

Infection rates of natural psyllid populations with ‘*Candidatus Phytoplasma mali*’ in South Tyrol (Northern Italy)

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Abstract

Apple proliferation is a severe disease of apple trees spreading in many European apple growing areas. It is caused by ‘*Candidatus Phytoplasma mali*’ that was shown to be transmitted through infected grafting material, via natural root grafts and by sap-sucking insects. Two psyllid species, *Cacopsylla picta* and *C. melanoneura*, that are recognised as the vectors of the disease, occur in orchards of South Tyrol (Northern Italy). The aim of this study was to assess the infection rates of natural populations of these insect species with ‘*Ca. P. mali*’. Two additional psyllid species (*C. mali* and *Triozia urticae*), which are frequent in some apple orchards of South Tyrol, were also investigated. A total of 801 specimens from 18 orchards was analysed using a real-time PCR procedure. While no specimen of *T. urticae* was found to be infected with ‘*Ca. P. mali*’, the mean infection rate of *C. melanoneura* and *C. mali* was below 1 %. The highest infection rate was found for *C. picta*, with a mean value of 11 % and peaking at 33%. Based on these results, it can be concluded that *C. picta* plays the major role as the vector of apple proliferation in South Tyrol.

Keywords: apple proliferation, *Cacopsylla mali*, *Cacopsylla melanoneura*, *Cacopsylla picta*, pathogen transmission, *Triozia urticae*

Introduction

Apple proliferation (AP), a disease of apple trees caused by ‘*Candidatus Phytoplasma mali*’ (Seemüller and Schneider 2004), has been spreading over the last decade in many European apple growing areas (Frisinghelli et al. 2000; Tedeschi et al. 2003; Carraro et al. 2008; Mayer et al. 2009). Apart from proliferation of auxiliary shoots (witches' brooms), enlarged stipules, chlorosis, yellowing or early leaf reddening, the pathogen can induce symptoms of economic relevance such as decreased size, quality and overall yield of fruit (Kartte and Seemüller 1988). The AP phytoplasma was shown to be transmitted through grafting of infected propagation material (Kartte and Seemüller 1988), via natural root grafts (Baric et al. 2008; Ciccotti et al. 2008) and by sap-sucking insects. So far, two psyllid species, *Cacopsylla picta* and *C. melanoneura*, were identified as the vectors of AP phytoplasma (Frisinghelli et al. 2000; Tedeschi and Alma 2004). In addition, the leafhopper *Fieberiella flori* was suggested as a further vector of this disease (Krczal et al. 1989; Tedeschi and Alma 2006). Since there is no therapy available to cure infected trees, the only possibility to control the disease is to prevent it from spreading by planting healthy material, uprooting diseased plants and vector control.

In order to propose a strategy for insect vector control in the Autonomous Province of South Tyrol (Northern Italy), the psyllid fauna present in the apple orchards was monitored during the vegetation period of 2006 (Walch 2006). The study revealed the presence of 13 psyllid species: *C. melanoneura*, *C. picta*, *C. mali*, *C. pyri*, *C. pyricola*, *C. brunneipennis*, *C. nigrita*, *C. affinis*, *C. crataegi*, *C. pruni*, *Psylla alni*, *Triozia urticae* and *Bactericera albiventris* (Walch 2006), though at different frequencies. The highest number of collected individuals was assigned to two species, *C. melanoneura* (80 %) and *C. picta* (6 %), while the occurrence of *C. mali* was notable in abandoned orchards with up to 8.9 individuals per branch (Walch 2006). The aim of the present study was to assess the infection rates with ‘*Ca. P. mali*’ of the most common psyllid species in South Tyrolean orchards in order to provide additional data about their potential role for the propagation of AP in this region.

Material and methods

Psyllids were captured by beating tray sampling in orchards distributed all over the apple production area of South Tyrol from March until June 2006 (Walch 2006). After species determination, insects were preserved in absolute ethanol. 801 specimens comprising four different species from 18 AP affected apple orchards were selected for DNA analysis (Table 1 and Figure 1). Preference was given to samples with higher specimen numbers to more reliably estimate the proportion of AP infected psyllids per orchard. The orchards considered in the study were mainly managed according to the principles of integrated production, while one orchard was organic and four abandoned. Nucleic acid was isolated from individual insects by following the procedure of Marzachi et al. (1998). Each DNA isolate was analysed in duplicate applying a highly sensitive TaqMan real-time PCR approach for the specific detection of AP phytoplasma (Baric and Dalla Via 2004; Baric et al. 2006).

Tab. 1 Number of specimens of four psyllid species from different localities in South Tyrol (Northern Italy) tested for the presence of AP phytoplasma by real-time PCR

Apple orchard locality	<i>Cacopsylla picta</i>		<i>Cacopsylla melanoneura</i>		<i>Cacopsylla mali</i>		<i>Trioza urticae</i>	
	N analysed	N AP positive (%)	N analysed	N AP positive (%)	N analysed	N AP positive (%)	N analysed	N AP positive
1 Mals/Malles	n.p.		47	0	n.p.		n.p.	
2 Glurns/Glorenza	n.p.		31	0	2	0	n.p.	
3 Latsch/Laces	14	0	24	1 (4.2)	n.p.		n.p.	
4 Naturns/Naturno ^a	18	1 (5.6)	113	1 (0.9)	n.p.		n.p.	
5 Saltaus/Saltusio	2	0	n.p.		n.p.		n.p.	
6 Schenna/Scena ^a	n.p.		n.p.		113	0	n.p.	
7 Tschermes/Cermes-A ^a	15	1 (6.7)	84	0	111	2 (1.8)	n.p.	
8 Tschermes/Cermes-B	27	9 (33.3)	n.p.		n.p.		n.p.	
9 Tschermes/Cermes-C	16	1 (6.3)	n.p.		n.p.		n.p.	
10 Tschermes/Cermes-D	3	0	n.p.		n.p.		n.p.	
11 Lana-A	12	0	n.p.		n.p.		n.p.	
12 Lana-B	58	7 (12.1)	5	0	n.p.		n.p.	
13 Lana-C	5	0	n.p.		n.p.		n.p.	
14 Tisens/Tesimo	28	2 (7.1)	n.p.		n.p.		n.p.	
15 Nals/Nalles ^b	10	2 (20.0)	8	0	n.p.		n.p.	
16 Eppan/Appiano-A	n.p.		31	0	n.p.		n.p.	
17 Eppan/Appiano-B	n.p.		3	0	n.p.		n.p.	
18 Salurn/Salorno ^a	n.p.		n.a.		n.p.		21	0
Total	208	23 (11.1)	346	2 (0.6)	226	2 (0.9)	21	0

n.p., no specimens present in the beating tray sample; n.a., not analysed because of small sample size

^a abandoned orchard; ^b organically managed orchard

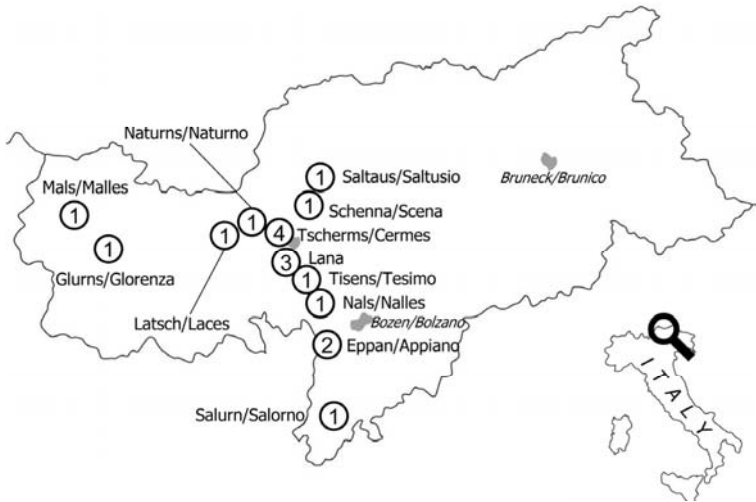


Fig. 1 Map of South Tyrol (Northern Italy) showing the locations where psyllid samples were collected. The number of orchards sampled at each location is given in the circles.

Results

The AP phytoplasma was detected in 27 out of the 801 specimens tested with the real-time PCR assay (see Table 1). The highest number of infected individuals (N = 23) was found for *C. picta*, the infection rates in the seven positively tested orchards ranging from 5.6 to 33.3 % (Table 1). Orchards lacking infected *C. picta* specimens were generally represented by low sample numbers. Two *C. melanoneura* individuals, each from a different orchard, were tested AP

positive, resulting in infection rates of 0.9 and 4.2 %. The same number of AP positive individuals was found for *C. mali* which was particularly abundant in two of the three sampling sites where this species was present (Table 1). Both infected specimens originated from the same orchard (Tscherms/Cermes-A). *Trioxa urticae* was found in higher numbers in a single orchard in the very south of the investigation area and none of the 21 individuals analysed was found to carry 'Ca. *P. mali*' (Table 1).

Discussion

Apple trees with symptoms of AP have been observed in the intensive orchards of South Tyrol since 1998. In the first years, however, the disease was mainly confined to orchards situated on the slopes of the low mountain range (Österreicher and Thomann 2003). The highest number of AP symptomatic apple trees, amounting to 520,000, was ultimately reached in 2006 (Mair 2009). Although by that time the disease had spread all over South Tyrol, the hot-spot was located in the district of Burggrafenamt/Burgraviato, where in some orchards more than 30% of the apple trees showed pronounced AP symptoms (Österreicher and Unterthurner 2006).

The recent explosive outbreak of the disease in South Tyrol could be related to the occurrence of *C. picta* in this area. This species was first noticed in 2004 in a single orchard (Wolf and Zelger 2006) and has since been observed in the entire apple production area except in the district of Eisacktal/Valle Isarco (Unterthurner and Österreicher 2008). Again, the highest number of orchards harbouring *C. picta* was located in the district of Burggrafenamt/Burgraviato (Wolf and Zelger 2006; Unterthurner and Österreicher 2008), even if the maximum density did not exceed 1.8 individuals per branch (Walch 2006).

Cacopsylla picta had been proven as an efficient vector of 'Ca. *P. mali*' in several transmission trials (Frisinghelli et al. 2000; Jarausch et al. 2003; Seemüller et al. 2004; Carraro et al. 2008). Furthermore, various studies have demonstrated that a high percentage of individuals occurring in apple orchards can carry the AP phytoplasma and the average natural infection rate of 11.1 % in South Tyrol is absolutely comparable with the results from Germany, Northern France and Switzerland (Jarausch et al. 2007) as well as the neighbouring Italian Region of Trentino (Cainelli et al. 2004). In contrast, the mean infection rate of *C. melanoneura* was almost 20-fold lower (0.6 %). Even though this species was shown to be able to acquire 'Ca. *P. mali*' (Pedrazzoli et al. 2007), extensive transmission experiments performed with *C. melanoneura* in Germany (Mayer et al. 2009) and South Tyrol (Wolf et al. 2003) failed to infect healthy test plants, while in Trentino the pathogen was successfully transmitted in only one of the 278 trials performed over a six-year period (Mattedi et al. 2008). In northeastern Italy, however, *C. melanoneura* is considered the main vector of 'Ca. *P. mali*' (Tedeschi and Alma 2004), since its transmission to healthy test plants was successful with naturally and experimentally infected specimens. In addition, the pathogen was commonly detected in natural populations of overwintered adults with maximum infection rates of 3.5 % (Tedeschi et al. 2003). The differences in vectoring ability found for *C. melanoneura* in different studies may be caused by the existence of distinct populations varying in their capacity to acquire and transmit the AP phytoplasma (Mayer et al. 2009). On the other hand, 'Ca. *P. mali*' was found to be genetically highly variable (Schneider and Seemüller 2009) and one could speculate that particular strains may be transmitted by *C. melanoneura* while others may be vectored by *C. picta*. However, this speculation needs to be further investigated by typing 'Ca. *P. mali*' isolates from different geographic regions and vector populations.

Low natural infection rates with 'Ca. *P. mali*' of 0.9 % were determined for *Cacopsylla mali*. This species is known to occur abundantly in untreated and abandoned apple orchards (Seemüller et al. 2004), which was also the case in South Tyrol (Walch 2006). While *C. mali* was shown to carry the pathogen, it has not been confirmed as a vector of 'Ca. *P. mali*', although transmission experiments involved a large number of individuals (Seemüller et al. 2004). Our findings seem to confirm that the ability of Ca. *P. mali* to acquire the phytoplasma is poor, since all the specimens tested were collected from an area with a high number of AP-affected apple trees.

Based on the relatively high infection rates of natural *C. picta* populations, the frequent occurrence of this species in many commercial apple orchards and its proven vectoring ability, we conclude that this species carries the largest risk for the spread of 'Ca. *P. mali*' in South Tyrol. Therefore, management strategies for vector control should focus on this species. However, further research on insect vectors is necessary to explain the dissemination of AP in areas where this species has so far never been observed.

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Literature

- Baric, S.; Dalla Via, J.; 2004: A new approach to apple proliferation detection: a highly sensitive real-time PCR assay. *Journal of Microbiological Methods* **57**, 135-145.
- Baric, S.; Kerschbamer, C.; Dalla Via, J.; 2006: TaqMan real-time PCR versus four conventional PCR assays for detection of apple proliferation phytoplasma. *Plant Molecular Biology Reporter* **24**, 169-184.
- Baric, S.; Kerschbamer, C.; Vigl, J.; Dalla Via, J.; 2008: Translocation of Apple Proliferation Phytoplasma via natural root grafts – a case study. *European Journal of Plant Pathology* **121**, 207-211.
- Cainelli, C.; Bisognin, C.; Vindimian, M.E.; Grando, M.S.; 2004: Genetic variability of AP phytoplasmas detected in the apple growing area of Trentino (North Italy). *Acta Horticulturae* **657**, 425-430.
- Carraro, L.; Ferrini, F.; Ermacora, P.; Loi, N.; Labonne, G.; 2008: Infectivity of *Cacopsylla picta* (Syn. *Cacopsylla costalis*), vector of 'Candidatus *Phytoplasma mali*' in North East Italy. *Acta Horticulturae* **781**, 403-407.
- Ciccioiti, A.; Bianchedi, P.L.; Bragagna, P.; Deromedi, M.; Filippi, M.; Forno, F.; Mattedi, L.; 2008: Natural and experimental transmission of Candidatus *Phytoplasma mali* by root bridges. *Acta Horticulturae* **781**, 459-464.
- 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.; 2003: First report of *Cacopsylla picta* as a vector of apple proliferation phytoplasma in Germany. *Plant Disease* **87**, 101.
- Jarausch, B.; Fuchs, A.; Schwind, N.; Krczal, G.; Jarausch, W.; 2007: *Cacopsylla picta* as most important vector for 'Candidatus *Phytoplasma mali*' in Germany and neighbouring regions. *Bulletin of Insectology* **60**, 189-190.
- Karte, S.; Seemüller, E.; 1988: Variable response within the genus *Malus* to the apple proliferation disease. *Journal of Plant Diseases and Protection* **95**, 25-34.
- Krczal, G.; Krczal, H.; Kunze, L.; 1989: *Fiebertella florii* (Stal), a vector of apple proliferation agent. *Acta Horticulturae* **235**, 99-106.
- Mair, K.; 2009: Apfetribsucht – Befallsituation 2008. *Obstbau Weinbau* **46**, 13-15.
- Marzachi, C.; Veratti, F.; Bosco, D.; 1998: Direct PCR detection of phytoplasmas in experimentally infected insects. *Annals of Applied Biology* **133**, 45-54.
- Mattedi, L.; Forno, F.; Cainelli, C.; Grando, M.S.; Jarausch, W.; 2008: Research on *Candidatus* *Phytoplasma mali* transmission by insect vectors in Trentino. *Acta Horticulturae* **781**, 369-374.
- Mayer, C.J.; Jarausch, B.; Jarausch, W.; Jelkmann, W.; Vilcinskas, A.; Gross, J.; 2009: *Cacopsylla melanoneura* has no relevance as vector of apple proliferation in Germany. *Phytopathology* **99**, 729-738.
- Österreicher, J.; Thomann, M.; 2003: Apfetribsucht in Südtirol. *Obstbau Weinbau* **40**, 305-307.
- Österreicher, J.; Unterthurner, M.; 2006: Problemkrankheit Apfetribsucht. *Obstbau Weinbau* **43**, 355-357.
- Pedrazzoli, F.; Gualandri, V.; Forno, F.; Mattedi, L.; Malagnini, V.; Salvadori, A.; Stoppa, G.; Ioriatti, C.; 2007: Acquisition capacities of the overwintering adults of the psyllid vectors of 'Candidatus *Phytoplasma mali*'. *Bulletin of Insectology* **60**, 195-196.
- Schneider, B.; Seemüller, E.; 2009: Strain differentiation of *Candidatus* *Phytoplasma mali* by SSCP and sequence analyses of the *hflB* gene. *Journal of Plant Pathology* **91**, 103-112.
- Seemüller, E.; Schneider, B.; 2004: 'Candidatus *Phytoplasma mali*', 'Candidatus *Phytoplasma pyri*' and 'Candidatus *Phytoplasma prunorum*', the causal agents of apple proliferation, pear decline and European stone fruit yellows, respectively. *International Journal of Systematic and Evolutionary Microbiology* **54**, 1217-1226.
- Seemüller, E.; Dickler, E.; Berwarth, C.; Jelkmann, W.; 2004: Occurrence of psyllids in apple orchards and transmission of apple proliferation by *Cacopsylla picta* (syn. *C. costalis*) in Germany. *Acta Horticulturae* **657**, 533-537.
- 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.
- Tedeschi, R.; Alma, A.; 2004: Transmission of apple proliferation phytoplasma by *Cacopsylla melanoneura* (Homoptera: Psyllidae). *Journal of Economic Entomology* **97**, 8-13.
- Tedeschi, R.; Alma, A.; 2006: *Fiebertella florii* (Homoptera: Auchenorrhyncha) as a vector of "Candidatus *Phytoplasma mali*". *Plant Disease* **90**, 284-290.
- Unterthurner, M.; Österreicher, J.; 2008: Die Überwachung des Sommerapfelblattsaugers 2007. *Obstbau Weinbau* **45**, 54-55.
- Walch, R.; 2006: Systematische Erhebung der Blattsauger in Apfelanlagen Südtirols. *Obstbau Weinbau* **43**, 245-247.
- Wolf, M.; Zelger, R.; Baric, S.; 2003: Die Rolle des Weißdornblattsaugers bei der Übertragung der Apfetribsucht in Südtirol. *Obstbau Weinbau* **40**, 315-317.
- Wolf, M.; Zelger, R.; 2006: Auftreten von Sommerapfelblattsauger in Südtirol. *Obstbau Weinbau* **43**, 14-17.