

## Investigation on European stone fruit yellows in experimental apricot orchards in the province of Trento (Italy)

Carlo POGGI POLLINI<sup>1</sup>, Laura BIANCHI<sup>1</sup>, Flavia FORNO<sup>2</sup>, Sergio FRANCHINI<sup>3</sup>, Luciano GIUNCHEDI<sup>1</sup>, Marino GOBBER<sup>3</sup>, Luisa MATTEDI<sup>2</sup>, Paolo MIORELLI<sup>3</sup>, Daniela PIGNATTA<sup>1</sup>, Davide PROFAIZER<sup>3</sup>, Claudio RATTI<sup>1</sup>, Nazareno REGGIANI<sup>1</sup>

<sup>1</sup>Dipartimento di Scienze e Tecnologie Agroambientali, Patologia vegetale, Università di Bologna, Italy

<sup>2</sup>Istituto Agrario S. Michele all'Adige (IASMA), Trento, Italy

<sup>3</sup>IASMA, Centro di Assistenza Tecnica, Trento, Italy

### Abstract

A severe outbreak of European stone fruit yellows (ESFY) was reported recently in apricot (*Prunus armeniaca*) orchards in the province of Trento. The spreading of the disease has been monitored since 2005 in 4 experimental orchards, planted with ESFY-free material (cv. Bergeron on Wavit) and treated with different chemical sprays.

The first results demonstrate that most of the applications have no efficacy in controlling the disease. Studies on the possible transmission of ESFY via root bridges have also been included in this research.

**Key words:** *Prunus armeniaca*, real time PCR, 'Candidatus Phytoplasma prunorum', control strategies, *Cacopsylla pruni*.

### Introduction

Surveys have been conducted, since 2004, in different apricot orchards to determine the current status of European stone fruit yellows (ESFY) disease in the province of Trento.

In this area a constant spread of the disease has been noted in recent years leading to partial or total tree die-back causing major economic losses to the growers (Pignatta *et al.*, 2007).

The presence of the vector of 'Candidatus Phytoplasma prunorum', of the psyllid *Cacopsylla pruni* (Scopoli), as well as of wild reservoirs of the phytoplasma (especially *Prunus spinosa* (blackthorn), *Prunus mahaleb* and wild *Prunus domestica*) have also been demonstrated (Pignatta *et al.*, 2007).

Recently, new approaches have been developed for the detection of fruit tree phytoplasmas in plant material and in vectors using a highly sensitive real-time PCR assay, useful for large-scale analysis (Baric and Dalla Via, 2004; Pignatta *et al.*, 2007).

The objective of this research was to study the epidemiology of the disease by monitoring the spread of the causal agent in 4 experimental orchards (about 180 plants each) of cv. Bergeron grafted on Wavit, where 25% of the propagation material had been previously checked for ESFY infection before planting in 2005. All the material tested was found to be ESFY-free (Pignatta *et al.*, 2007).

These orchards were treated with different pesticides in 2006 to control the vector; the presence of ESFY-like symptoms was monitored during the different seasons with field observations and subsequent molecular analysis.

Trials conducted in the same areas under controlled conditions demonstrated that the agent of apple proliferation ('Candidatus Phytoplasma mali') can be transmitted by root grafting (Ciccotti *et al.*, 2007). For this reason the transmission of 'Ca. P. prunorum' by natural root bridge was also investigated.

### Materials and methods

Visual inspections for typical ESFY-symptoms (early budbreaks in winter, premature leafroll in summer and autumn) were performed in the experimental fields at least three times a year.

Samples of trees with typical and doubtful symptoms were taken and tested for 'Ca. P. prunorum' presence via real time PCR using a multiplex procedure for simultaneous detection of the pathogen and host DNA, to avoid false negatives due to PCR inhibition.

Total DNA was extracted from plants and insects collected in the fields with a phytoplasma enrichment procedure (Marzachi *et al.*, 1999). All the reagents used in real-time PCR were from Applied Biosystems (AB) with the exception of the primers, obtained from Invitrogen Life Technologies. A multiplex reaction was carried out in AB PRISM 7000 SDS; 4 µl of template DNA were added to 16 µl of real-time PCR mix containing 1X TaqMan Buffer A, 5.5 mM MgCl<sub>2</sub> solution, 0.5 U AmpliTaq Gold DNA Polymerase, 0.8 mM dNTPs, 750 nM of qAP-16S primers, 150 nM of qMd-cpLeu primers, 160 nM of qESFY-16S probe and 80 nM of qMd-cpLeu probe. PCR conditions were: 10 min at 94 °C, followed by 40 cycles of 15 s at 94 °C and 1 min at 60 °C (Baric and Dalla Via, 2004; Pignatta *et al.*, 2006).

Chemical sprays were performed with 3 different protocols as described in table 1. Two Bergeron/Wavit orchards from the same nursery, planted in 2003 and 2004 were used as untreated controls. All the insecticides used are known to provide good activity against apple psyllids.

To study the possible translocation of 'Ca. P. prunorum' through root bridges, a systemic herbicide (Glyphosate) was applied during winter 2006-2007 exclusively on the cut surface of some ESFY-infected trees.

**Table 1.** Number and time of insecticide applications in the apricot experimental fields in 2006.

Date	BALBIDO 1 and 2 Treatment no. 1	Date	PERGINE Treatment no. 2	Date	SALOBBI Treatment no. 3
2/4	Chlorpyrifos	13/4	Etofenprox	22/4	Neem + oil
8/4	Malathion	3/5	Phosalone	19/5	Rotenone + oil
28/4	Chlorpyrifos	6/5	Etofenprox		
21/5	Phosalone				

**Table 2.** Effects of insecticide applications.

	BALBIDO 1	BALBIDO 2	PERGINE	SALOBBI	untreated
ESFY-infected plants in autumn 2006	6.6%	4.1%	0.5%	4.4%	5-7%
Plants with budbreaks in spring 2007	1.2%	1.2%	NO	NO	3%
New generation of <i>C. pruni</i> (eggs and nymphs).	YES	YES	NO	YES	YES

## Results

Table 2 shows how the disease increased in 2006 and also in spring of 2007 in most of the different experimental fields with no differences as compared with the non-sprayed fields.

Only in one location (Pergine) the ESMY spread was extremely low (1 plant with severe decline in summer 2006 out of 200). The infected plants were promptly eradicated.

The new generation of *C. pruni* was found in most of the orchards examined (table 2).

After Glyphosate applications no herbicide symptoms were found in neighboring trees. Furthermore, these apricots did not show any expression of ESMY symptoms in spring 2007.

## Discussion

The potential epidemic threat posed by '*Ca. P. prunorum*' in stone fruit orchards is confirmed by the annual increase in the number of infected trees in 4 experimental fields of three year old cv. Bergeron on Wavit.

Real-time PCR tests performed in the 25% of propagation material suggested that in our conditions new infection of trees is presumably due to the transmission of '*Ca. P. prunorum*' by the vector rather than to the contamination of propagation material, as reported in other epidemiological studies.

As regards control measures, the results obtained after only one year show that most of the insecticide applications have no efficacy in controlling the disease as compared with unsprayed orchards.

ESFY spread was extremely low in only one experimental field (Pergine), but this situation could be due to environmental factors which can influence psyllid fitness in different areas. In 2007, however, all treatments in the experimental fields were performed following the second protocol (treatment no. 2 in table 1).

In medium-aged and old apple orchards in Trentino, natural root bridges are thought to play an important role in disease spread (Ciccotti *et. al.*, 2007). On the contrary, the results obtained in our research with herbicide treatments did not confirm this type of transmission for '*Ca. P. prunorum*' in neighboring apricot trees.

## Acknowledgements

This research was supported by Provincia autonoma di Trento (Progetto triennale: "Verifica dello stato sanitario e studio di possibili strategie di contenimento del Gi-allume Europeo delle Drupacee").

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**Corresponding author:** Carlo POGGI POLLINI (e-mail: carlo.poggipollini@unibo.it), *Alma Mater Studiorum* Univer-sity of Bologna, DiSTA, Patologia vegetale, viale G. Fanin 42, 40127 Bologna, Italy.