Bulletin of Insectology **60** *(2): 195-196, 2007*
ISSN 1721-8861

Acquisition capacities of the overwintering adults of the psyllid vectors of '*Candidatus* Phytoplasma mali'

Federico PEDRAZZOLI[1], Valeria GUALANDRI[1], Flavia FORNO[1], Luisa MATTEDI[1], Valeria MALAGNINI[1], Antonella SALVADORI[1,2], Gabriele STOPPA[2], Claudio IORIATTI[1]
[1]*IASMA Research Centre, Plant Protection Department, San Michele all'Adige (TN), Italy*
[2]*University of Trento, Computer and Management Sciences Department, Trento, Italy*

Abstract

The acquisition of '*Candidatus* Phytoplasma mali' by the overwintering adults of *Cacopsylla melanoneura* and *Cacopsylla picta* was studied by feeding the insects on infected plants for different periods (1-2, 4 and 6 days). After the acquisition, some individuals were directly frozen prior analysis and some others were put on healthy plants to assess the multiplication of the pathogen. The phytoplasma titre in the insects was quantified by real-time PCR (SYBR™ Green technology).

The analyses indicated that the two species did not differ in the mean phytoplasma titre found during the acquisition. In both species the multiplication of the pathogen within the insect started few days after the acquisition, with a significant increase in the titre detected by PCR. However, the maximum level of phytoplasma was already reached after 4 days of acquisition. After the multiplication period on healthy plants only some individuals of *C. picta* showed an important increase in phytoplasma concentration indicating an efficient phytoplasma multiplication in these individuals.

Key words: apple proliferation, *Cacopsylla melanoneura*, *Cacopsylla picta*, real-time PCR.

Introduction

Apple proliferation is a phytoplasma disease seriously affecting apple trees in many fruit growing regions across Europe. The etiological agent, '*Candidatus* Phytoplasma mali', is transmitted in a circulative, propagative manner by two species of psyllids (Homoptera: Psyllidae): *Cacopsylla melanoneura* (Förster) and *Cacopsylla picta* (Förster).

In Trentino (Italy) *C. melanoneura* is present mainly in bottom valleys, while *C. picta* is typically diffused in hill environments (Tomasi *et al.*, 2000). These species spend only few months in the orchard, accomplishing one generation per year, and then migrate on forest shelter plants.

C. melanoneura, which has been demonstrated to be vector only in Italy, shows a variable transmission efficiency in the different Italian areas: it is the main vector of '*Ca.* P. mali' in Aosta Valley and Piedmont (Tedeschi *et al.*, 2002; Tedeschi and Alma, 2004), transmitting in all vital stages. On the other hand, a very low transmission efficiency was found in Trentino (Mattedi *et al.*, 2007), even though important percentages of infected individuals have been found in the orchards of other northern Italian regions (Poggi Pollini *et al.*, 2002).

The trials conducted in Italy confirmed that *C. picta* is a vector, transmitting the pathogen as neanid/nymph and new generation adult (Frisinghelli *et al.*, 2000; Mattedi *et al.*, 2007) or also as overwintering adult (Carraro *et al.*, 2007). Also in Germany, all the vital stages of this species have been demonstrated to be efficient vectors of the disease (Jarausch *et al.*, 2004).

The aim of this work was to study the acquisition dynamics of the overwintering adults of both species. Indeed, only a low percentage of the individuals appeared infected at the beginning of the migration, while this number can increase with the time spent on the infected trees (Tedeschi *et al.*, 2003).

Materials and methods

The experiments were conducted in 2005 on single plants, kept in plastic pots (27 cm in height by 10.5 cm in diameter), and under controlled conditions (photoperiod 16L: 8D; temperature 20 °C max./15 °C min.). The plants used in the acquisition experiments were micropropagated Golden Delicious apple-plants.

Individuals of the two psyllid species were collected in experimental fields at the beginning of the oviposition. The insects were put in groups of 10-15 individuals on plants infected with '*Ca.* P. mali' strain PM6.

Different acquisition periods were chosen: 1-2 days, 4 days and 6 days. Some individuals were frozen just after the acquisition, and some others were moved to healthy plants and kept there as long as they survived (up to 3-4 weeks) to allow the multiplication of the phytoplasma inside the vector.

At the end of the experiments, each insect, kept at -80 °C, was lyophilised and then homogenised. The total DNA was extracted with the CTAB procedure described by Doyle and Doyle (1990).

A real-time PCR was performed on the extracted DNA in order to have an absolute quantification of the phytoplasma titre in the insects, following the method developed by Jarausch *et al.* (2004).

A non-parametric statistical analysis (Kruskal-Wallis test) was performed on the data collected.

Results and discussion

Data collected are illustrated in figure 1. *C. melanoneura* reached only in few cases phytoplasma titres higher than 400,000 copies/insect; only few *C. picta* showed titres higher than 1,000,000 copies/insect. However, even though *C. picta* seemed to reach phytoplasma titres higher than *C. melanoneura*, the statistical analysis did

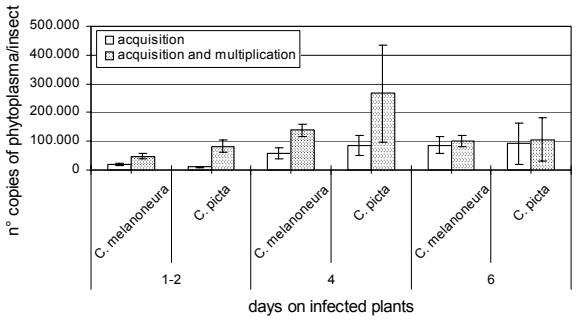

Figure 1. Phytoplasma titre (mean value ± SE) in the two psyllid species after different acquisition periods: comparison between acquisition only and acquisition plus multiplication.

not point out significant differences between the global values in the two species (P=0.197).

Regarding the comparison between the different acquisition periods, for *C. melanoneura* a highly significant difference (P=0.000) in the mean phytoplasma level emerged between 1-2 and 4 days of acquisition but no further increase in phytoplasma titre occurred between 4 and 6 days. For *C. picta* a similar trend of the titre could be observed, with the maximum peak reached at 4 days, but the differences between the acquisition periods were not significant (P=0.774).

The phytoplasma titre increased in both species when the psyllids were kept after the acquisition for a further period on healthy test plants. For *C. melanoneura* this increase was significant for the acquisition periods of 1-2 days and 4 days (P=0.018 and P=0.001, respectively). However, even after a long multiplication period the maximum phytoplasma concentration in an individual of *C. melanoneura* never exceeded the level of 400,000. Contradictory results were observed for *C. picta.* Although statistically significant for the acquisition period of 1-2 days (P=0.001), the increase in phytoplasma concentration after the multiplication period was rather low for the 1-2 and 6 days acquisition periods. By contrast, higher values were found for individuals with a 4 days acquisition period. This result is due to the few individuals in the latter group which showed phytoplasma concentrations higher than 1,000,000 copies after the multiplication period.

It can be hypothesised that the low titre found after short acquisitions represents the amount of pathogen ingested with the phloem sap. A first multiplication may happen in the gut (Weintraub and Beanland, 2006). Only in few individuals the pathogen reaches the specific regions inside the insect where the main multiplication takes place and, thus, the titre increases. In the present experiment, this was only observed for *C. picta* which underlines the previous results that this species is the main vector of AP in Trentino.

Acknowledgements

This work was funded by the SMAP2 Project (Scientific Coordinator Wolfgang Jarausch). The authors thank Rosaly Zasso for the help with the molecular analyses.

References

CARRARO L., FERRINI F., LABONNE G., ERMACORA P., LOI N., 2007.- Infectivity of *Cacopsylla picta* (Syn. *Cacopsylla costalis*), vector of '*Candidatus* Phytoplasma mali' in North East Italy.- *Acta Horticulturae*, in press.

DOYLE J. J., DOYLE J. L., 1990.- A rapid total DNA preparation procedure for fresh plant tissue.- *Focus*, 12: 13-15.

FRISINGHELLI C., DELAITI L., GRANDO M. S., FORTI D., VINDIMIAN M. E., 2000.- *Cacopsylla costalis* (Flor, 1861), as a vector of apple proliferation in Trentino.- *Journal of Phytopathology*, 148: 425-431.

JARAUSCH B., SCHWIND N., JARAUSCH W., KRCZAL, G., 2004.- Overwintering adults and springtime generation of *Cacopsylla picta* (Synonym *C. costalis*) can transmit apple proliferation phytoplasmas.- *Acta Horticulturae*, 657: 409-413.

JARAUSCH W., PECCERELLA T., SCHWIND N., JARAUSCH B., KRCZAL G., 2004.- Establishment of a quantitative Real-Time PCR assay for the quantification of apple proliferation phytoplasmas in plants and insects.- *Acta Horticulturae*, 657: 415-420.

MATTEDI L., FORNO F., CAINELLI C., GRANDO M. S., JARAUSCH W., 2007.- Research on '*Candidatus* Phytoplasma mali' transmission by insect vectors in Trentino.- *Acta Horticulturae*, in press.

POGGI POLLINI C., ZELGER R., WOLF M., BISSANI R., GIUNCHEDI L., 2002.- Indagine sulla presenza di psillidi infetti dal fitoplasma degli scopazzi del melo (AP= apple proliferation) in provincia di Bolzano, pp. 607-612. In: *Atti Giornate fitopatologiche*, vol. 2. Baselga di Pinè, Trento, Italy, 7-11 April 2002.

TEDESCHI R., ALMA A., 2004.- Transmission of apple proliferation phytoplasma by *Cacopsylla melanoneura* (Homoptera: Psyllidae).- *Journal of Economic Entomology*, 97 (1): 8-13.

TEDESCHI R., BOSCO D., ALMA A., 2002.- Population dynamics of *Cacopsylla melanoneura* (Homoptera: Psyllidae), a vector of apple proliferation in northwestern Italy.- *Journal of Economic Entomology*, 95: 544-551.

TEDESCHI R., VISENTIN C., ALMA A., BOSCO D., 2003.- Epidemiology of Apple Proliferation (AP) in northwestern Italy: evaluation of the frequency of AP-positive psyllids in naturally infected populations of *Cacopsylla melanoneura* (Homoptera: Psyllidae).- *Annals of Applied Biology*, 142: 285-290.

TOMASI F., BRANZ A., GRANDO M. S., FORNO F., FORTI D., VINDIMIAN M. E., 2000.- Individuazione di fitoplasmi del gruppo AP nelle psille presenti nei frutteti.- *L'Informatore Agrario*, 38: 51-54.

WEINTRAUB P. G., BEANLAND L., 2006.- Insect vectors of phytoplasmas.- *Annual Review of Entomology*, 51: 91-111.

Corresponding author: Federico PEDRAZZOLI (e-mail: federico.pedrazzoli@iasma.it), IASMA Research Centre, Plant Protection Department, via E. Mach 1, 38010 S. Michele all'Adige (TN), Italy.