PROBLEMS OF EUROPEAN STONE FRUIT YELLOWS PHYTOPLASMA IN THE CZECH REPUBLIC

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Summary

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. The ESFY phytoplasma was identified in hosts: *Prunus persica*, *P. domestica*, *P. salicina* x *cerasifera*, *P. amygdalus* x *cerasifera*, *P. spinosa*, *Cerasus avium*, and *C. vulgaris*. Psyllid *Cacopsylla pruni* was confirmed as a vector of ESFY phytoplasma.

Full text of the paper will be published in Acta Horticulturae (2004).

Key words: p*runus* sp., Apricot chlorotic leaf roll, *Cacopsylla pruni*, vector

Introduction

Apricot chlorotic leaf roll - ACLR (Morvan, 1977), associated with European stone fruit yellows (ESFY) phytoplasma (Lorenz et al., 1994), 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).
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 (Blattný, 1977; Navrátil et al., 1998).

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). Twigs from stone fruit trees (*Prunus armeniaca*, *P. persica*, *P. domestica*, *P. spinosa*, *P. salicina* x *cerasifera*, *P. amygdalus* x *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.
Insect. The catching 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.*
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).
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). 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 amplimers by *Rsa*I, *Bfm*I, and *Alu*I (Fermentas) restriction endonucleases. 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). 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. However, the proportion of infected apricot trees per analysed plots varied from 0 to 35%. 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 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 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. Population density of the *C. pruni* and *C. melanoneura* 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 black thorns, 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. 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 confirm the presence of ESFY phytoplasma. Moreover, some adults were found to carry phytoplasmas belonging to the Aster yellows group. Two *C. pruni* individuals were positive for the presence of apple proliferation phytoplasma.
In adults of new generation of *C. pruni* that developed on ESFY positive apricot tree high incidence (93%) of ESFY phytolasma 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 (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.

Acknowledgement

The research was supported by the grant of Ministry of Agriculture of the Czech Republic No. QD1048.

References

AHRENS, U., SEEMÜLLER, E. 1992. Detection of DNA of plant pathogenic mycoplasmalike organisms by a polymerase chain reaction that amplifies a sequence of the 16S rRNA gene. Phytopathology 82:828-832.
BLATTNÝ, C. 1977. Syndrome of apricot witches´broom. Biologia Plant. (Prague) 19:313-315.
CARRARO, L., OSLER, R., LOI, N., ERMACORA, P. and REFATTI, E. 1998. Transmission of European stone fruit yellows phytoplasma by *Cacopsylla pruni*. Journal of Plant Pathology 80(3):233-239.
CARRARO, L., OSLER, R., LOI, N., ERMACORA, P., and REFATTI, E. 2001. Fruit tree phytoplasma diseases diffused in nature by psyllids. Acta Horticulturae 550:345-349.
JARAUSCH, W., JARAUSCH-WERHEIM, B., DANET, J.L., BROQUAIRE, J.M., DOSBA, F., SAILLARD, C. and GARNIER, M. 2001a. Detection and identification of European stone fruit yellows and other phytoplasmas in wild plants

in the surroundings of apricot chlorotic leaf roll-affected orchards in southern France. European Journal of Plant Pathology 107:209-217.
JARAUSCH, W., DANET, J.L., LABONNE, G., DOSBA, F., BROQUAIRE, J.M., SAILLARD, C. and GARNIER, M. 2001b. Mapping the spread of apricot chlorotic leaf roll (ACLR) in southern France and implication of *Cacopsylla pruni* as a vector of European stone fruit yellows (ESFY) phytoplasmas. Plant Pathology 50:782-790.
JARAUSCH, W., LANSAC, M., SAILLARD, C., BROQUAIRE, J.M. and 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.
LEE, I.-M., BERTACCINI, A., VIBIO, M. AND GUNDERSEN, D.E. 1995. Detection of multiple phytoplasmas in perennial fruit trees with decline symptoms in Italy. Phytopathology 85:728-735.
LORENZ, K.H., SCHNEIDER, B., AHRENS, U. AND SEEMÜLLER, E. 1995. Detection of the apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and nonribosomal DNA. Phytopathology 85:771-776.
MORVAN, G. 1977. Apricot chlorotic leaf roll. EPPO Bull. 7 (1):37-55.
NAVRÁTIL, M., VÁLOVÁ, P., FIALOVÁ, R., FRÁNOVÁ, J., VORÁČKOVÁ, Z. and KAREŠOVÁ, R. 1998. Occurrence of fruit tree phytoplasmas in the Czech Republic. Acta Horticulturae 472:649-654.
RICHTER, S. 2002. Susceptibility of Austrian apricot and peach cultivars to ESFY. Plant Protection Science 38 (Special Issue 2):281-284.
SCHNEIDER, B., SEEMÜLLER, E., SMART, C.D., AND KIRKPATRICK, B.C. 1995. Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas. p. 369-380. In: S. Razin and J.G. Tully (eds.), Molecular diagnostic procedures in mycoplasmology. San Diego, CA: Academic Press.