Bulletin of Insectology ***64*** *(Supplement): S143-S144, 2011*
ISSN 1721-8861

Population dynamics of *Cacopsylla pruni* and '*Candidatus* Phytoplasma prunorum' infection in North-Eastern Italy

Paolo ERMACORA, Francesca FERRINI, Nazia LOI, Marta MARTINI, Ruggero OSLER
Department of Agriculture and Environmental Sciences, University of Udine, Udine, Italy

Abstract

A two year surveys was conducted in Friuli Venezia Giulia region (north-eastern Italy) with the aims to improve knowledge about biological characteristics of *Cacopsylla pruni* Scopoli, and to ascertain its role in the spread of different '*Candidatus* Phytoplasma prunorum' strains. Insects were captured starting from March till the end of April in apricot orchards with high European stone fruit yellows incidence. For phytoplasma detection a nested PCR protocol based on *ace*F gene was adopted. Results confirmed that the percentage of phytoplasma positive insects increased from March to April, because they fed on infected trees. During the reimmigration season the percentage of females was higher than that of males, especially after coupling time. Besides a very high percentage of infected *C. pruni*, no significant differences related to the sex were observed. '*Ca*. P. prunorum' strains of *ace*F-A, *ace*F-C and *ace*F-E subgroups, were mainly found in the captured *C. pruni* population.

Key words: *ace*F gene, nested PCR, European stone fruit yellows.

Introduction

Cacopsylla pruni (Scopoli) is the vector of *'Candidatus* Phytoplasma prunorum', associated with European stone fruit yellows (ESFY) disease (Carraro *et al*., 1998). *C. pruni* is a univoltine psyllid widespread in Europe. In spring eggs of *C. pruni* are laid by reimmigrant adults on *Prunus* spp.; individuals of new generation develop on *Prunus* spp. and new adults abandon the original host in summer to overwinter on conifers (Thébaud *et al.,* 2009). In Friuli Venezia Giulia (FVG) region, north–eastern Italy, several *Prunus* species were reported as natural host of ESFY (Carraro *et al.*, 2002) and different strains of '*Ca*. P. prunorum' with different virulence were observed (Ermacora *et al.*, 2010). Recently, in order to investigate phytoplasma strains variability, molecular tools for '*Ca*. P. prunorum' detection and characterization were developed (Danet *et al.*, 2008, Martini *et al.*, 2010). Although in our conditions *C. pruni* population density usually does not cause direct damages to the crops their ability to transmit '*Ca*. P. prunorum' requires a correct orchard management in order to prevent the spread of ESFY. With the aim to monitor the presence of *C. pruni* and evaluate their infectivity for a correct integrated pest management, an extensive survey was conducted during 2010 and 2011 in an area with high ESFY disease pressure. Another aim of this research was to acquire more knowledge about the biology of *C. pruni*.

Materials and methods

During 2010 and 2011, from March till the end of April, a total of 383 individuals of reimmigrant *C. pruni* were collected in apricot orchards located in areas with high ESFY disease pressure. In the monitored orchards ESFY incidence was very high (about 90% of PCR positive plants) and management program was insecticide free in order to avoid interferences with the natural dynamic of *C. pruni* population. Insects were captured by the beating method, and immediately stored in 80% ethanol. *C. pruni* were individually observed under stereomicroscope for proper identification and sex characterization. Nucleic acids were extracted from the insects according the protocol proposed by Doyle and Doyle (1990) with slight modifications.

Protocol adopted for '*Ca*. P. prunorum' detection in the samples was based on *aceF* gene, with a direct PCR with primers AceFf1/AceFr1 followed by nested-PCR with primers pair AceFf2/AceFr2 (Danet *et al.*, 2008). RFLP of nested-PCR amplicons using endonucleases *Bpi*I, *Hae*III and *Tsp*509I was adopted for characterization of '*Ca*. P. prunorum' strains (Martini *et al.*, 2010).

Results

During the two considered years, presence of reimmigrant *C. pruni* was detected from March 22 till the end of April, with a peak of population in the period 12-16 April. Mating period lasting from the beginning till the 20th of April. Male and female percentages varied in the considered period with a substantial reduction of male presence. Percentage of insects that hosted '*Ca*. P. prunorum' increased during their presence on apricots without significant differences between males and females. Considering the characterization of '*Ca*. P. prunorum' strains present in the insect analysed population, a clear prevalence of strains of *ace*F-A, *ace*F-C and *ace*F-E subgroups was reported, *ace*F-B strains were rare and *ace*F-D strains were not detected; in 19% of the cases we found mixed infections.

Table 1. Presence of males and females of *C. pruni* during the sampling period and their infectivity.

	Capture period					
	22-25 March	30 March-02 April	05-09April	12-16 April	19-22 April	26-30 April
% males	34.6	10.5	38.9	36.1	25.0	11.5
% females	65.4	89.5	61.1	63.9	75.0	88.5
% males '*Ca*. P. prunorum' positive	57.9	75.0	42.3	71.4	100.0	33.3
% females '*Ca*. P. prunorum' positive	55.6	61.8	63.7	84.6	80.0	82.6

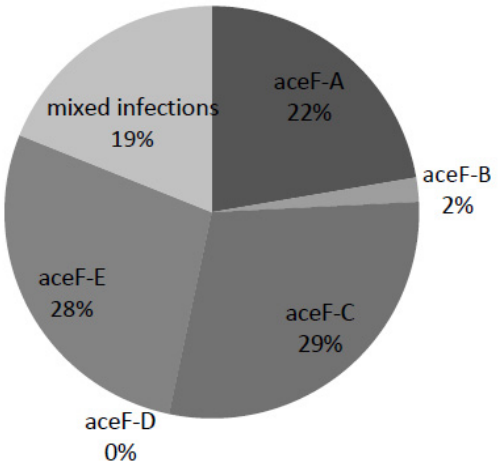

Figure 1. Characterization of '*Ca*. P. prunorum' strains in *C. pruni* based on *aceF* gene.

Discussion

This survey confirmed the presence of *C. pruni* and its high infectivity in the FVG region, where ESFY disease is endemic and affects wild and cultivated *Prunus*. As reported by Carraro *et al.* (2004) it was confirmed that overwintering adults infectivity increase after their re-immigration on infected *Prunus* spp.. In our case the infection rate of the first capture reimmigrants was 56.4% and reached a plateau slightly exceeding 80% in the last two captures. Results obtained on the male/female percentages when the reimmigrant individuals are present in the orchards, evidenced the longevity of females that at the end of the monitored period represented 88.5% of the population. The longevity of females could be explained from an evolutionary point of view considering the crucial role of the females for the species survival. Another hypothesis to explain the variability of the male presence during the monitoring period and the changes in their mean infectivity could be a mortality of the males after the copulation. Concerning '*Ca*. P. prunorum' molecular characterization, the obtained results indicated mainly the presence of strains of AceF-A,-C, and -E subgroups and a sporadic presence of AceF-B strains. Mixed infections were also detected and explained with the presence of the same strains in plants of *Prunus* spp. in the same area.

Acknowledgements

The authors thank Alberto Loschi, Alfeo Agosti and the farm "La Miduna", Tauriano, Pordenone.

References

CARRARO L., OSLER R., LOI N., ERMACORA P., REFATTI E., 1998.- Transmission of the European stone fruit yellows phytoplasma by *Cacopsylla Pruni*.- *Journal of Plant Pathology,* 80(3): 233-239.

CARRARO L., FERRINI F., ERMACORA P., LOI N., 2002.- Role of wild *Prunus* species in the epidemiology of European stone fruit yellows.- *Plant Pathology,* 51: 513–517.

CARRARO L., FERRINI F., LABONNE G., ERMACORA P., LOI N., 2004.- Seasonal infectivity of *Cacopylla pruni*, vector of European stone fruit yellows phytoplasma.- *Annals of Applied Biology*, 144: 191-195.

DANET J. L., BAHRIZ H., CIMERMAN A., FOISSAC X., 2008.- New molecular typing tools to monitor fruit tree phytoplasma variability in the 16SrX taxonomic group.- *Acta Horticolturae*, 781: 343-349.

DOYLE J. J., DOYLE J. L., 1990.- Isolation of plant DNA from fresh tissue.- *Focus*, 12: 13-15.

ERMACORA P., LOI N., FERRINI F., LOSCHI A., MARTINI M., OSLER R., CARRARO L., 2010.- Hypo- and hyper- virulence in apricot trees infected by European stone fruit yellows.- *Julius-Kühn-Archiv*, 427: 197-200.

MARTINI M., FERRINI F., DANET J.C., ERMACORA P., GULSEN S., DELIC D., LOI N., FOISSAC X., CARRARO L., 2010.- PCR/RFLP based method for molecular characterization of '*Candidatus* Phytoplasma prunorum' strains using *acef* gene.- *Julius-Kühn-Archiv*, 427: 386-391.

THÉBAUD G., YVON M., ALARY R., SAUVION N., LABONNE G. 2009.- Efficient transmission of '*Candidatus* Phytoplasma prunorum' is delayed by eight months due to a long latency in its host-alternating vector.- *Phytopathology,* 99: 265-273.

Corresponding author: Paolo ERMACORA (e-mail: paolo.ermacora@uniud.it), Department of Agriculture and Environmental Sciences, Via delle Scienze 208, 33100 Udine, Italy.