

'Candidatus Phytoplasma mali' infected *Cacopsylla picta* found in apple orchards in South-Western Finland

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Abstract

Apple orchards in four localities in southwestern Finland were surveyed in May 2009 and 2010 to monitor the occurrence of *Cacopsylla picta* (Förster) and *C. melanoneura* (Förster), vectors of apple proliferation phytoplasma. Both psyllid species were present in apple orchards, but *C. picta* prevailed. The number of individuals varied considerably in 2009-2010. The total number of captured overwintering *Cacopsylla* species from the same orchards was 395 in 2009 and 45 in 2010. Individuals of *C. picta* infected with 'Candidatus Phytoplasma mali' were found from single orchards in Lohja and Parainen. In 2010 the incidence of 'Ca. P. mali' in *C. picta* individuals collected from apples (*Malus domestica*) was 11.4%.

Key words: apple proliferation, *Malus*, psyllids, PCR, RFLP.

Introduction

Apple proliferation (AP) phytoplasma, classified as 'Candidatus Phytoplasma mali' (Seemüller and Schneider, 2004) was first described in Italy in the 1950s (Rui *et al.*, 1950) and currently causes severe epidemics and economic losses in many pome fruit growing areas in Europe, including Italy and Germany (Carraro *et al.*, 2008; Jarausch *et al.*, 2004).

The pathogen is transmitted through infected grafting material, via natural root grafts (Ciccotti *et al.*, 2007) and sap-sucking insects. *Cacopsylla picta* (Förster) has proved to be the most important vector of 'Ca. P. mali' in Germany and neighbouring regions (Jarausch *et al.*, 2007), whereas *C. melanoneura* (Förster) was reported as the main vector of 'Ca. P. mali' in Northwestern Italy (Tedeschi *et al.*, 2002).

C. picta has been known to occur on apple in Southern Finland (Ossiannilsson, 1992) and *C. melanoneura* was found in Finland in the 1990s (Albrecht *et al.*, 2003). In the 2000s *C. melanoneura* spread northwards to a greater extent than *C. picta* (Mattila and Söderman, 2011). *C. melanoneura* occurs mainly on hawthorn, but has been found also on apple and pear (Ossiannilsson, 1992). Both psyllid species are presumed to overwinter on conifers in Fennoscandia (Ossiannilsson, 1992).

Apple cultivation has been predicted to expand in Finland in the future as a result of global warming (Kaukoranta *et al.*, 2010). The main area for commercial apple cultivation in Finland is in the south and southwestern parts of Finland, and in the Åland Islands. Apple proliferation phytoplasma has not been surveyed or reported either from apple trees or insects in Finland. The aim of the present study was to confirm the occurrence of *C. picta* and *C. melanoneura* in apple orchards in southern Finland and to determine the incidence of 'Ca. P. mali' in *C. picta*.

Materials and methods

In May 2009, 18 psyllid samples were collected in Southwestern Finland (Parainen, Piikkiö, Lohja, Jokioinen) from apple (*Malus domestica*) in commercial orchards, research stations and in a few home gardens.

In May 2010, 19 samples were collected in the same orchards. Overwintering adult psyllids were collected using a beating net with an opening of 0.25 m². In commercial orchards, one branch from each of 33 randomly selected apple trees was beaten for each sample. Insects were separated, identified and counted by examining male and female terminalia (Ossiannilsson, 1992).

Specimens were stored in 95% ethanol until DNA extraction. In some of the later samples, 1st-3rd nymph stages of *Cacopsylla mali* (Schmidb.) were also present. *C. mali* overwinters as eggs and is the most common psyllid on apple in Finland (Mattila and Söderman, 2011).

Total DNA from adult *Cacopsylla* species was extracted from all individual psyllids from single specimens in 2010, whereas DNA from a third of the psyllids collected in 2009 was extracted from 26 batches of five individuals.

DNA extraction was done using a DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's instructions. Apple proliferation phytoplasma was detected in the DNA extracts through the use of PCR amplification with AP-specific primers AP5/AP4 (Jarausch *et al.*, 1994).

DNA from AP phytoplasma positive samples was analysed further for subtypes by PCR/RFLP using primers AP13/AP10 (Jarausch *et al.*, 2000) and amplicons were digested using enzymes *HincI* and *RcaI* (New England BioLabs) following the instructions of the manufacturer.

Results

In 2009 a total of 395 *Cacopsylla* adults were collected in the four localities: 294 were identified as *C. picta* and 101 as *C. melanoneura*. In 2010 a total of 45 *Cacopsylla* spp. were collected from the same localities and orchards. Of the 45 identified individuals 44 were *C. picta* and one was *C. melanoneura*.

In 2009, *C. picta* was present in all collected samples, the numbers varying from 1 to 41 per sample, 56% of the 294 were females. *C. melanoneura* was found in half of the samples, 1 to 46 per sample, 65% of the 101 were females. In 2010, *C. picta* was found in 12 samples, 1 to 16 per sample, and 82% were females. *C. melanoneura* was present in only one sample as a single female.

'*Ca. P. mali*' was detected in four groups of the selected 26 groups of *C. picta* samples (4/26) collected in 2009. The characteristic amplicon (483 bp) was amplified from insects using specific primers AP5/AP4. All the positive samples were from one orchard in Parainen.

'*Ca. P. mali*' was detected in five of 45 individuals collected in 2010, all from the same orchard (Lohja). The occurrence of '*Ca. P. mali*' in the specimens of *C. picta* in that orchard was 20.8% (5/24), whereas the incidence in all *C. picta* specimens collected in 2010 was 11.4% (5/44).

AP13/AP10 amplicons and RFLP analyses confirmed that the '*Ca. P. mali*' subtypes present in Finnish orchards were AP and AT-2.

Discussion

Results of this preliminary survey confirmed the incidence of both psyllid vector species of '*Ca. P. mali*', *C. picta* and *C. melanoneura*, in Finnish apple orchards. The number of individuals of both species varied in 2009-2010, but *C. picta* prevailed in both years. It is interesting that the reduction in numbers of *C. melanoneura* after the hard winter in 2010 was relatively much higher compared with that for *C. picta*. As *C. melanoneura* is considered to be a newcomer in Finland (Vänninen *et al.*, 2011), it may be present here at its northernmost border.

Occurrence of '*Ca. P. mali*' in *C. picta* was determined in samples collected in two localities. One of these orchards (Parainen) represented organic farming, where numerous imported foreign apple varieties were planted. The other orchard (Lohja) represented an old conventional orchard. Observation of abnormal symptoms in apple orchards have not been reported by growers or advisors. '*Ca. P. mali*' infected apple trees have yet to be found and therefore any conclusions based on the present distribution of '*Ca. P. mali*' in Finnish apple orchards are premature.

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