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'*Candidatus* Phytoplasmas mali': identification of potential insect vectors in Spanish apple orchards

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Abstract

Samplings realized in apple plots of different geographical areas of Spain indicated the presence of the two species of *Cacopsylla* reported as vectors of the apple proliferation disease. The population evolution of *Cacopsylla picta* and *C. melanoneura* was followed during two years in the two regions of Spain where these species were identified. The population showed two peaks, one for adults re-immigrants, which in the two years occurred in early April and other for new generations between June and July.

Key words: Apple proliferation, '*Candidatus* Phytoplasma mali', *Cacopsylla picta, Cacopsylla melanoneura*.

Introduction

Apple proliferation (AP) disease is associated with '*Candidatus* Phytoplasma mali' and it is one of the most important phytoplasma diseases in Europe causing considerable economic losses in apple orchards. This phytoplasma is present in many countries of Europe such as, Germany, France, Switzerland and Italy. Some of the symptoms of AP are witches' broom, elongated stipules, chlorosis and early leaf reddening. The fruits have lower size and worse quality. In Spain it has been found in commercial plots of different varieties of apple in Asturias and the Basque Country and also in plant material from several nurseries.

In the last years there has been a significant increase of the disease in Europe. This new outbreak (Danet *et al*., 2011) may be due to the emergence of new isolates or to the presence of more effective vectors. For this reason, the identification and control of vectors and a good understanding of the population dynamics, insect infectivity and the host plants for both, phytoplasma and vectors in a specific region are of great importance.

In Europe two psyllid species have been described as vectors of the phytoplasma: *Cacopsylla picta* (syn. *C. costalis*) and *Cacopsylla melanoneura*. However, their distribution, infectivity and transmission capacity vary between different geographical areas (Jarausch *et al*., 2007; Tedeschi *et al*., 2003; Mayer *et al*., 2009). During two years, the search of psyllids, vectors of '*Ca.* P. mali', and of other potential vectors was extended to different geographical areas of Spain.

Materials and methods

The insects were captured on yellow sticky traps (10 x 16 cm) placed in apple orchards of different geographical areas of Spain (Catalonia, Asturias and Basque Country), during 2010 and 2011. The traps were replaced every 15 days. The insect capture was done from February until the beginning of August. All specimens captured were separated and classified for DNA extraction and PCR analysis.

DNA from insects was extracted by grinding 1-5 insects, depending on the species, following the methods used in previous studies (Garcia-Chapa *et al*., 2005).

Results

In the sampled plots were caught several species of psyllids and leafhoppers were caught. The two species of *Cacopsylla* reported as vector of the disease have been identified in the Basque Country and Asturias. In a plot located in Oberlaún (Basque Country), with a higher incidence of the disease *Cacopsylla picta* and some individuals of *C. melanoneura* have been identified. In Aduna (Basque Country) and Siero (Asturias) C. *melanoneur*a has been identified. The population evolution of both these species showed two population peaks, one for adults re- immigrants, which for both years occurred in early April and another one for new generations between June and July (figure 1). The higher population of these species, know as vectors of the '*Ca*. P. mali', has been found in the most affected plot (Oberlaún, Guipúzcoa) (figure 1, table 1).

Other species of cicadellidae known as potential vectors of phytoplasmas were captured in the sampled plots (table 1). In the plots of Catalonia located very close to plum, peach and pear plots, other *Cacopsylla* species such *as C. pruni* and *C. pyri* were captured (table 1).

The PCR analyses of the two species cited as vectors of the phytoplasma (*C. picta* and *C. melanoneura*) are being carried out with the aim to determine the percentage of individuals carrying the phytoplasma.

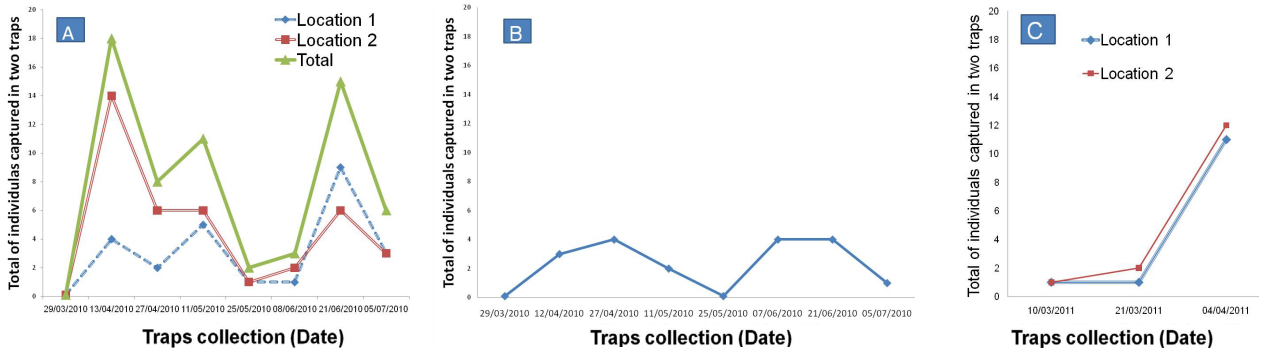

Figure 1. Population evolution of *C. picta* in (A) Oberlaún (Basque Country) and of *C. melanoneura* in (B) Siero (Asturias) in 2010. (C) Population evolution in 2011 in Oberlaún (Basque Country). (In colour at www.bulletinofinsectology.org)

Table 1. Species of cicadelidae and psyllidae captured in different apple orchards of Basque Country, Asturias and Catalonia.

Species	Basque Country	Asturias	Catalonia
Agallia sp.	0	0	2
Cacopsylla picta	72	1	0
Cacopsylla melanoneura	9	12	0
Cacopsylla sp.	0	0	24
Cacopsylla crataegui	0	0	1
Cacopsylla pruni	0	0	72
Cacopsylla pulchella	0	0	41
Cacopsylla pyri	0	0	44
Cercopidae sp.	0	6	0
Ciixidae sp.	8	0	1
Empoasca sp.	44	3	0
Erythroneura sp	10	1	0
Euscelidius variegatus	0	2	0
Jassidae	2	10	0
Macrosteles sp.	1	4	0
Psillidae	1	2	15
Triozidae	0	0	6
Zygina	28	8	0

Discussion

In Spain *C. picta* is also the insect species that can be related with a higher apple proliferation incidence in the sampled plots such as happens in other European areas (Jaraush *et al*., 2007). In other plots where the disease is present but has a lower incidence, *C. melanoneura* was identified. In the other sampled areas of Spain where the disease is not present, these species have not been identified. The absence of vectors can explain why the disease is not present, despite that the phytoplasma was sporadically identified in some cases also in certified plant material (Batlle *et al*., 2004).

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