

# '*Candidatus Phytoplasma mali*': the current situation of insect vectors in northwestern Italy

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## Abstract

The two acknowledged vectors of '*Candidatus Phytoplasma mali*' in north-western Italy, the psyllid *Cacopsylla melanoneura* and the leafhopper *Fieberiella florii*, have been compared to highlight their role in the apple proliferation epidemiology in relation to their infectivity, transmission efficiency and permanence in apple orchards and in wild vegetation, predominantly hawthorn formations. The early permanence of *C. melanoneura* on apple trees is characterised by a high population density and a low infectivity rate. On the contrary, *F. florii* colonizes apple orchards belatedly and the higher infection rate is balanced by a very low population density. Hawthorn plants proved to be a source of inoculum for the phytoplasma as well as a host plant for the two species. Moreover, another psyllid species, *Cacopsylla peregrina*, known as a typical hawthorn feeder, proved to be positive to '*Ca. P. mali*'.

**Key words:** *Cacopsylla melanoneura*, *Fieberiella florii*, apple proliferation, hawthorn.

## Introduction

In the last years, the insect vectors of '*Candidatus Phytoplasma mali*' achieved the attention of many researchers because of the fast spreading of the phytoplasma infections and the serious damages caused by the disease in central Europe and in particular in Italy. In this country, because of the relevant economic losses, compulsory measures have been introduced by a Ministerial Decree against this pest. For this reason the vector control strategies gain further in importance and a good overall view of the insect vector situation, concerning the population dynamics, the infectivity and the host plants, in a country or a region, is a matter of greatest importance.

The population dynamics and the infectivity of the two species reported in north-western Italy: the psyllid *Cacopsylla melanoneura* (Förster), originally known as a hawthorn psyllid (Minucci *et al.*, 1996; Tedeschi *et al.*, 2002; Tedeschi *et al.*, 2003; Tedeschi and Alma, 2004) and the leafhopper *Fieberiella florii* (Stål) (Krczal *et al.*, 1988; Tedeschi and Alma, 2006) have been described and compared to assess their real role in the phytoplasma spread in apple orchards and in wild vegetation areas. In particular, the relationship between the vectors, the phytoplasma and hawthorn plants has been investigated. Moreover, the possibility of other psyllid species to carry the phytoplasma was tested.

## Materials and methods

Field samplings with yellow sticky traps and beat trays were carried out in apple orchards located in Piedmont and in the Aosta Valley, north-western Italy from 1999 to 2005 to assess the population dynamics of *C. melanoneura* and *F. florii*. Further samplings were carried out in wild formations of *Crataegus monogyna* Jacquin in the neighbourhood of apple orchards.

Transmission trials were carried out to prove the vec-

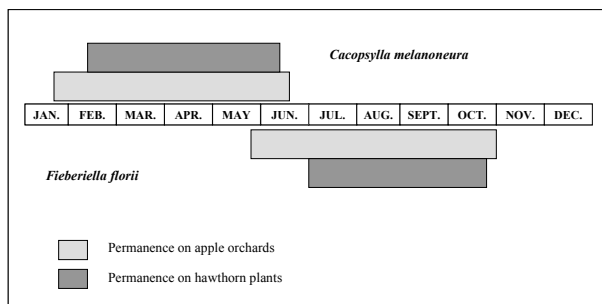
tor ability of both *C. melanoneura* and *F. florii*.

The presence of '*Ca. P. mali*' was determined from field collected insects and test plants by PCR assays and confirmed by RFLP. First the phytoplasma universal primers P1/P7 (Schneider *et al.*, 1995), then the AP-specific group primer fO1/rO1 (Lorenz *et al.*, 1995) in nested PCR were used. Amplicons were further digested with the restriction endonuclease *SspI* (Sigma-Aldrich Srl, St. Louis, MO, USA).

## Results

*C. melanoneura* completes one generation/year. Over wintered adults start to colonise apple orchards at the end of January and reach the population peak in mid-March (up to 212 specimens/orchard/week). Eggs are visible from the second half of March until mid-April, while nymphs are recorded from mid-April until mid-May. Newly emerged adults live on apple trees until the first half of June, and then they move onto alternative hosts (figure 1). The proportion of infected insects, estimated by its maximum-likelihood estimator, ranged between 3-4% and 0.8% for over wintered and newly emerged adults, respectively. The probability of transmission by a single *C. melanoneura* is 0.7% with springtime adults after experimental acquisition. In contrast, naturally infected over wintered adults yielded higher proportions of transmission, ranging from 1.4% to 8.4%, in relation of the infection rate of the source apple. On hawthorn formations, the permanence period of *C. melanoneura* is similar, but a little bit delayed; it arrives around mid-February and stays until the beginning of June (figure 1). The proportion of infected insects was 2.4%.

*F. florii* completes one generation/year, over winters as egg, nymph or adult and starts to colonise apple orchards at the end of May reaching the population peak around the second half of September and the beginning of October (3-9 specimens/orchard/week) (figure 1).



**Figure 1.** Presence period of *Cacopsylla melanoneura* and *Fieberiella florii* in apple orchards and on hawthorn bushes.

The percentage of infected leafhoppers collected in apple orchards was 5.2%, while the probability of transmission by a single *F. florii* experimentally inoculated ranges between 0.7% and 2.2%. On hawthorn, also in the case of *F. florii*, the arrival of the insect is delayed (beginning of July) comparing with apple orchards, but the peak is always around the second half of September and the beginning of October (figure 1).

In this biotope the infection percentages were higher, reaching 20%.

'*Ca. P. mali*' was detected also in plant samples of *C. monogyna* as well as in another psyllid species known as a typical hawthorn feeder, *Cacopsylla peregrina* (Förster), with a proportion of infection around 1%.

## Discussion

As *C. picta* was never found in northwestern Italy, *C. melanoneura* was considered for many years the only insect vector of '*Ca. P. mali*' in these regions (Minucci *et al.*, 1996; Tedeschi *et al.*, 2002; Tedeschi *et al.*, 2003; Tedeschi and Alma, 2004). The confirmation of the role of *F. florii* as a vector of AP phytoplasma and its finding in apple orchards and in wild vegetation areas (Tedeschi and Alma, 2006) opens new perspectives for the study of the disease epidemiology and in particular for control strategies. Although *C. melanoneura*, because of the higher population density, is still considered as most worrying vector, the role of *F. florii* should not be underestimated, because it colonizes the apple orchards during a period in which the psyllid is on alternative hosts and when the titre of phytoplasmas in apple trees is higher. Moreover, the high polyphagy of *F. florii* makes the leafhopper a dangerous spreader of the phytoplasma.

The presence of the two species, during the same periods also on wild vegetation, in particular *C. monogyna* plants, should be taken into account for control strategies in situations in which wild hawthorn formations are very close to apple orchards.

The detection of '*Ca. P. mali*' in hawthorn plants in the field, could re-evaluate the role of those plants not only as natural hosts of the vectors, but also as phytoplasma reservoir. The possibility of other psyllid species such as *C. peregrina* to carry '*Ca. P. mali*' should be further investigated to assess their possible role as phytoplasma vectors.

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