

# **Epidemiology of European Stone Fruit Yellows Phytoplasma in the Czech Republic**

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## **Abstract**

**Apricot chlorotic leaf roll (ACLR), associated with European stone fruit yellows (ESFY) phytoplasma, is one of the most important decline diseases of apricots in southern and central Europe. The paper surveys results of ESFY phytoplasma studies carried out during the period 1993–2003 in the Czech Republic. The special emphasis was devoted to apricots (*Prunus armeniaca*) as the most frequent and economically important host species of the phytoplasma. All of main apricot cultivars grown in the Czech Republic were found infected with ESFY phytoplasma. The ESFY phytoplasma was also identified in other hosts: *Prunus persica*, *P. domestica*, *P. salicina* × *cerasifera*, *P. amygdalus* × *cerasifera*, *P. spinosa*, *Cerasus avium*, and *C. vulgaris*. Psyllid *Cacopsylla pruni* was confirmed as a vector of ESFY phytoplasma. It was demonstrated that *C. pruni* is able to accomplish the regular development on three species of host plants: *Prunus armeniaca*, *P. persica*, and *P. spinosa*.**

## **INTRODUCTION**

Apricot chlorotic leaf roll - ACLR (Morvan, 1977), associated with *European stone fruit yellows* (ESFY) phytoplasma (Lorenz et al., 1995), is one of the most serious important decline diseases of apricots in southern and central Europe, including the Czech Republic. Leaf yellowing and roll, sparse foliage, and decline are considered as characteristic symptoms of the disease. Recently, a new period of the disease study has been started with identifying of ESFY phytoplasma specific vector psylla *Cacopsylla pruni* in Italy (Carraro et al., 1998). Subsequently, few studies concerning with epidemiological aspects of ESFY were conducted in France (Jarausch et al., 1998, 2001a, b) and in Italy (Carraro et al., 2001).

An occurrence of apricot and other *Prunus* trees showing symptoms attributable to phytoplasmas in the Czech Republic indicates that the disease may became a problem in our fruit tree growing areas (Blatný, 1977; Navrátil et al., 1998). The aim of the work was to contribute to a knowledge of the disease status in the Czech Republic studying: the ESFY phytoplasma host range with attention to cultured *Prunus* species, occurrence and population dynamics of potential phytoplasma vectors, with main emphasis to phenology and natural infectivity of *Cacopsylla pruni*.

## MATERIAL AND METHODS

### Plants

To identify incidence and plant host range of ESFY phytoplasma visual symptom observations were conducted in apricot and peach orchards, experimental plantations, and private gardens in growing area of south Moravia (Czech Republic) between 1996 and 2002. Twigs from stone fruit trees (*Prunus armeniaca*, *P. persica*, *P. domestica*, *P. spinosa*, *P. salicina* × *cerasifera*, *P. amygdalus* × *cerasifera*, *Cerasus avium*, and *C. vulgaris*) exhibiting yellows, leaf rolling or decline symptoms and from non-symptomatic ones were cut for phytoplasma tests during the vegetative seasons.

### Insects

The catching of psyllids was carried out from March to May between 2000 and 2003 in three localities of south Moravia: Lednice (an experimental plantation with apricot and peach trees), Velké Bílovice (a commercial apricot orchard with *Prunus spinosa* and *Crataegus* sp. in a neighbourhood), and Bulhary (*P. spinosa* at the edge of the forest complex, 15 km far from the nearest apricot orchard). Population density was estimated as frequency of individuals caught on 50 branches using limb-jarring Steiner. Adults of *Cacopsylla pruni* were subjected to phytoplasma detection. To study the development of *C. pruni*, adults captured in nature were introduced into isolators on branches of *P. armeniaca*, *P. spinosa*, and *P. persica*. In a case, adults of the species caught in Lednice locality in 2002 were reared in the isolator on an ESFY positive apricot branch.

### Detection and Identification of Phytoplasmas

The total plant DNA was extracted from axial phloem preparations using the phytoplasma enrichment procedure according to Ahrens and Seemüller (1992). The DNA extracts from individuals of *C. pruni* were obtained using commercial kit (Wizard Genomic DNA Purification Kit, Promega, USA).

DNA extracts from both plants and insects were subjected to PCR analysis with phytoplasma universal primer pairs derived from 16S rRNA gene. Nested-PCR assays were performed with primer pair R16F1/R0, R16F2/R2 (Lee et al., 1995), and fU5/rU3 (Lorenz et al., 1995). DNA preparations from plants of *Vinca rosea* infected with AT isolate of apple proliferation phytoplasma served as a positive control. Tubes with reaction mixture devoid of DNA templates were included as a negative control. PCR products were analysed by electrophoresis through a 1.5% agarose gel, stained with ethidium bromide and visualized with an UV transilluminator.

Identification of phytoplasmas was carried out by RFLP analyses of R16F2/R2 amplicons. Fifteen µl of the products were digested separately with *Rsa*I, *Bfm*I, and *Alu*I (Fermentas, Lithuania) restriction endonucleases according to the instruction of the manufacturer. The restriction products were separated by electrophoresis through 3% MetaPhor agarose (FMC, USA) gel, stained with ethidium bromide and visualized with an UV transilluminator.

### Sequencing

PCR products covering P1/P7 (Schneider et al., 1995) segment of 16S rRNA gene from an apricot (ESFY-CZ isolate) were sequenced on automated DNA ABI PRISM 310 sequencer (Perkin Elmer Applied Biosystems, Lincoln, USA). Sequence data were read and analysed by DNASTAR programme package (Lasergene, USA). Multiple sequence alignment was performed using the www service ClustalW (<http://www.ddbj.nig.ac.jp>) and tree was drawn with the TreeView 1.6.6 software.

## RESULTS AND DISCUSSION

PCR detection confirmed the presence of ESFY phytoplasma in all tested stone fruit species from monitored growing area. From an economical point of view, apricots

were of the main interest in the testing. All of main apricot cultivars grown in the Czech Republic ('Velkopavlovická', 'Bergeron', 'Maďarská', 'Karola', 'Veecot') were found affected with the phytoplasma. However, the proportion of infected apricot trees per analysed plots varied from 0 to 35% (rarely reached 82%). Samples from apricot trees with typical ACLR symptoms usually gave positive reaction. In addition, positive tests were obtained in samples from trees without visual symptoms in all mentioned cultivars. It is noteworthy that phytoplasma affected apricot trees with strong symptoms do not usually live more than one year. The situation of the relatively high level of ESFY phytoplasma infection within apricots seems to be similar to this in other European countries as in France (Jarausch et al., 1998, 2001a) or in Austria (Richter, 2002).

Peach trees ('Redhaven', 'Cresthaven', 'Fairhaven') were found less affected by the ESFY phytoplasma, when proportion of infested trees in plots varied from 0 to 12%.

A sequence of a representative phytoplasma isolate from an apricot tree showing typical symptoms of ACLR was deposited in the GenBank database under accession number Y11933. The dendrogram (Fig. 1) shows the phylogenetic position of the isolate (ESFY-CZ) within the apple proliferation phytoplasma group (16SrX). The detailed sequence comparison revealed the close relationship with the ESFY phytoplasma (X68374) isolated from peach tree in Germany and '*Candidatus* Phytoplasma prunorum' (AY029540).

*Cacopsylla pruni* and *C. melanoneura* were dominant psyllid species in the localities pursued during the monitoring of potential vectors of phytoplasma. *Cacopsylla pyrisuga*, *C. saliceti*, *C. crataegi*, *C. affinis*, *Stictocephala bisonia*, *Allygus mixtus*, and *Dictyophara europea* were sporadically found. No individual of *Fieberiella* sp. was recorded.

Data concerning with population dynamics of *C. pruni* are given in Table 1. Population density of the species can be considered high in the area studied in spite of differences among localities.

Phenological aspects of *C. pruni* in the area were followed. The species has one generation per year. The over-wintering adults occur from the half of March till the beginning of June on apricots, rarely on peaches. In March-April period adults suck and from the beginning of May they lay eggs during three-four next weeks. Larvae occur from May till the half of June, when the adults of new generation appear. They pass aestivation, following by hibernation.

*Cacopsylla pruni* prefers black thorn (*Prunus spinosa*) for its development. It migrates from apricot orchards to reach the host during April. A smaller part of over-wintering generation of *C. pruni* remains within orchard, where the laying eggs and the development of the new generation run. During summer the adults of *C. pruni* colonize conifers to over-winter. Preliminary observations indicate that another plant host on which they can over-winter probably exists. We proved experimentally that *C. pruni* is able to develop on three species of host plants: black thorn - the most important one, apricot tree, and peach tree.

Results of phytoplasma detection and identification in *C. pruni* adults captured from south Moravia sites during season of 2002 are surveyed in Table 2. The presence of ESFY phytoplasma was proved in individuals from all localities. Moreover, some adults were found to carry phytoplasmas belonging to the Aster yellows group. Two *C. pruni* individuals from Velké Bílovice locality were positive for the presence of apple proliferation phytoplasma. However, no case of mixed infection was found out.

In adults of new generation of *C. pruni* that developed on ESFY positive apricot tree high incidence (93%) of ESFY phytoplasma positive individuals was found out compared to 13% infectivity determined in the natural population introduced into the isolator.

ESFY infected individuals of the species were identified in samples captured on apricots and black thorns. Simultaneously, black thorn was detected as symptom-less carrier of the phytoplasma in the study. Black thorn seems be important for a persistence and spread of the ESFY phytoplasma in the Czech Republic. Likewise, a role of wild

*Prunus* species in epidemiology of ESFY and other phytoplasmas was demonstrated by Jarausch et al. (2001a).

In view of the economical importance of ACLR, further investigations concerning epidemiological aspects of the disease are necessary to conduct including control of wild phytoplasma resources in the vicinity of orchards and monitoring of an occurrence and an activity of vectors.

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## Tables

Table 1. The estimate of population density of *Cacopsylla pruni* in sampling sites of south Moravia. Number of adults caught on 50 branches using limb-jarring Steiner is expressed.

Locality	2000	2001	2002	2003
	March - April	March - April	March - April	March - April
Lednice	3 - 4	2 - 4	1 - 2	2 - 3
Velké Bílovice	1 - 2	1 - 2	0.2	0.1
Bulhary	nt	6 - 10	4 - 6	6 - 8

Table 2. Natural infectivity of *C. pruni* adults collected in south Moravia sites in 2002.

Locality	Collection date	No. of tested individuals	No. of positive individuals (%)	Phytoplasma identity ESFY / AY / AP
Lednice	March 28	15	5 (33.3)	2 / 3 / 0
Velké	March 28	14	9 (64.3)	3 / 5 / 1
Bílovice	April 17	54	18 (33.3)	15 / 2 / 1
Bulhary	March 28	17	2 (11.8)	2 / 0 / 0
	April 17	57	8 (14.0)	7 / 1 / 0
Total	-	157	42 (26.7)	29 / 11 / 2

## Figures

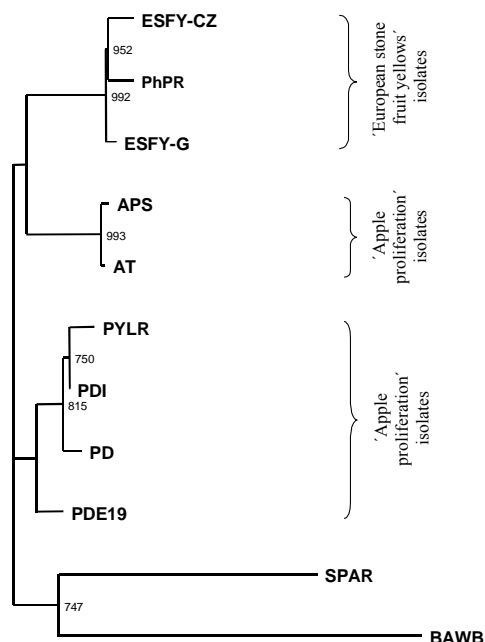


Fig. 1. Phylogenetic dendrogram of apple proliferation group phytoplasma isolates. The scale bar represents a phylogenetic distance of 1%. The numbers on the branches are bootstrap values. Phytoplasmas and GenBank accession are as follows: ESFY-CZ: ESFY/Czech Republic (Y11933), PhPR: *Candidatus* Phytoplasma prunorum (AY029540), ESFY-G: ESFY/Germany (X68374), APS: *Candidatus* Phytoplasma mali (X76426), AT: apple proliferation (X68373), PYLR: peach yellow leaf roll (Y16394), PDI: pear decline/Italy (Y16392), PD: pear decline/Germany (X76425), PDE19: *Candidatus* Phytoplasma pyri (AF319552), SPAR: *Spartium* witches'-broom (X92869), BAWB: buckthorn witches'-broom (X76431).