

# Problems in detection of European stone fruit yellows phytoplasma in apricot trees in the Czech Republic

Jaroslav POLÁK, Jaroslav SALAVA, Marcela BRYXIOVÁ, Jiří SVOBODA

Department of Virology, Division of Plant Health, Crop Research Institute, Prague, Czech Republic

## Abstract

Collection of thirty eight trees of four apricot cultivars grew in orchard where ESFY symptoms appeared was evaluated during 2004-2007 years. The presence of ESFY was evaluated by inspection of symptoms, PCR detection, and indexing on the peach indicator GF-305. Ten trees showed typical ESFY symptoms during the period of evaluation.

Typical symptoms of ESFY, such as leaf yellowing and rolling, little leaves and sparse foliage are a reliable diagnostic criterion for this diseases. Apricot trees with ESFY symptoms died out either within one year, or during the next vegetation period. Twenty five trees were without symptoms in the years 2004-2007, from which two trees tested positive by nested PCR in four years, two trees were positive in three years, four trees in two years, seven trees in one year only, and ten trees were negative during the period 2004-2007. Results of biological indexing of twenty five symptomless apricot trees were negative in 2004-2007. Nine apricot trees died of ESFY, and three of apoplexy; symptoms on one tree disappeared; and 25 trees remained without any symptoms after four years of evaluation of 38 apricot trees. The biological indexing on GF-305 is not reliable enough, as well as nested PCR.

**Key words:** phytoplasma; European stone fruit yellows; detection; evaluation of symptoms; biological indexing; nested PCR.

## Introduction

The presence of European stone fruit yellows (ESFY) phytoplasma was confirmed in the Czech Republic in 1990s (Navrátil *et al.*, 1998). The presence of ESFY was detected by PCR and fluorescence microscopy and all the phytoplasma infected apricot trees died within two years after appearance of symptoms. Phytoplasmal infection was verified only in symptomatic apricot trees (Navrátil *et al.*, 2001).

In 2002 ESFY phytoplasma was detected in symptomless apricot trees from an orchard in Southern Moravia. The trees never showed symptoms of phytoplasma infection and they were not dying. Results of nested PCR analysis were compared with the biological indexing on GF 305. Results of biological indexing were negative. The results of nested PCR were completely different from the biological test (Polák *et al.*, 2006). Therefore, another experiment was established in apricot orchard in 2004 where typical symptoms of ESFY were observed. Visual evaluation of symptoms, PCR testing and biological indexing were used for detection of ESFY phytoplasma.

## Materials and methods

### Plant material, symptom evaluation, sampling

The experiment was established in an apricot orchard of the SEVA-FLORA Valtice Company in Úvaly in 2004. A cluster of 38 trees of apricot cultivars Velkopavlovická, Goldrich, Bergeron and Bocucia Liscia was selected. Eight trees showed ESFY symptoms and 30 were without symptoms in spring 2004. Visual evaluation of symptoms was performed four times during vegetation period from June till October in the years 2004-2007.

Two-year shoots were collected from tested trees in

November. The first part of shoots was used for the detection of ESFY by nested PCR, the second part for biological indexing.

### Molecular analyses

The detection of ESFY by nested PCR was carried out immediately after collecting samples. All trees were tested by nested PCR in every year 2004-2007. The first step of nested PCR was carried out with the primers R16F1/R16R0 (Lee *et al.*, 1995). The second step of nested PCR was carried out with two different pairs of primers, R16F2/R16R2 and fU5/rU3I (Lorenz *et al.*, 1995). The protocol according to Navrátil (modified protocol of Ahrens and Seemüller, 1992) was used for DNA isolation.

### Biological indexing

Shoots sampled in November were stored at 4 °C. Buds taken from sampled shoots of tested tree were grafted in March on the wooden indicator GF-305 in 3 replicates for each tested tree. Symptoms on the indicator plants were evaluated for the first time one month after the chip-budding and then in the interval of two weeks, and after three months on four weeks till the end of November.

## Results

Of 38 trees of apricot, cvs. Velkopavlovická, Goldrich, Bergeron and Boccuccia Liscia ten trees showed typical symptoms of ESFY phytoplasma: leaf yellowing and rolling, smaller leaves and sparse foliage. No differences in type of symptoms were observed among different cultivars. Six trees with ESFY symptoms died out within several months in 2004, so biological indexing was not possible. Two symptomatic trees died in 2005, results of biological testing were negative. However

ESFY was detected by nested PCR only in three trees, results of detection in five trees were negative. In two cases ESFY symptoms appeared in 2005. One of these trees was dead in spring 2006; symptoms on the second one disappeared in 2006. This tree was without symptoms in 2007 too, and results of nested PCR and biological testing were negative in 2004-2007.

Further two trees died out during the winter 2005/2006 and third one in spring 2006 showing wilting of leaves. Apoplexy probably was the reason of dieback, ESFY symptoms were never observed.

Twenty five apricot trees remained without symptoms in the years 2004-2007 and results of biological indexing were negative. On the other hand results of nested PCR in samples from two healthy trees were positive in the all four years, samples from two trees were positive in three years, from four trees in two years, from seven trees in one year only, and results of nested PCR of samples from ten trees were negative in the years 2004-2007. Some of positive results of nested PCR in one year were negative in the next year and opposite. Results were obtained with primers R16F2/R16R2. In case the second step of nested PCR was carried out with primers fU5/rU3I even more positive reactions appeared. Obtained results indicate false positive reactions of nested PCR.

## Discussion

Trees of different apricot cultivars, which will display typical symptoms of ESFY phytoplasma, usually die out either in one year or during next growth period. Typical symptoms of ESFY, leaf yellowing and rolling, little leaves and sparse foliage are a reliable diagnostic criterion for this disease. The results of nested PCR showed high sensitivity, however also the occurrence of false positive reactions and disagreement of these results with the long-term absence of symptoms of the disease on tested trees. The biological indexing on GF-305 is not reliable enough, as well as molecular diagnostics based on PCR.

## Acknowledgements

The work was supported by the Ministry of Agriculture of the Czech Republic, Grant No. MZe 0002700603.

## References

- ÄHRENS U., SEEMÜLLER E., 1992.- Detection of DNA of plant pathogenic mycoplasma-like organisms by a polymerase chain reaction that amplified a sequence of the 16S rRNA gene.- *Phytopathology*, 82: 823-832.
- LEE I.-M., BERTACCINI A., VIBIO M., GUNDERSEN D. E., 1995.- Detection of multiple phytoplasma in perennial fruit trees with decline symptoms in Italy.- *Phytopathology*, 85: 728-735.
- LORENZ K. H., SCHNEIDER B., AHRENS U., SEEMÜLLER E., 1995.- Detection of the apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and nonribosomal DNA.- *Phytopathology*, 85: 771-777.
- NAVRÁTIL M., VÁLOVÁ P., FIALOVÁ R., FRÁNOVÁ J., VORÁČKOVÁ Z., KAREŠOVÁ R., 1998.- Occurrence of fruit tree phytoplasmas in the Czech Republic.- *Acta Horticulturae*, 472: 649-653.
- NAVRÁTIL M., VÁLOVÁ P., FIALOVÁ R., PETROVÁ K., PONCAROVÁ-VORÁČKOVÁ Z., FRÁNOVÁ J., NEBESÁŘOVÁ J., KAREŠOVÁ R., 2001.- Survey for stone fruit phytoplasmas in the Czech Republic.- *Acta Horticulturae*, 550: 377-382.
- POLÁK J., SALAVA J., BRYXIOVÁ M., SVOBODA J., 2006.- Diagnostics of ESFY in symptomless apricot trees – disagreement between molecular and biological assays, pp. 105-110. In: *Proceedings of XVII. Czech and Slovak Plant Protection Conference*, Prague, 12-14 September 2006. Czech Agricultural University.

**Corresponding author:** Jaroslav POLÁK (polak@vurv.cz), Department of Virology, Division of Plant Medicine, Crop Research Institute, Drnovská 507, 16106 Prague, Czech Republic.