Occurrence and Epidemiology of *European Stone Fruit Yellows* Phytoplasma in Spain

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Abstract

The results of studies carried out in two fruit producing areas of Spain (Catalonia and Extremadura) have shown that phytoplasmas related to the *European stone fruit yellows* group are harmful to plum, peach, apricot, nectarine and cherry trees. The occurrence of affected plum trees was very high in some fruit fields of Baix Llobregat (Catalonia) (25-78 %), whereas in Extremadura the incidence of affected trees in the surveyed plots was low (1-5%). The *European stone fruit yellows* phytoplasma was the only one detected in the tests on the plum, peach, cherry, apricot and nectarine samples. The identification of vectors for the European stone fruits yellows was done in the Baix Llobregat area (Catalonia), studying species known to be liable to transmit phytoplasmas. Two species of *Cacopsylla* were captured in all affected fruit fields, *Cacopsylla pruni* and *Cacopsylla pulchella*. PCR analyses showed that most individuals of *C. pruni* were carriers of the phytoplasma, therefore *C. pruni* could also transmit the phytoplasma in this area.

INTRODUCTION

Phytoplasmas are considered to be the causal agent of several stone fruit disorders. Their etiology is associated with genetically similar phytoplasmas, for which the name *European stone fruit yellows* phytoplasma has been adopted (Lorenz et al., 1994). This phytoplasma belongs to the Apple proliferation group (Seemüller et al., 1998). These diseases have been known in Europe for many years. The first published description of them was in Italy by Goidanich (1933). In Spain the stone fruit disorders were reported mainly in plums and apricots in Catalonia (Sala, 1935; Torres et al., 2002) and Valencia (Sanchez Capuchino and Forner, 1973, Llacer et al., 1986). In plum trees the symptoms are early blooms and shoots during January and February, responsible for a lack of fructification and substantial losses of production. In peach trees there is early fructification and premature blushing as well as chlorosis irregularly distributed in the tree during autumn.

Control strategies against phytoplasma-borne diseases should be based on sound knowledge of the phytoplasma infections present in the area and their epidemiology. The identity of the vector or vectors of the ESFY phytoplasma was unknown until 1998 when Carraro and his collaborators demonstrated the transmission of ESFY through *Cacopsylla pruni* to Japanese plum in Italy.

Surveys carried out in Spain and France in areas where the disease was widespread showed a great number of cicadelidae species which are potential vectors of phytoplasmas. Among the species linked to the disease were: *Austroagallia sinuata, Euscelis lineolatus, Neoaliturus fenestratus, Neoaliturus haematoceps* and *Psammotettix striatus* in Spain (Llacer et al., 1986). A large number of cicadellidae, delphacidae and cixiidae species, which were captured in infected plots in France were analyzed by PCR. None of the species was ESFY positive except one individual of *Synophropsis lauri*

(Jarausch et al., 2001).

The aim of this study was to ascertain the occurrence and relative importance of ESFY in different fruit producing areas of Spain. Furthermore, the study includes the identification of the vector or potential vectors in severely affected plum plots in Catalonia.

MATERIAL AND METHODS

Survey and Phytoplasma Detection

A survey was conducted in the late summer of 2001 and 2002, in different fruit producing areas of Spain; in Catalonia and Extremadura. In Catalonia the areas sampled were Baix Llobregat (Barcelona), Lleida and Tarragona. In each area, different plots of plums, peach, apricot, cherries and nectarine trees were sampled. Phytoplasma disease incidence (% of plants with phytoplasma symptoms) was estimated for each plot by visual inspection of 100 plants. Samples from plants with phytoplasma infection symptoms were collected to identify the phytoplasma involved.

Insect Capture

The identification of vectors of the European stone fruits yellows was done in the Baix Llobregat area (Catalonia) studying those species known to be liable to transmit the phytoplasma. Insects were captured in three ESFY-infected plots of plum on sticky yellow traps of 20 x 20 cm placed within or near the plots. The traps were placed 0.5 and 1 m above the soil surface and were replaced weekly from February to July.

Phytoplasma Detection in Plants and Insects by PCR

PCR was used for phytoplasma detection in plant tissues and insects. DNA was isolated from approximately 1g of fresh plant material, leaf midribs, buds or stems, using the phytoplasma-enrichment procedure of Ahrens and Seemüller (1992). DNA from insects was extracted by grinding 1-5 insects as described by Daire et al. (1992).

Nested PCR was used for specific detection of the phytoplasma. The universal primers P1/P7 (Smart et al., 1996) located at the 16S rDNA and 23S rDNA gene respectively, were used in the first step. The second step was performed with the fO1/rO1 specific primers for the Apple proliferation (AP) group (Lorenz et al., 1995). The samples giving negative results with the specific primers were analyzed in the second step with the universal primer pair fU5/rU3 to determine the presence of phytoplasmas different from the AP group. Ten µl of the mixture containing the DNA amplified in the second step was directly digested overnight at 37ºC with 1 unit of T*ru* I and R*sa* I enzyme.

RESULTS

Survey

The results of the survey showed that the occurrence, distribution and relative incidence of phytoplasma infections varied between different growing regions (Table 1). In the Llobregat area (Barcelona) we found the highest number of affected plots belonging to different plum cultivars, native as well as Japanese plums. The incidence of symptomatic trees in the affected plum plots was between 25 and 78%. A plot with peach trees affected by the phytoplasma was identified in the same area. Affected plots of peach with an incidence of between 5 and 10% were identified in Lleida, while in Tarragona, affected plots of apricot and plum were found (Table 1).

In Extremadura the affected plum plantations belonged mainly to the cultivars Son Gold and Black Diamond, where the incidence of the disease in the affected plots was 1-5%. Diseased peach and nectarine trees were also identified (Table 1).

Nested PCR with universal and specific primers, showed that all samples from the symptomatic trees selected were positive when fO1/rO1 primers were used, and a 1,050 bp amplification product was obtained (Figure 1). Patterns obtained from RFLP of

amplified sequences from symptomatic trees were indistinguishable from each other, and were identical to those of the ESFY phytoplasma strain (Marcone et al., 1996) (Fig. 1).

Identification of Vectors

The species of insects captured in the three plantations are listed in Table 2. Different species of leafhoppers and planthoppers were identified in the affected fruit fields, among them *Zygina flamigera, Idiocerus* sp. and *Empoasca* sp. (Table 2). Two species of *Cacopsylla* were captured in all affected fruit fields in the Baix Llobregat area, *Cacopsylla pruni* and *Cacopsylla pulchella*. PCR analyses showed that most individuals of *C. pruni* were carriers of the ESFY phytoplasma. This specie was present from March to June in the three plots studied (Fig. 2). *C. pruni* was first detected in plot 3-C which is a plantation close to a forest. PCR analyses also showed that some individuals of *C. pulchella* were positive of the phytoplasma.

DISCUSSION

In the Llobregat area (Catalonia) we found affected trees belonging to different plum varieties, old varieties such as *Prunus cerasifera* 'Llevador', 'Vallespir', 'Valentins', as well as new Japanese varieties, although the severity of the symptoms are greater in the latter, as has been mentioned by other authors (Desvignes and Cornaggia, 1982, Giunchedi et al., 1982). The severity in the new Japanese plum plantations is most probably due to the fact that the disease has been widespread in the zone for a long time, and the old varieties are more tolerant to the disease and act as an reservoir for the phytoplasma. Furthermore, local cultivation practice such as that of cleftgrafting onto the same rootstocks where the variety is dead due to the disease, does not favour its elimination.

In Lleida (Catalonia) most of the analyzed peach trees with symptoms were also positive by PCR. There is the possibility of confusion of symptoms with iron chlorosis, which is prevalent in this area, although peach trees affected by phytoplasmas can be distinguished by their reduced growth, curling and deformation of the leaves and irregular distribution of the symptoms on the tree.

The disease occurrence in Extremadura is minor mainly due to the fact that the plantations are new and mainly certified materials have been used. However, the evolution of the disease incidence as well as monitoring the presence of the vector of the ESFY in this area is necessary to avoid the disease extending further.

The study carried out in Catalonia for the identification of the vector or vectors of the ESFY in this territory has shown the existence of *C. pruni* from March to June. *C. pruni* is known to be a vector of ESFY in other countries, therefore it could also transmit the phytoplasma in this area. During March the population of *C. pruni* was higher in the plantations close to a forest and the main individuals were captured in wild *Prunus*. This data is in agreement with other authors who mention the preference of *C. pruni* for the wild *Prunus*, most probably an in-between host being the preferred host such as a conifer or other perennial plant (Jarausch et al., 2001, Carraro et al, 1998, Labone et al. 2000). The role of the other *Cacopsylla* captured, *C. pulchella*, in the spread of the disease is still to be studied.

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Tables

Table 1. Occurrence of ESFY on stone fruit samples collected from fields surveyed in Catalonia and Extremadura. DNA phytoplasma was detected by nested PCR with P1/P7 and fO1/rO1 primers.

Extremadura	Cultivar	Sampling site	% of symptomatic trees in surveyed plot	PCR detection/ Phytoplasma
Prunus salicina	Son Gold	San Benito (Badajoz)	3%	ESFY
Prunus salicina	Black Diamond	Valdelacalzada(Badajoz)	1-5%	ESFY
Prunus salicina	Black Diamond	La Encomienda (Badajoz)	1-3%	ESFY
Prunus persica	Spring Lady	Valdelacalzada(Badajoz)	2%	ESFY
Prunus persica	Catherine	Valdelacalzada(Badajoz)	1%	ESFY
Prunus persica	Spring Lady	Zurbaran (Badajoz)	1-2%	ESFY
Prunus persica	Snow Queen	San Benito (Badajoz)	2%	ESFY
Prunus persica	Silvery	Valdelacalzada(Badajoz)	1%	ESFY
Catalonia				
Prunus cerasifera	Vallespir	Llobregat (Barcelona)	25-78%	ESFY
Prunus cerasifera	Llevador	Llobregat (Barcelona)		ESFY
Prunus cerasifera	Valentins	Llobregat (Barcelona)		ESFY
Prunus salicina	Juliana	Llobregat (Barcelona)		ESFY
Prunus salicina	Rosella	Llobregat (Barcelona)		ESFY
Prunus persica	Large White	S.Vicenç (Barcelona)	1%	ESFY
Prunus salicina	Black Diamond	Ginestar (Tarragona)	1%	ESFY
Prunus salicina	Fortuna	Ginestar (Tarragona)	1%	ESFY
Prunus salicina	Angeleno	Granja d'Escarp (Lleida)	1-5%	ESFY
Prunus persica	Catherine	Alcarras (Lleida)	5-15%	ESFY
Prunus avium	Prime Giant	Seròs (Lleida)	5%	ESFY
Prunus armeniaca	Moniqui	Benissaret (Tarragona)	2%	ESFY

Table 2. Number of insects and trapping data of the most abundant species of cicadellidae and psyllidae in three affected plots of Llobregat area (Catalonia)

Plot 1-O		14-3	25-3	1-4	8-4	16-4	5-5	14-5	23-5	2-6	13-6	2-7	Total
Cacopsylla	*pruni*	6	2	2	3	6	1			9	16		45
Cacopsylla	*pulchella*							1		2			3
Empoasca	*sp*	20	5	23	3	3	6	19	27	60	183	253	602
Idiocerus	*sp*				1								1
Oncopsis	*sp*			1									1
Zygina	*flamigera*	4		1									5
Zygina	*sp*		5	11			1				11	5	33
Zyginidia	*scutellaris*				5								5
Total		30	12	38	12	9	8	20	27	71	210	258	695

Plot 2-S		14-3	25-3	1-4	8-4	16-4	5-5	14-5	23-5	2-6	13-6	2-7	Total
Cacopsylla	*pruni*		7	3	2	3	3	1		11	8		38
Cacopsylla	*pulchella*						24	12	4	6			46
Empoasca	*sp*	12	2	10	10	10	21	54	34	114	243	72	582
Idiocerus	*sp*	3			1								4
Zygina	*flamigera*	4								47			51
Zygina	*sp*		4	5			2				87		98
Total		19	13	18	13	13	50	67	38	178	338	72	819

Plot 3 - C		14-3	25-3	1-4	8-4	18-4	24-4	3-5	14-5	2-6	13-6	Total
Cacopsylla	*pruni*	18	26	31	42	11	14	8	5	4	11	170
Cacopsylla	*pulchella*						5	172	7	1	3	188
Agallia	*laevis*								2			2
Artianus	*sp*									1		1
Euscelidius	*variegatus*					1						1
Empoasca	*sp*	20	24	15	19	9	22	26	6	37	162	340
Zygina	*flamigera*			10								10
Zygina	*sp*	3	5		14	5			1			28
Total		41	55	56	75	26	41	206	21	43	176	740

Figures

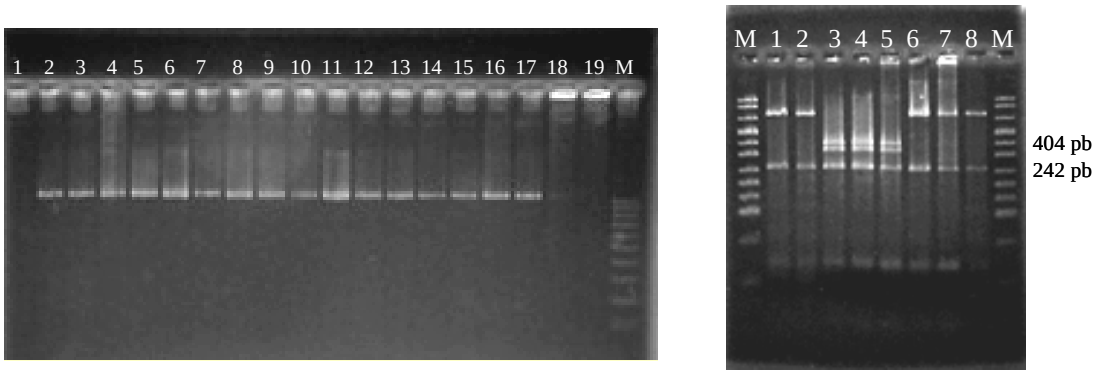

Fig. 1. Nested PCR amplification of phytoplasma ribosomal DNA from symptomatic plums (2-10), nectarine trees (11-14), apricot trees (15-16), peach trees (16 and 17) and 1(healthy control), using the universal primers, P1/P7 followed by the specific primers, fO1/rO1. Right, R*sa* I restriction profiles of 16S rDNA amplified with primer pair fO1/rO1 (1,2,6,7 and 8: pear samples with PD. 3,4 and 5: symptomatic plums). M:Marker pUC 8 mix

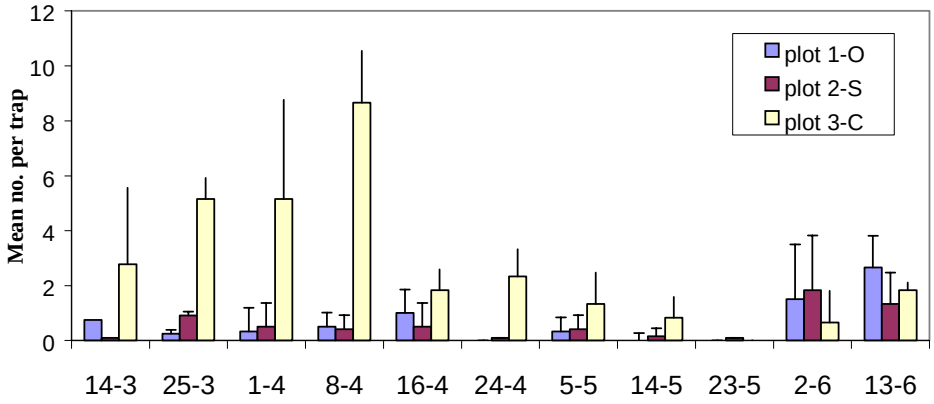

Fig. 2. Population dynamics of *Cacopsylla pruni* in three affected plots of plum in Llobregat area (Catalonia)