

Detection and distribution of European stone fruit yellows (ESFY) in apricot cv. 'Bergeron' and epidemiological studies in the province of Trento (Italy)

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Abstract

The aim was to investigate the performance of 'Bergeron' on 'Wavit' in 4 experimental fields, in the province of Trento (Italy), where European stone fruit yellows (ESFY) caused by "Candidatus *Phytoplasma prunorum*" has been constantly spreading since 2000.

This included visual inspections for typical symptoms (early bud-break during dormancy and premature leaf-roll) and a highly sensitive Real time-PCR (Rt-PCR) assay. 25 % of the propagation material was checked with this method and found to be healthy, before planting in 2005.

The epidemiology of the disease was also studied by focusing on: the presence of the vector *Cacopsylla pruni* (Scopoli) on conifers, the detection of "Ca. *P. prunorum*" in psyllid eggs and the transmission efficiency at different stages. This was done by exposing apricot trees in 2 locations, during 2 periods from January to July, to the overlapping presence in the orchards of the re-immigrants and the new generation of *C. pruni*.

The results obtained demonstrated that 'Bergeron' seems to be highly susceptible to ESFY: typical bud-break was rarely observed, but up to 20-30% of the plants showed premature leaf-roll, fruit deformation and dieback. *C. pruni* was caught only once on *Picea abies* during winter; "Ca. *P. prunorum*" was found in 4 egg samples from 2 locations and the preliminary results on the exposed trees confirmed that the re-immigrants could be the most efficient vectors at least on apricot.

Keywords: *Prunus armeniaca*, cultivar 'Bergeron', Real time-PCR, "Candidatus *Phytoplasma prunorum*", epidemiology.

Introduction

Surveys have been conducted, since 2004, in different apricot orchards to determine the current status of European Stone Fruit Yellows (ESFY) in the province of Trento (Italy). In this area a constant progression of the disease, caused by "Candidatus *Phytoplasma prunorum*", has been noted in recent years leading to partial or total tree dieback causing major economic losses to growers. To prevent the disease spreading, four experimental orchards of cv. 'Bergeron' grafted on 'Wavit' have been established since 2005. 25% of the propagation material was checked with a highly sensitive Real time-PCR (Rt-PCR) assay, useful for large-scale analyses and found to be "Ca. *P. prunorum*"- free (Pignatta et al., 2006).

Surveys have been conducted in these experimental fields including visual inspections for typical symptoms (early bud-break during dormancy and premature leaf-roll) and Rt-PCR assays. The epidemiology of the disease was also studied by focusing on some points in the life cycle of "Ca. *P. prunorum*" vector, the psyllid *Cacopsylla pruni* (Scopoli), such as the presence of *C. pruni* on conifers during winter, the detection of "Ca. *P. prunorum*" in psyllid eggs, and transmission efficiency at different life stages of the vector.

Material and methods

Field surveys: Visual inspections for typical ESFY-symptoms (early bud-break during dormancy, fruit deformation and premature leaf-roll in summer and autumn) have been performed in the experimental fields at least three times a year since 2006. The presence of *C. pruni* on conifers has been investigated by searching for the insect in different places, especially on *Abies alba* and *Picea abies* (Thebaud et al., 2006), in the province of Trento since winter, 2007. The eggs were carefully removed from the leaf surfaces of apricots and blackthorn (*Prunus spinosa*) with a needle during May, 2008, and, after identification, processed in groups of five.

Detection of “Ca. *P. prunorum*”: Samples of trees with typical and dubious symptoms, insects and eggs, were taken and tested for the presence of “Ca. *P. prunorum*” via Rt-PCR. A multiplex procedure was used for simultaneous detection of the pathogen and host DNA, to avoid false negatives due to PCR inhibition, as previously described (Pignatta et al., 2006). Total DNA was extracted from apricot phloem and insects with a phytoplasma enrichment procedure (Marzachi et al., 1999).

Studies on transmission efficiency: The vector transmission efficiency, at different stages of its life, was studied by exposing apricots of the same cultivar, in 2 different locations (Balbido and Calavino) during 2 periods (80 plants in all), to the overlapping presence of re-immigrants (adults that have overwintered) (from 13/3/2007 to 16/5/2007) and the new generation of *C. pruni* in the orchards (from 16/5/2007 to 3/7/2007). All plants were tested individually with Rt-PCR before exposure and found to be phytoplasma-free. After each exposure period, the test plants were treated with insecticide, kept for at least one year in an insect-proof greenhouse, inspected for ESFY-symptoms and finally individually tested with Rt-PCR.

Results

Field surveys and detection of “Ca. *P. prunorum*”: The results obtained demonstrated that ‘Bergeron’ seems to be highly susceptible to ESFY: typical bud-break was rarely observed, but up to 20-30% of the plants showed premature leaf-roll, fruit deformation and dieback causing economic losses (Table 1). Amplification was always obtained from symptomatic plants, but no phytoplasma were found in asymptomatic and healthy apricots kept in an insect-proof greenhouse as healthy controls. Regarding the epidemiological studies, *C. pruni* was caught only once on *P. abies* during winter, 2007, very far from the orchards (Monte Bondone). Moreover, the 4 adults captured (re-immigrants) were individually tested and found not to be infected by “Ca. *P. prunorum*”. On the other hand, the phytoplasma was found in 4 egg samples from 2 different locations, on blackthorn and on apricot leaves respectively (Table 2).

Tab. 1 progression of ESFY-infection in 4 experimental fields.

	Location			
	Bleggio Balbido Crosina (170 Trees)	Bleggio Balbido Farina (172 Trees)	Pergine S. Caterina Biasi (200 Trees)	Val Di Non Salobbi Pisani (180 Trees)
Number of trees with symptoms and positive by Rt-PCR (2006-2007)	20 (11.8%)	17 (9.9%)	3 (1.5%)	15 (8.3%)
Number of trees with symptoms and positive by Rt-PCR (2008)	37 (21.8 %)	51 (29.6 %)	9 (4.5 %)	19 (10.5 %)

Tab. 2 results of phytoplasma detection by Rt-PCR on *C. pruni* eggs.

Location*	Specie	Positive/Tested (groups)
Balbido	<i>Prunus armeniaca</i>	1/25**
Calavino	<i>P. spinosa</i>	3/25**

*eggs were collected in May, 2008. ** 5 eggs each sample

Table 3 shows the results of Rt-PCR assays on the exposed apricot trees. First typical symptoms were noted at least 15 months after the field exposure.

Tab 3 natural spread of “Ca. *P. prunorum*” in the field.

Location	Exposure Periods	Symptoms Observed	Positive/Tested
Balbido - Crosina	I° : 13/3/07 – 16/5/07	Premature leaf-roll (Sept 08); dieback (Feb 09) (1 plant)	1/20** (5%)
Calavino - Chemelli	I° : 13/3/07 – 16/5/07	Premature leaf-roll (Sept 08); dieback (Feb 09) (1 plant)	1/20** (5%)
Balbido - Crosina	II°: 16/5/07 – 3/7/07	/*	0/20
Calavino – Chemelli	II°: 16/5/07 – 3/7/07	/	0/20

* no symptoms observed. **the 2 symptomatic plants were positive by Rt-PCR.

Discussion

The first aim was to investigate the field performance of the cultivar ‘Bergeron’ grafted on ‘Wavit’ in the environmental conditions of the province of Trento, where ESFY has been constantly spreading since 2000. The results obtained

revealed an important annual progression of infected trees (Table 1), expressed by severe foliar and fruit symptoms, decline and total dieback. Latent infections on apparently healthy apricot trees (especially cv. 'Luizet') have been reported in Valais (Western Switzerland) elsewhere (Genini and Ramel, 2004). Our results, however, confirmed a strict association between symptoms - especially premature leaf-roll and dieback, but not typical bud-break that was rarely observed on this cultivar - and the presence of "Ca. *P. prunorum*". A dramatic increase in the disease was noted especially in 2 experimental fields located in Balbido. ESFY spread was, however, lower in the experimental field of Pergine, but this situation could be due to environmental factors which can influence psyllid fitness in different areas.

Rt-PCR tests performed before planting on 25% of propagation material suggested that under our conditions new tree infections are due to the transmission of "Ca. *P. prunorum*" by the vector rather than to contaminated propagation material, as reported in other epidemiological studies (Ramel and Gugerli, 2004). The high proportion of insects and of blackthorn hedges found to be infected by "Ca. *P. prunorum*" in this area (Pignatta *et al.*, 2006) and the lack of efficacy of insecticide applications in controlling the disease (Poggi Pollini *et al.*, 2007) justify all efforts to better understand ESFY epidemiology. During this study some insights were gained into the insect overwintering sites that remain unknown. Only once a few adults were captured on *P. abies* at a great distance from the orchards. Large migration movements of this insect have, however, been clearly demonstrated (Sauvion *et al.*, 2007).

"Ca. *P. prunorum*" was found in 4 egg samples from 2 different locations. It should be noted that the proportion of infected eggs is quite substantial, especially on blackthorn (Table 2). This strongly suggests that under our conditions this species could provide an efficient pathogen-source for *C. pruni*. Previous reports indicate that an epidemiological cycle of ESFY can be achieved in blackthorn even in the absence of *Prunus* orchards (Yvon *et al.*, 2004). Moreover, the possibility of transovarial transmission, recently demonstrated for this pathogen (Tedeschi *et al.*, 2006), has important implications for disease management.

The results of Rt-PCR assays on the exposed apricot trees (Table 3) confirmed that the re-immigrants, infected the previous year, can be the most efficient vectors of "Ca. *P. prunorum*" at least on apricot. These data are consistent with the research recently performed on apricot in France that demonstrated how most re-immigrants can be infectious and are able to inoculate susceptible plants when they return and reproduce on *Prunus* (Thebaud *et al.*, 2006).

The potential epidemic threat posed by ESFY to stone fruit orchards is confirmed by the annual increase in the number of infected trees in 4 experimental fields of five-years-old 'Bergeron' on 'Wavit'. Our data suggest that some new, more tolerant, varieties are therefore necessary for apricot orchards in the province of Trento. Further investigation is necessary to complete and clarify the epidemiology of the disease and examine possible control with phytosanitary treatments.

➤ This research was supported by the Provincia autonoma di Trento.

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