Detection of *European Stone Fruit Yellows* Phytoplasma (ESFYP) in *Homoptera* Insects and in Wild Stone Fruit Trees Collected in Peach Orchards in Northern Italy

C. Poggi Pollini, R. Bissani
and L. Giunchedi
Dipartimento di Scienze e Tecnologie
Agroambientali (DiSTA)
40126, Bologna
Italy

N. Mori
Dipartimento di Agronomia Ambientale
e Produzione Vegetale (DAAPV)
35020, Legnaro (PD)
Italy

D. Dradi
Centrale Ortofrutticola (CREA)
47020, Martorano di Cesena (FO)
Italy

T. Visigalli
Servizio Fitosanitario Regionale
37060, Buttapietra (VR)
Italy

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Abstract

***European stone fruit yellows* (ESFY) has been reported in Italy with increasing frequency in peach orchards in the provinces of Forlì-Cesena, Ravenna, Rimini and Verona. The discovery of the natural vector of ESFY phytoplasma, the psyllid *Cacopsylla pruni,* prompted us to investigate the presence of psyllids infected by ESFYP, as well as other potential vectors and alternative hosts, in various orchards in these provinces. The molecular diagnostic method used (PCR-ELISA) made it possible to detect several *C. pruni* psyllids infected by ESFYP in this area, but no important secondary vectors were found in the peach orchards. Molecular tests were also made on wild plants surrounding the orchards, leading to ESFYP identification in wild *Prunus* alone.**

INTRODUCTION

In Europe, several syndromes caused by European Stone Fruit Yellow Phytoplasma (ESFYP), a member of the Apple Proliferation (AP) group (Lee et al, 1998; Seemüller et al., 1998) have been described in stone fruit species. This pathogen can be spread naturally by the psyllid *Cacopsylla pruni* Scopoli (Carraro et al., 2001).

Prior to 1995, ESFYP was only sporadically reported in peach tree orchards, but recent studies have indicated a spreading of this syndrome in Northern Italy. In the orchards inspected, 1-4% of trees were affected, most of which grafted on cv. G.F. 677 (Poggi Pollini et al., 2001a).

This article reports further investigation of ESFYP epidemiology to evaluate the possible existence of alternative hosts or other potential vectors.

MATERIALS AND METHODS

In the two year period 2000-2002, to obtain greater information about the spreading of ESFYP in peach orchards, leafhoppers and psyllids were captured in the Forlì-Cesena, Ravenna, Rimini and Verona provinces. The insects were captured using the "frappage" method or chromotropic traps placed on cultivated and wild stone fruit trees (Table 1), and, during the winter, on conifers and other wild plants surrounding the areas where an increase had been noted in the disease in recent years (Table 2).

Tests were also made on other wild rosaceous plants (*Prunus cerasifera, P. mahaleb, P. spinosa, Rosa canina, Rubus fruticosus),* generally asymptomatic or showing off-season growth in winter, in the vicinity of the affected orchards. Several common wild plants (*Celtis australis, Cornus sanguinea, Corylus avellana, Cupressus leylandii,*

Fraxinus excelsior, Laurus nobilis) were also analysed for the presence of ESFYP or other phytoplasmas. Previous reports indicated that some were ESFYP-hosts (Jarausch et al., 2001a). Out of 20 *C. australis* trees, 8 showed leaf chlorosis and 8 leaf roll. All the *C. sanguinea* shrubs exhibited leaf reddening; the other species were symptomless.

Total DNA was extracted from groups of insects (2-5), after classification, or from individual plants according to a phytoplasma enrichment procedure (Marzachì et al., 1999). ESFYP was identified with immunoenzymatic detection of PCR products (PCR-ELISA), using primer pair fOI/rOI and an ESFYP-specific probe as described previously (Poggi Pollini et al., 2001a). PCR products, initially amplified with universal ribosomal primer pair R16F2/R2, were diluted to 1:40 and 2 µl aliquots were used as templates in nested-PCR reactions primed by one of the group-specific primer pairs (I, III, V, X) (Lee et al., 1995) to evaluate the presence of other phytoplasmas alone or in association with ESFYP.

RESULTS AND DISCUSSION

The results obtained showed that numerous groups of *C. pruni*, captured in all provinces on different stone fruit trees *(P. cerasifera, P. persica* and *P. spinosa*) were infected by ESFYP. Other psyllids, one from each group of *Bactericera curvatinervis, Laurotrioza alacris* and *Trioza urticae,* were also found to be infected by ESFYP. In the winter traps placed on conifers, only one group of *C. pyrisuga* (Forster), captured on *C. leylandii* in the Verona province in 2002, was positive for ESFYP.

Out of the numerous leafhoppers tested, there were only two positive groups of *Macrosteles cristatus* (Ribaut), collected from peach trees in the summer of 2000 in the Verona province (Table 3). Further tests on these leafhoppers in subsequent years did not however confirm these results. The occasional finding of ESFYP in leafhoppers has already been reported (Jarausch et al., 2001b).

The results obtained indicate that PCR-ELISA is reliable and can also be used for specific detection of ESFYP in insects, as previously demonstrated for AP and pear decline (PD) phytoplasmas in its vectors. Furthermore, it offers a greater sensitivity than gel electrophoresis (Poggi Pollini et al., 2001b). No phytoplasmas belonging to other groups (I, III, V) or other phytoplasmas of the same AP-group (X) were detected in the insects examined.

As regards the wild plants examined, various *P. cerasifera* and *P. spinosa* trees and occasionally other wild *Prunus* were found to be infected, although most were asymptomatic. The largest number of infected psyllids were captured precisely on these trees (Table 4 and 5). No phytoplasmas were found, even using nested-PCR, in the other plants with suspicious phytoplasma symptoms or in symptomless plants.

It can be concluded that individual infected *C. pruni* were present in the peach orchards of all the provinces examined. At present the intensive testing of insects suggests there is no important secondary vector of ESFYP in peach orchards in Northern Italy; however the role of other psyllids in ESFYP transmission will be evaluated.

As regards the wild plants surrounding the peach orchards, only wild *Prunus* were found to be infected by ESFYP. The possible role of additional plant hosts in the spread of the disease, as suggested in France (Jarausch et al, 2001a), was not confirmed by our research, since all the methods used failed to detect ESFYP in these species.

Wild *Prunus* around the peach orchards certainly play a role in the epidemiology of the disease. As already indicated in the past, they represent good hosts for ESFYP, without showing symptoms, as well as for the insect vector (Carraro et al., 2001; Jarausch et al., 2001b). This indicates the importance of including these plants in a control program to eliminate the vector psyllid.

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Tables

Table 1. List of *Homoptera* caught in peach orchards or on wild *Prunus*.

Genus and species	Nb. of groups (ins./group)	Caught periods	Caught locations (province)
Cacopsylla pulchella	48 (2) 96	Ap/June 2000	FC-RA-RM
Neoaliturus fenestratus	46 (5) 230	June/Aug 2000	FC-RA-RM
C. pruni	61 (2) 122	June 2001	FC-RA-RM
C. pruni	20 (2) 40	Mar/Jun 2002	RA-RM
Laodelfax striatellus	24 (5) 120	May/June 2000/01	VR
Macrosteles cristatus	48 (5) 240	May/Aug 2000/01	VR
Psammotetix spp.	20 (5) 100	May/Aug 2000	VR
Asimmettrasca spp.	7 (3) 21	July/Aug 2000	VR
C. pruni	8 (2) 16	May/June 2001	VR
C. crataegi	7 (2) 14	Jan/Mar 2002	VR
Trioza remota	3 (2) 6	Jan/Ap 2002	VR
C. pruni	52 (2) 104	Feb/June 2002	VR
C. pulchella	19 (2) 36	Feb/June 2002	VR
C.pyrisuga	4 (2) 8	Feb/Mar 2002	VR
C.affinis	2 (2) 4	Mar/Ap 2002	VR
Laurotrioza alacris	1 (2) 2	May 2002	VR
Trioza urticae	1 (2) 2	May 2002	VR
Bactericera curvatinervis	1 (2) 2	June 2002	VR
Others	34 (3) 102	Ap 2000 - June 02	VR

Table 2. List of *Homoptera* caught during winter on conifers or other perennial plants surronding peach areas.

Genus and species	Nb.of groups (ins./group)	Caught plants	Periods and locations
Empoasca spp.	51 (3) 153	Conifers, *R. fruticosus*	Winter 01/RA
Trioza spp.	7 (3) 21	*Pinus nigra*	Winter 01/RA
Empoasca spp.	26 (3) 78	Conifers, *R.fruticosus*	Winter 02/RA
Trioza spp.	4 (3) 12	*P. nigra, R. fruticosus*	Winter 02/RA
Cacopsylla affinis	5 (2) 10	Hedge 1	Winter 02/VR
Cacopsylla crataegi	3 (2) 6	Hedge 1	Winter 02/VR
Cacopsylla pyrisuga	3 (2) 6	*Cupressus leylandii*	Winter 02/VR
Cacopsylla pyrisuga	4 (2) 8	Hedge 1 or 2	Winter 02/VR
Cacopsylla pulchella	2 (2) 4	Hedge 1	Winter 02/VR
Bactericera curvatinervis	2 (2) 4	Hedge 2	Winter 02/VR
Trioza urticae	3 (2) 6	Hedge 2	Winter 02/VR

Hedge 1: *C. sanguinea, Crataegus oxyacantha, Pyracantha coccinea, P. cerasifera, R. fruticosus;*
Hedge 2: *Hedera helix, Platanus acerifolia, Robinia pseudoacacia, R. fruticosus*.

Table 3. ESFYP detection from the insects caught**.**

Species	Nb.infected/ tested groups	Caught in (periods and locations)	Caught on (species)
Macrosteles cristatus	2/48	Ag 00 - Pescantina (VR)	*P.persica*
Bactericera curvatinervis	1/3	Giu 02 - S. Lucia (VR)	*P. persica*
Laurotrioza alacris	1/1	Mag 02 - S.G.Lupatoto (VR)	*P. persica*
Trioza urticae	1/4	Mag 02 - S.G.Lupatoto (VR)	*P. persica*
Cacopsylla pruni	6/45	Giu 01 - Forlimpopoli (FC), Vallecchio (RM)	*P.spinosa*
	1/4	Mar 02 - Faenza (RA)	*P.cerasifera*
	4/16	Giu 02 - Longiano (RM)	*P.spinosa*
	2/8	Giu 01 - Arcè (VR)	*P. persica*
	5/10	Feb-Ap 02 - Castelnuovo d/G e S.G. in Salici (VR)	*P.cerasifera*
	3/9	Feb-Ap 02 - C.nuovo d/G e S.G.Lupatoto (VR)	*P. persica*
	4/30	Giu 02 - C.nuovo d/G (VR)	*P.cerasifera*
Cacopsylla pyrisuga	1/11	Feb 02 - S.G. Lupatoto (VR)	*C. leylandii*

Table 4. ESFYP detection from the wild perennial plants collected.

Species and nb. of examinated plants	Nb.infected/ tested plants	Locations of infected plants
Celtis australis	0/20	/
Cornus sanguinea	0/8	/
Corylus avellana	0/8	/
Cupressus leylandii	0/5	/
Fraxinus excelsior	0/3	/
Laurus nobilis	0/10	/
Prunus cerasifera	4/22	Castelnuovo d/G(3) (VR); Faenza(1) (RA)
Prunus mahaleb	1*/10	Castelnuovo d/G. (VR)
Prunus spinosa	6**/32	Forlimpopoli (2) (FC); Longiano (2) e Vallecchio (2) (RM)
Prunus spp.	1***/3	Castelnuovo d/G. (VR)
Rosa canina	0/12	/
Rubus fruticosus	0/12	/

*: plant with witches'broom symptoms;
**: 3 plants with off-season growth symptoms;
***: plant with leaf reddening symptoms.

Table 5. Correlation of the number of infected insects with the wild infected plants collected in the same areas.

Locations	Nb.infected/tested groups (insects)	Nb.infected/ tested plants
PRUNUS CERASIFERA		
Faenza (RA)	1/4	1/3
Forlimpopoli (FC)	0/26	0/13
Castelnuovo del Garda (VR)	8/35	3/6
PRUNUS SPINOSA		
Faenza (RA)	/	0/5
Forlimpopoli (FC)	4/9	2/5
Longiano (RM)	4/16	2/6
Borgo Tulliero (RA)	0/16	0/5
Vallecchio (RM)	2/8	2/8