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Marina Barba, Francesco Faggioli, Vincenza Ilardi,
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CRA - Centro di Ricerca per la Patologia Vegetale
Via C.G. Bertero, 22 I-00156 - Roma, *Italy*
Tel. +39.06.82070220 Fax +39.06.86802296
E-mail: petria.pav@entecra.it
sito Web: www.ispave.it

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RECOVERY PHENOMENA IN APRICOT TREES CV. BERGERON INFECTED BY EUROPEAN STONE FRUIT YELLOWS IN THE PROVINCE OF TRENTO (ITALY)

C. Poggi Pollini[1], S. Franchini[2], M. Gobber[2], C. Lanzoni[1], C. Ratti[1]

[1]Dipartimento di Scienze e Tecnologie Agroambientali (DISTA), Alma Mater Studiorum, Bologna, Italy
[2]Istituto Agrario S. Michele all'Adige (IASMA), Trento, Italy

E-mail: carlo.poggipollini@unibo.it

Abstract

Recovery phenomena have been intensively studied for apple proliferation and grapevine yellows. Several recent findings in apricot orchards in Friuli-Venezia Giulia indicate the importance of this strategy also for the control of European Stone Fruit Yellows (ESFY) infections, caused by '*Candidatus* Phytoplasma prunorum'. The aim of this work was to investigate the occurrence of recovery in 2 experimental orchards of apricot cv. Bergeron on 'Wavit', located in the province of Trento, where the disease has been spreading since 2000. This included visual inspections for typical symptoms and a diagnostic method based on a Spot Real-Time Reverse Transcription PCR assay. The results obtained showed that there were three different groups of plants infected by ESFY: many plants with typical symptoms, some recovered and some symptomless infected plants. To date no interesting plant genotypes showing resistance to ESFY are available, moreover most of the insecticide applications have no efficacy in controlling the vector than the disease. The use of both pathogen-free propagation material and resorting to natural recovery could be adopted as an integrated approach for the control of this phytoplasma disease. For these reasons the research will continue with the aim of quantifying the concentration of the pathogen in the recovered plants.

Introduction

'*Candidatus* Phytoplasma prunorum' is associated with the quarantine phytoplasma European stone fruit yellows (ESFY) disease that generally induces yellows, tree decline or die-back and vegetative disorders with typical symptoms such as an early bud break and leaf rolling on most wild and cultivated *Prunus* species (Poggi Pollini *et al*, 2001).

ESFY is mainly known in Europe, but has also been reported in Turkey and Azerbajdzhan (Marcone *et al*., 2010). Due to the high efficiency of its natural vector *Cacopsilla pruni* and the use of non symptomatic infected plant material, '*Ca.* P. prunorum' can spread rapidly in stone fruit cultivated areas. In apricot orchards the

number of infected trees can double in a few years, especially on susceptible varieties such as 'Bergeron'(Ramel and Gugerli, 2004, Poggi Pollini *et al.,* 2010).

To date, all approaches applied to plants already infected by phytoplasmas have not reduced the incidence of these epidemiological diseases (Osler *et al*., 2012).

As a consequence, great relevance has been recently attributed to a well known phenomenon called recovery, the spontaneous remission of symptoms in diseased plants, intensively studied in apple and grapevine affected by phytoplasma and also known to occur in apricot infected by ESFY (Carraro *et al.,* 2004; Maixner, 2006a). Recovery can be natural or promoted by the application of abiotic stress, resistance inducers and several endophytic fungi and bacteria (Conti and Faoro, 2012); the involvement of a hypovirulent strain of '*Ca*. P. prunorum' has also been suggested (Morvan *et al.*, 1986; Ermacora *et al.*, 2010).

In apricot orchards in Friuli-Venezia Giulia (Italy) under high ESFY infection pressure after several years of observation, three different groups of plants infected by ESFY were identified: symptomatic, symptomless and recovered (Osler *et al*, 2012).

The aim of our work was to investigate the occurrence of recovery in 2 experimental orchards of apricot cv. Bergeron grafted on 'Wavit', located in the province of Trento (Italy), (Bleggio-Crosina and Bleggio- Farina). Both orchards were originally planted using ESFY-free material, but in the area ESFY has been spreading since 2000 leading to partial or total tree dieback and causing major economic losses to growers (Poggi Pollini *et al*, 2010).

Materials and Methods

Visual inspections for typical ESFY-symptoms (early bud breaks during dormancy, premature leaf-roll, fruit deformation and die-back) have been performed in the experimental orchards at least three times a year since 2006. All data were properly recorded appropriately.

Eighty-two apricot samples were analyzed using a Spot Real-Time Reverse Transcription-PCR Taqman assay, recently developed for 'Ca. P. prunorum' detection on stone fruit trees samples (Minguzzi *et al*, 2010). Many samples showed typical ESFY-symptoms in late summer 2011;in contrastome did not show any visible symptoms. Five apricots infected by ESFY and 5 healthy apricots, grown in an insect-proof screenhouse, were also chosen as positive and negative controls, respectively. All molecular tests were performed during September-December 2011. All the samples, consisting of 1 g of phloem from woody shoots of apricot trees were placed in extraction bags (Bioreba, Reinach, Switzerland) and immediately tested or stored at -20C° until used. Analyses were performed using a rapid extraction method adapted from previously published works (Osman and Rowhani, 2006, Minguzzi *et al.,* 2010).

All the primers and probes employed in this study were designed using Primer Express software (Applied Biosystems, Branchburg, NJ, USA). Using sequence alignment of the 16S rRNA gene region of nine different phytoplasma strains (Baric and Dalla-Via, 2004), a specific '*Ca*. P. prunorum' primer and MGB probe assay was designed.

A previously published assay (Osman *et al.,* 2007) was slightly modified in order to obtain a DiSTA 18s MGB probe and DiSTA 18s-F / DiSTA 18s-R primer pair.

Fluorogenic probes were 5'modified with FAM (6-carboxyfluorescein) (*ESFY-16S*) and VIC *(DiSTA 18s*). The multiplex RT-PCR reaction was performed in a total volume of 25 µL containing 2 µL of template, using an automated ABI PRISM 7000 Sequence Detection System (Applied Biosystems) in MicroAmp optical 96-well plates.

Results

The results of field observations showed that three different groups of plants were present in the experimental fields. Many plants (57) expressed severe foliar symptoms, fruit deformation, progressive decline and sometimes total die-back in the last 2 years (2010-2011); according to previous results off-season growth was rarely observed (only 4 plants during winter 2011) (Poggi Pollini *et al*., 2010). Some plants (13) showed ESFY-symptoms only in 2006-2007, but a spontaneous remission of symptoms has been occurring since 2009. The remaining 12 plants did never shown any visible ESFY-symptoms. Moreover in the last 2 groups no undersized fruits were observed and a good production was always achieved.

The distribution of the 3 groups in the 2 experimental orchards is summarized in Tab. 1.

As regards molecular tests, specific amplification was always obtained from the positive controls, from 57 symptomatic plants, from 13 recovered plants and surprisingly from 3 out of the 12 apparently healthy plants. No phytoplasma were found in the other 9 plants in the third group nor in samples from the healthy controls (Tab. 2).

Tab. 1 - different groups of plants collected in the experimental fields, during Sept-Dec 2011.

LOCATION AND NUMBER OF PLANTS	SYMPTOMATIC TREES	TREES WITH SYMPTOMS REMISSION	SYMPTOMLESS TREES
BLEGGIO – CROSINA (170)	26	6	6
BLEGGIO – FARINA (172)	31	7	6
TOTAL	57/342 – 16,7 %	13/342 – 3,8 %	12/342 – 3,5 %

Tab. 2 - results of phytoplasma detection by RT-PCR in different groups of trees.

LOCATION AND NUMBER OF PLANTS	SYMPTOMATIC TREES	TREES WITH SYMPTOMS REMISSION	SYMPTOMLESS TREES
BLEGGIO – CROSINA (170)	26/26*	6/6	1/6
BLEGGIO – FARINA (172)	31/31	7/7	2/6
TOTAL	57/57	13/13	3/12

* number of samples positive by RT-PCR/total number of samples

Discussion

The aim of our work was to select individual apricot with acquired tolerance in an area with a high ESFY-infection pressure.

Our research, based on a six-year plan of field inspections and on a rapid, sensitive and reliable diagnostic method (Spot Real-Time Reverse Transcription-PCR Taqman assay) clearly demonstrated for the first time the occurrence of recovery phenomena in 2 experimental apricot orchards in the province of Trento. Thirteen plants – 3.8% of the apricots in the experimental plots – stably recovered from the symptoms but not from the infection revealing acquired resistance. It is worth noting that usually the phytoplasma infection in the cv. Bergeron significantly reduces fruit size and causes fruit deformations leading to economic losses (Poggi Pollini *et al*., 2010). In contrast these recovered plants did not show any fruit symptoms and produced a yield like the healthy plants. Interestingly three individual plants – less than 1% of the total-have remained symptom-less since 2006 even if infected; these plants can be considered tolerant to ESFY. All the studies conducted in Friuli-Venezia Giulia in apricot orchards provided clear evidence that '*Ca*. P. prunorum' infections

persist in the aerial parts of recovered apricot trees (Osler *et al*., 2012).

The potential epidemic threat posed by '*Ca*. P. prunorum' in apricot orchards is confirmed by the dramatic increase in the number of infected trees, especially when a highly susceptible cultivar, like 'Bergeron', is grown under high European stone fruit yellows infection pressure (Poggi Pollini *et al.*, 2010). Like other phytoplasma, direct protection of trees from '*Ca*. P. prunorum' cannot be achieved by chemical control measures. Moreover all the measures used to prevent ESFY spread such as sanitary selection, use of clean propagating material, removal of the infected plants and vector control with insecticide treatments have so far not reduced the progression of the disease in apricot orchards in Italy, (Poggi Pollini *et al*, 2007; Osler *et al*, 2012). Thus the possibility to develop new control strategies based on recovery phenomenon have recently become of practical interest (Conti and Faoro, 2012)

Recovery can be a temporary or a permanent phenomenon, thus it has been recently proposed that an originally infected plant can be considered as having recovered only after a minimum of 3 consecutive years without symptoms (Maixner, 2006b). As regards apricot and ESFY, in infective areas the infected but tolerant plants behave much better than healthy ones, when grafted on tolerant rootstocks. Moreover recovered plants are less likely to be re-infected than plants that have never been previously infected (Osler *et al.,* 2012). Despite in the fact that the physiological basis of recovery is still not completely understood and the interaction between hypo- and hyper-virulent strains of '*Ca.* P. prunorum' in mixed infections is still under evaluation, the presence of recovered ESFY-infected plants has indicated the importance of this strategy also for the control of this infection (Ermacora *et al*, 2010; Osler *et al,* 2012).

In the next few years our research will focus on ascertaining the behavior of young plants derived from mother plants with acquired tolerance, grafted on appropriate rootstocks. A Real Time quantification method will be applied to confirm the correlation between low phytoplasma concentration and the absence of symptoms in the aerial parts of the trees (Ermacora *et al.*, 2010). The molecular identification of protectant strains which can be used for cross protection will also be performed.

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