus collected during the winter period from the sites, we can consider the location as an important overwintering site for psyllids in the Czech Republic conditions. It must be mentioned that the location is situated far (several tens of kilometres) from the main area of stone fruit tree production. However, it is proposed that psyllids can migrate for a long distance. Concordantly, overwintering sites of *C. pruni* have been referred to be located in mountainous sites far away from the area with *Prunus* orchards (Yvon *et al*., 2004). The authors still remarked that *C. pruni* has not been found to overwinter on conifers in the lower altitude area with a blackthorn hedge. Likewise, Poggi Pollini *et al*. (2004) in Italy collected no specimens of the species during the winter captures on conifers surrounding the areas where the disease has been found in recent years.

Concerning phytoplasma infection of the species captured in the studied apricot plantations, our results show that individuals of *C. pruni* positive for ´*Ca*. P. prunorum´ are present in the overwintering as well as in the new generation. These results fit with the findings of other studies that both generations are infected but some authors (Thébaud *et al.*, 2007) considered the overwintering generation as more important in the phytoplasma transmission.

In conclusion, our findings indicate that in the Czech conditions the psyllid *C. pruni* can act as a reservoir of ´*Ca*. P. prunorum´ during the growing season as well as during the winter.

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