Bulletin of Insectology ***60*** *(2): 189-190, 2007*
ISSN 1721-8861

Cacopsylla picta as most important vector for '*Candidatus* Phytoplasma mali' in Germany and neighbouring regions

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Abstract

A survey of the distribution of *Cacopsylla picta* and *Cacopsylla melanoneura*, the putative vectors of '*Candidatus* Phytoplasma mali', and their natural infection with the agent has been conducted between 2002 and 2007 in Germany, northern France (Alsace) and northern Switzerland (Aargau, Solothurn). It could be demonstrated that both psyllid species are present in all investigated regions but only individuals of *C. picta* carried the phytoplasma. Except for northern Germany, phytoplasma-infected *C. picta* were found in all geographic areas. During transmission trials under controlled conditions it was proven that only *C. picta* was able to transmit the pathogen while *C. melanoneura* had no importance as vector for '*Ca*. P. mali' in the studied regions.

Key words: Psyllid vectors, *Cacopsylla picta, Cacopsylla melanoneura*, apple proliferation, natural infection, transmission trials.

Introduction

'Candidatus Phytoplasma mali' is actively spread in nature by insects. Two psyllids, *Cacopsylla picta* (Foerster) (syn. *C. costalis*) and *Cacopsylla melanoneura* (Foerster) are vectors of the disease agent (Frisinghelli *et al*., 2000; Tedeschi *et al*., 2002; Jarausch *et al*., 2003). However, their distribution, natural infection rate and transmission capacity is heterogeneous among different geographic regions. In most of the studied areas both species are present (Carraro *et al*., 2001; Jarausch *et al*., 2005; Delic *et al*., 2005; Mattedi *et al*., 2005), in others only *C. melanoneura* has been found (Tedeschi *et al*., 2002). In northwest Italy, Tedeschi *et al*. (2003) estimated that 3,5% of overwintering *C. melanoneura* are infected and 0,8% of the springtime generation; in northeast Italy, the natural infection rate of *C. picta* was found to be 9% and 13%, respectively, for overwintering and offspring adults (Carraro *et al*., 2007). In southwest Germany, about 10% of overwintering *C. picta* and 0.2% of overwintering *C. melanoneura* were naturally infected with the pathogen (Jarausch *et al*., 2005). Despite some estimation about the incidence and development of apple proliferation in selected apple orchards, concrete information on the epidemiology of the disease is still missing and more precise data are necessary to describe a relationship between a defined vector and the spread of the disease.

The objective of this study was to collect data about the epidemiology and spread of apple proliferation in different apple growing regions north side of the Alps.

Material and methods

Regular insect captures in selected apple orchards in Germany were conducted since 2002 using the beat tray method. Since 2005, the same procedure was applied to selected apple orchards with apple proliferation incidence in northern France (Alsace) and two different departments in northern Switzerland (Aargau, Solothurn). Mixed insect samples were frozen at -20 °C and then investigated for species specification. Total DNA from individual insects as well as from plants was extracted using a CTAB method (Jarausch *et al*., 2004). PCR amplification of phytoplasma 16S rDNA as well as of a non-ribosomal fragment was carried out as described by Jarausch *et al*. (1994; 2004). Samples with positive signals were quantified for the phytoplasma titre by real-time PCR as described by Jarausch *et al*. (2004).

Transmission trials with overwintering adults collected from infested apple orchards and with young imagines of both species from rearings on healthy Golden Delicious plants were carried out as described by Jarausch *et al*. (2004).

Results and discussion

The long year's survey by regular insect captures in different German regions, in northern France and northern Switzerland showed that *C. picta* and *C. melanoneura* were present in all investigated apple growing areas. In Germany, the population densities of *C. melanoneura* were always 3 - 10 times higher than for *C. picta* reaching total numbers between 150 and 1,500 individuals per year of study. For *C. picta* total numbers varied between 15 and 500 overwintering adults collected in all investigated orchards per year. Among the different German regions the distribution was heterogeneous: the highest populations were found in southwest Germany while in the North of Germany only 1 single *C. picta* and in the South a total of 11 individuals were found from 2002 to 2006. In comparison, the number of *C. picta* collected in orchards in northern France corresponded to the German regions with high populations while in the Swiss departments, namely Solothurn, the population density was lower.

The long year's survey in the same orchards revealed an important variation of the population density of

both species among the different years. This variation was observed for all regions in a similar way and might be due to the climatic conditions that influence the remigration of the overwintering adults, the larval development and finally the migration of the new imagines.

The natural infection rate was calculated from the results of the PCR tests of all field collected individuals of *C. picta* and *C. melanoneura* from all investigated areas. Interestingly, a homogeneous infection rate of about 10% was found for *C. picta* in Germany as well as in northern France and Switzerland. Furthermore, the quantification of the phytoplasmas in the field-collected infected individuals showed that comparatively high titres were found in all investigated areas. Only in the year 2006, no infected *C. picta* at all were found which might be due to the lowest population densities ever measured during this study.

Despite up to ten times higher numbers of *C. melanoneura* collected, only two overwintering adults naturally infected with '*Ca*. P. mali' were detected during the whole study. In contrast, a good relationship between population density, natural infection rate and the spread of the disease in the investigated orchards was observed for *C. picta*. E.g. only very low numbers of non-infected *C. picta* were captured during several years in the far northern and southern parts of Germany where nearly no infestation with apple proliferation was observed in the orchards.

In order to characterize the vector capacity of *C. picta* and *C. melanoneura*, transmission trials were conducted under controlled conditions. During 2002 and 2006, transmission rates with overwintering adults of *C. picta* were between 8 and 45% of all test plants. Under our conditions, no transmission of '*Ca*. P. mali' with overwintering adults of *C. melanoneura* occurred. Acquisition feeding of young imagines of *C. picta* and *C. melanoneura* resulted in an infection of 5-25% of healthy test plants only with *C. picta* while a small percentage of *C. melanoneura* was at least able to acquire the agent but was not able to transmit it to healthy test plants. First results of the quantification of phytoplasmas in infected *C. picta* showed that those individuals collected from a successfully infected test plant bear phytoplasma DNA copies in the range of 10^6–10^8 in both generations of the insect while infected psyllids without transmission success had often only a low titre. This analysis proposes a minimum threshold value of the phytoplasma concentration in the insect to be infective.

Our data demonstrate that *C. picta* occurs in rather low populations but with a constantly important percentage of individuals naturally infected with '*Ca*. P. mali' while the natural infection of the more numerous *C. melanoneura* was negligible. Transmission trials under controlled conditions proved that only *C. picta* was able to transmit the agent to healthy test plants but not *C. melanoneura*. We therefore conclude that under our climatic conditions *C. picta* is the only important and a highly efficient vector for '*Ca*. P. mali'.

Acknowledgements

The authors thank Uwe Harzer, Arno Fried, Elfie Schell, Hubertus Gernoth, Hubert Schneider, Hervé Bentz, Othmar Eicher, Josef Brägger for psyllid collections and information about the spread of apple proliferation in selected orchards. A special thank is addressed to Dr. Pavel Lauterer for his help in psyllid determination. The survey was funded by Rhein-Pfalz-Wohnen GmbH (FELR), the province of Trentino (project SMAP) and the European Community (INTERREG IIIA).

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