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Detection of different types of phytoplasmas in stone fruit orchards in northern Italy

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Abstract

Phytoplasmas belonging to the ribosomal subgroup 16SrIII-A (X disease and related phytoplasmas) were identified in two plants of declining cherry during the monitoring of two experimental plum orchards. The same phytoplasmas were identified also in *Philaenus spumarius* samples collected in yellow sticky traps located inside the plum orchards. '*Candidatus* Phytoplasma prunorum' was detected in one declining cherry plant and in samples of *Fieberiella florii.* Among the insect species tested for phytoplasma presence *Cacopsylla pruni* was only captured once, and the sample resulted negative to the molecular tests.

Key words: Cherry decline, PCR/RFLP analyses, insects, epidemiology.

Introduction

Vignola is one of the most important plum and cherry growing areas in northern Italy. From the early thirties a disease called "leptonecrosis" affecting plum, cherry and apricot (Goidanich, 1933; 1934) was reported in the area. This disease now named European stone fruit yellows (ESFY) is associated with '*Candidatus* Phytoplasma prunorum' (Seemüller and Schneider, 2004). Severe outbreaks were observed over the years, mainly after Japanese plum varieties were introduced into the area. Recently, two experimental plum orchards where tolerance/resistance to ESFY phytoplasma is studied were monitored. Surveys were extended to verify phytoplasma presence also in cherry plants growing nearby. Symptomatic cherry plants, about 10 year's old, showed poor vegetation, reduced leaf size, upward curled leaves with discolorations ranging from yellow to red. Insects collected on yellow sticky traps located in the experimental plum orchards were also tested in order to verify phytoplasma presence in the environment.

Materials and methods

Leaf and small branch samples were collected from six cherry plants (3 symptomatic and 3 asymptomatic) near the experimental plum fields. A total of 12 samples were employed for nucleic acid extraction using a chloroform/phenol method (Prince *et al*., 1993).

During 2005-2006 insect monitoring was carried out from March to the end of July with yellow sticky traps randomly distributed in the two experimental plum orchards. Insects collected from traps were maintained in alcohol 100% at 4 °C for classification; nucleic acid extraction from interesting species was then carried out with a rapid CTAB-based procedure (Angelini *et al*., 2001) on batches of 3 insects per species.

Direct PCR with P1/P7 primer pair (Deng and Hiruki, 1991; Schneider *et al.*, 1995) and nested amplification with primers R16F2/R2 and/or R16(X)F1/R1 (Lee *et al*., 1995) were performed on both plant and insect samples following a described protocol (Schaff *et al.,* 1992). Reference phytoplasma strains were employed as positive controls. Amplicons obtained with R16F2/R2 and R16(X)F1/R1 primer pairs were subjected to RFLP analyses with *Tru*I, *Rsa*I and *Ssp*I restriction enzymes to verify phytoplasma identity.

Results and discussion

Only samples from both phloem and leaf midribs of symptomatic cherry plants gave positive results in nested PCR amplifications. RFLP analyses indicate the presence of '*Ca.* P. prunorum' in one of the trees and of phytoplasmas belonging to ribosomal subgroup 16SrIII-A in the other two symptomatic cherry plants (figure 1).

Several potentially phytoplasma vectors were captured in yellow sticky traps (table 1). *Cacopsylla pruni* Scopoli, vector of '*Ca*. P. prunorum' (Carraro *et al*., 1998) was only captured once and was negative to molecular tests. Nested PCR amplification detected phytoplasma presence in 4 out of the 6 species tested. RFLP analyses with *Tru*I showed restriction profiles of phytoplasmas related to ribosomal group 16SrV (Elm yellows) in *Anoplotettix* spp. and *Metcalfa pruinosa* Say. Ribosomal group 16SrX (Apple proliferation) and subgroup 16SrIII-A (Peach X disease) were respectively detected in *Fieberiella florii* (Stål) and *Philaenus spumarius* L.. Further RFLP analyses on R16(X)F1/R1 amplicons from *F. florii* with *Rsa*I and *Ssp*I restriction enzymes allowed the identification of '*Ca.* P. prunorum' (table 1).

The finding of '*Ca.* P. prunorum' in cherry trees indicates an epidemic inside and around the experimental plum orchards. These phytoplasmas were detected also in a number of plum plants under evaluation for ESFY resistance/tolerance.

The finding of 16SrIII-A phytoplasmas in two declining cherry trees represent the first evidence of this phytoplasma presence in cherry in Italy. Previous reports of 16SrIII phytoplasmas in cherry only refers to ribosomal

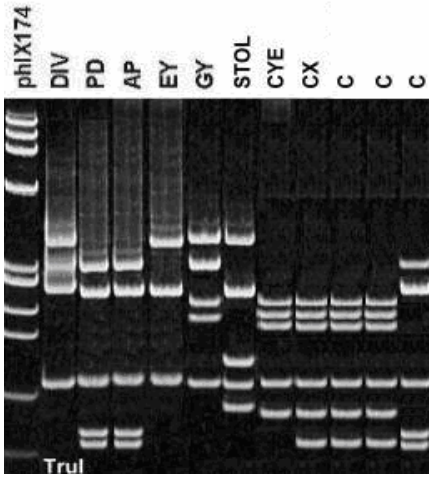

Figure 1. RFLP profiles on 5% polyacrylamid gel of phytoplasmas from symptomatic cherry trees (C) after amplification with primers R16F2/R2. Controls: DIV, *Diplotaxis* virescence (16SrI-B); PD, pear decline (16SrX-C); AP, apple proliferation (16SrX-A); EY, elm yellows (16SrV-A); GY, grapevine yellows from Germany (16SrI-C); STOL, stolbur from pepper from Serbia (16SrXII-A); CYE, clover yellow edge (16SrIII-B); CX, X disease of peach (16SrIII-A).

Table 1. Results of molecular tests on batches of 3 insects per species collected in experimental plum orchards.

INSECT SPECIES	% of phytoplasma infection (n. of tested batches)		RFLP identification
Fieberiella florii	100	(1)	16SrX-B
Anoplotettix sp.	7.32	(14)	16SrV
Philaenus spumarius	12.51	(3)	16SrIII-A
Metcalfa pruinosa	9.06	(4)	16SrV
Dicthiophara europaea	-	(9)	-
Empoasca spp.	-	(3)	-

subgroup 16SrIII-B phytoplasmas (Paltrinieri *et al*., 2001; 2007). The same phytoplasmas were identified in *P. spumarius* from yellow sticky traps confirming presence of this phytoplasma in the environment. However, the role of this insect as possible phytoplasma vector is under investigation in several different environments.

The scarcity of captures of *C. pruni* and the contemporeneous identification of '*Ca*. P. prunorum' in *F. florii* represent a finding that deserves extensive further investigations. It is necessary to reevaluate the insect vector pattern of ESFY especially in southern European stone fruit cultivation areas and in the warmest European regions.

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