

# Investigation on the apple proliferation epidemics in the orchards of the Pelion Mountain and preliminary observations on possible phytoplasma vectors

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## Abstract

The apple producing region in the Pelion Mountain (Magnesia prefecture, Thessaly, Greece) is suffering for more than 10 years from the apple proliferation disease. The orchards are basically planted with cv 'Starking Delicious', are grafted on seedling rootstocks and are older than 40 years. The presence of the '*Candidatus Phytoplasma mali*' was proven by PCR/RFLP analyses as well as by sequencing. '*Ca. P. pyri*' was also detected in at least three different orchards. First attempts to identify the vectors were carried out by monitoring the insect populations in 8 different orchards. One species of psyllids morphologically identified as *Cacopsylla pulchella* (Low) was the most present insect in the orchards all along the two-month monitoring (May/June). *Cacopsylla mali* (Schmidberger) was also present in the orchards but in lower numbers. The monitoring is continued and still in progress.

**Key words:** apple proliferation, psyllid vector, molecular identification, phytoplasmas.

## Introduction

The apple producing region in the Pelion Mountain (Magnesia prefecture, Thessaly, Greece) is suffering for more than 10 years from the apple proliferation disease. The disease was firstly described in Greece in late eighties (Rumbos, 1986) and the '*Candidatus Phytoplasma mali*' was recently detected and identified by molecular methods in infected material (Rumbou *et al.*, 2007).

The infected orchards are basically planted with cv Starking Delicious, are grafted on seedling rootstocks and are older than 40 years. Approximately 90% of the apple trees are infected and the yield losses range from 20-40% in the lower elevations to 70-100% in the higher elevations, where apple orchards neighbour beech forest. The last five years and because of the extended epidemics, infected trees are massively removed and replaced with new trees grafted on dwarf rootstocks and planted in palmettos. However, the pathogen has been detected also to those newly planted trees (Rumbou *et al.*, 2010a), therefore survey was undertaken to verify the disease presence as well as to identify the insect vector of the disease.

## Materials and methods

Samples from apple trees grown in different orchards in the Pelion Mountain and exhibiting apple proliferation symptoms were collected from September to November 2009 and were preserved at -80°C or -20°C. Screening of symptomatic apple material was carried out with total DNA extraction from 24 leaf and root samples from diseased apple trees. DNA extraction was performed in Julius Kühn Institute (Institute for Plant Protection in

Fruit Crops and Viticulture, Dossenheim, Germany) according to a modified protocol following Doyle and Doyle (1990). PCR amplification with P1/P7 was followed by nested PCR with the universal phytoplasma primers fU5/rU3 or with the fruit tree-specific primers fO1/rO1 (Lorenz *et al.*, 1995). The PCR products were cloned and sequenced. A second round of PCR amplifications was performed in Max-Planck Institute (Institute for Molecular Genetics, Berlin) with universal primers fU5/rU3. Sequencing was carried out using ABI3730XL capillary systems (ABI 3730xl) and resulted in 20-fold coverage. Sequence quality assessment and assembly were performed using MIRA assembly program and GAP4.

A first attempt to identify the vectors of the pathogen was done in 2006 by monitoring the insect populations in eight different orchards for the flying season.

## Results

Positive results were obtained mainly from root samples, in particular nine samples originating from eight different orchards were identified as '*Ca. P. mali*', while three samples originating from three different orchards were identified as '*Ca. P. pyri*'. Two samples from the same orchard were found to be infected by '*Ca. P. pyri*' and '*Ca. P. mali*', respectively (Rumbou *et al.*, 2010b).

From the insects trapped, two species of psyllids and one of Jassidae, possible phytoplasma vectors, were identified. From these one species of psyllids, morphologically identified as *Cacopsylla pulchella* (Low), was the most frequently trapped insect in the orchards with 50-1,660 adults/8 traps and flying mainly from 17/5-1/6, with the exception of an orchard in high altitude where

they were present until the 21/6. A non-identified species of Jassidae was the second insect for number of individuals (18-278 adults/8 traps) and present during the two-month monitoring (26/4-21/6). *Cacopsylla mali* (Schmidberger) was also present in the orchards but in lower numbers (1-59 adults/total traps).

## Discussion

The presence of the '*Candidatus Phytoplasma mali*' was firstly proven by molecular means in 2005. Lately, the first sequences from local strains were obtained with the use of phytoplasma universal and 16SrX-group specific primer pairs revealing that, apart from '*Ca. P. mali*' also '*Ca. P. pyri*', the agent associated with pear decline, is present in at least three different orchards in the surveyed region.

This year the insect monitoring is repeated and the identification will be done with more accurate tools. The detection of phytoplasma in the insects' body remains to be shown before the transmission trials will be initiated.

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