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Seasonal occurrence of '*Candidatus* Phytoplasma pyri' in pear trees in the Czech Republic

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Abstract

Seasonal detection of '*Candidatus* Phytoplasma pyri' was studied in three pear cultivars Rouge Red, Agata and Boscova Lahvice. Six pear trees of each cultivar were analysed every month from March 2003 to June 2007 by nested PCR. The higher detection rates by PCR were obtained in winter months during dormancy, i.e. from December to March and April. Results of testing throughout summer months indicate lower incidence of positive detection of phytoplasmas.

Key words: *Pyrus communis*, pear decline, seasonal detection, PCR.

Introduction

The phytoplasma associated with pear decline belongs to the apple proliferation group (Seemüller *et al*., 1998) and causes serious diseases in pear crops. The disease is widespread in many pear-growing areas of Europe and North America, and wherever the domestic European pear (*Pyrus communis* L.) is grown (Davies *et al*., 1994; Garcia-Chapa *et al*., 2003). The pear decline phytoplasma was newly denominated as '*Candidatus* Phytoplasma pyri' (Seemüller and Schneider, 2004). Molecular technologies based on PCR have improved the sensitivity and reliability of phytoplasma detection (Ahrens and Seemüller, 1992). However detection by PCR can be hampered by irregular distribution and low concentrations of '*Ca.* P. pyri' in the tree and because of the presence of PCR inhibitors which can vary throughout the year (Garcia-Chapa *et al*., 2003). Studies on the seasonal movement of phytoplasmas associated with pear decline are essential for the determination of the best times of the year for sampling to detect the disease in the climatic conditions of the Czech Republic.

Materials and methods

Pear trees from orchards of cultivars Rouge Red, Agata and Boscova Lahvice grafted on *P. communis* rootstocks were used. Six pear trees of each cultivar, that had shown symptoms of pear decline and that had been tested positive by PCR analysis, were chosen for this study. For each tree, samples from branches were taken every month from March 2003 to June 2007.

Phloem tissue of the branches was used for DNA extraction according to Lee *et al.* (1991). About 20 ng of each DNA preparation in water were added to the polymerase chain reaction (PCR) mix (Schaff *et al*., 1992) in a final reaction mixture volume of 25 µl.

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 (Lee *et al*., 1993, Gundersen and Lee, 1996;) and fU5/rU3 (Lorenz *et al*., 1995) primer pairs. Final R16F2/R2 amplicons (10 µl) were digested with *Rsa*I and *Bfm*I (Fermentas, Lithuania) 16 hours at 37 °C. The digests were mixed with SYBRGreen I before electrophoresis for visualization under UV light and were run in 3% agarose gels in TBE buffer.

Results

The results of five years survey carried out on the presence of phytoplasma infection in pear trees are shown in table 1. The higher detection rates by PCR were obtained in winter months during dormancy, i.e. from December to March and April. Results of testing throughout summer months indicate lower rates of phytoplasma detection. Our results show that the diagnosis of the disease, i.e., the pathogen detection, is possible throughout most part of the year and not only in summer and early autumn.

Discussion

These results are in agreement with results of Garcia–Chapa *et al.* (2003), Garcia–Chapa *et al.* (2004) and Errea *et al.* (2002) and with EPPO detection methods, which recommend detection of this disease in the period of dormancy. The presence of phytoplasmas in tree crowns during winter is important and dangerous especially from the point of view of pear propagation with infected dormant buds. In practice, grafting is usually performed in spring with dormant buds collected during the winter, when our PCR results show the presence of '*Ca.* P. pyri'.

Seemüller *et al.* (1984) stated the presence of the pathogen in branches especially in the period from spring to the end of autumn. Their detection was done with the use of fluorescence and grafting on woody indicators.

Table 1. Incidence of pear decline phytoplasma in pear trees in years 2003-2007.

	Number of tested samples and results of PCR in years									
	2003		2004		2005		2006		2007	
Months	total/positive	%	total/positive	%	total/positive	%	total/positive	%	total/positive	%
I. - II.	nt	nt	18 / 6	33.3	18 / 4	22.2	18 / 10	55.5	18 / 14	77.8
III. - IV.	18 / 7	38.8	18 / 5	27.7	18 / 4	22.2	18 / 7	38.9	18 / 12	66.7
V. - VI.	18 / 4	22.2	18 / 1	5.5	18 / 2	11.1	18 / 3	16.6	18 / 3	16.6
VII. - VIII.	18 / 4	22.2	18 / 2	8.3	18 / 3	16.6	18 / 4	22.2	nt	nt
IX. - X.	18 / 4	16.6	18 / 2	11.1	18 / 3	16.6	18 / 5	27.7	nt	nt
XI. - XII.	18 / 7	38.8	18 / 2	27.7	18 / 10	55.6	18 / 16	88.9	nt	nt

nt, not tested.

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