Characteristics of the Spread of Apple Proliferation by Its Vector *Cacopsylla picta*

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ABSTRACT

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The distribution and natural phytoplasma infection of *Cacopsylla picta* were investigated during a long-term field survey between 2002 and 2009 in commercial and abandoned apple proliferation-infected orchards throughout Germany, northern Switzerland, and eastern France. Comparable population dynamics were described for the different sites whereas considerable variations in the absolute population densities were observed among the years. Individual polymerase chain reaction (PCR) testing revealed, for each year, a rather stable natural infection rate with ‘*Candidatus Phytoplasma mali*’ of ≈10% for overwintered adults of *C. picta*. Both genders were equally highly infected although more females were caught. The overall male/female ratio was 1:1.5. No direct correlation was found between the infection status of the orchard and the

infection rate of overwintered *C. picta*. No influence of agricultural practices was seen. However, a relationship between the incidence of the disease and the vector population density became evident on a regional scale. Successful transmission of ‘*Ca. P. mali*’ occurred each year with overwintered individuals as well as with new adults. The transmission efficiency varied among the years within 8 to 45% for overwintered adults and 2 to 20% for individuals of the new generation. The load of single *C. picta* with ‘*Ca. P. mali*’ was determined by quantitative real-time PCR. High phytoplasma titers were measured in overwintered adults already at their first appearance in the orchards after remigration from their overwintering hosts. Thus, the data indicate the transmission of the disease on a regional scale by remigrant adults of *C. picta* and at a local scale within the same season by emigrant adults which developed on infected plants.

Additional keywords: epidemiology, gender balance.

Apple proliferation (AP) is one of the economically most important phytoplasma diseases in Europe which seriously impairs fruit size and quality, yield, and vigor of apple trees, rendering them unmarketable and thus causing a major economic loss (21). Typical symptoms are enlarged stipules and witches’ brooms in late summer or autumn, which allow a reliable identification of infected plants (36). AP was first reported in northern Italy (34) and has been detected since then in all European countries where apple is grown commercially. The highest incidences of AP are found in temperate climatic zones of Europe (21). Thus far, the geographic distribution of AP has been restricted to Europe and neighboring regions and it is not known whether this restriction is due to a missing vector or unsuitable temperatures northward or southward from this zone (15). However, only recently, its presence has been reported from outside Europe in the Asian part of Turkey (4), indicating the spread of this disease toward thus-far-unaffected areas. The causative agent of AP is ‘*Candidatus Phytoplasma mali*’ which, together with other economically important phytoplasmas on fruit trees, belongs to the phytoplasma 16Sr group X (38). The cell-wall-less and thus-far-uncultured phytoplasmas colonize the phloem tissue of their plant host as well as the body of their insect hosts. They are spread through grafting of infected plant material or they are naturally transmitted by phloem-feeding insects (25,44).

Psyllid species belonging to the taxon *Cacopsylla* Ossiannilsson 1970 (Hemiptera: Psyllidae) are considered to be the main vectors for European fruit tree phytoplasmas (15). Two *Cacopsylla* spp.

are recognized vectors of ‘*Ca. P. mali*’: *Cacopsylla picta* (Förster) (synonym *C. costalis*) is the vector for ‘*Ca. P. mali*’ in north-eastern Italy (11) and in Germany (17) while *C. melanoneura* (Förster) was identified as main vector of this disease in north-western Italy (41,42), even though the leafhopper *Fieberiella florii* (Stål) has also been reported as a vector in this region (39). In contrast to northwestern Italy, the German population of *C. melanoneura* is not able to transmit ‘*Ca. P. mali*’ (27) and thus, *C. picta* is regarded as the only vector. The single AP-transmission event with *F. florii* reported by Krczal et al. (20) could never be repeated since (G. Krczal, *personal communication*). *C. picta* has a palaearctic distribution and is monophagous on *Malus* spp. (22). The psyllid completes one generation per year and overwinters as an adult on conifers (8,30). At the end of winter (March or April), *C. picta* remigrants move from their overwintering sites to their main host apple for oviposition. Larval development (L1 to L5) takes 4 to 5 weeks; the newly hatched imagines (emigrants) remain in the orchards for ≈2 weeks before they leave the orchards in June or July to migrate to their overwintering sites (12,26,30). As far as is known, phytoplasmas are transmitted by leafhoppers (44) and psyllids (7,43) in a persistent manner. This can also be supposed for the transmission of ‘*Ca. P. mali*’ through *C. picta* because first remigrants of *C. picta* collected in apple orchards in early spring were shown to be highly infectious (5,16,17). In southwest Germany, ≈10% of overwintering *C. picta* were naturally infected with the pathogen and both generations of *C. picta* were able to transmit the pathogen under experimental conditions (16,17).

Despite some particular reports (5,9,10,14,26) on AP incidence and the presence of its vector *C. picta*, all-encompassing information about the epidemiology of this important disease is still missing. Hence, the objective of this study was to collect precise and repetitive data over a period of years in order to provide a

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complete description of the relationship between the spread of this disease and the presence, life cycle, natural infectivity, and transmission capacity of its main vector in Germany and adjacent regions, *C. picta*. The quantification by ‘*Ca. P. mali*’-specific real-time polymerase chain reaction (PCR) (18) and defined experimental transmission trials (16) were applied to assess the infectivity of individuals of *C. picta*.

MATERIALS AND METHODS

Collection of insect and plant material. Insect collections were undertaken in selected cultivated and uncultivated apple orchards (*Malus × domestica*) in different apple-growing regions of Germany, eastern France (Alsace), and northern Switzerland (Table 1). Overall, we investigated 52 different sites, including 31

commercial apple orchards managed according to the principles of integrated production (IP) (3), 6 commercial apple orchards managed according to the rules of organic production (2), and 15 untreated, abandoned or scattered orchards. The infection by ‘*Ca. P. mali*’ at the sampling sites was estimated visually by advisers and plant health inspectors by recording typical symptoms (witches’ brooms and enlarged stipules) for those years with important psyllid captures (Table 1). Wherever possible, visual diagnosis was confirmed by PCR tests for detection of ‘*Ca. P. mali*’. Because scattered sites rarely show visual symptoms but constitute important reservoirs for *C. picta*, a random sample of trees was analyzed by PCR for the presence of ‘*Ca. P. mali*’.

All insect collections were done using the beating tray method according to Muther and Vogt (31). The collected insects from all regions were counted, separated in vials, and frozen at –20°C for

TABLE 1. Apple orchards and their apple proliferation (AP) infection status in Germany, eastern France (Alsace), and Northern Switzerland (Aargau, Solothurn) in which psyllids have been captured during 2002 to 2008^a

Year of psyllid captures	Region	Site code	Orchard type	Year of monitoring data	AP infection (%) (visual diagnosis)	PCR (positive/total) ^b	AP infection class in Table 3 (%)
2003–2005	Schleswig-Holstein	SH1	Organic	2005	<1	nt	<1
2003–2005	Schleswig-Holstein	SH2	IP	2005	<1	nt	na
2002–2005	Rheinland	AW1	Organic	2005	5	nt	1–5
2002–2003	Rheinhessen	OP1	IP	2003	11	nt	10–20
2002–2003	Rheinhessen	OP2	Organic	2003	5	nt	1–5
2002–2005	Rheinhessen	OP3	IP	2003	5	nt	1–5
2003–2004	Rheinhessen	OP4	IP	2003	5	nt	1–5
2003–2005	Rheinhessen	OP5	IP	2004	12	nt	10–20
2003–2004	Vorderpfalz	Bio1	Organic	2003	5	3/3	1–5
2003	Vorderpfalz	Bio2	Organic	2003	5	nt	1–5
2002–2006	Vorderpfalz	HA1	Scattered	2001	nt	14/16 (88)	> 75
2005	Vorderpfalz	Klohr	Scattered	2005	25	nt	25–50
2007–2008	Vorderpfalz	MeStr	Scattered	2010	43	3/3	25–50
2002	Vorderpfalz	Mu	Scattered	2001	65	13/14	50–75
2007	Vorderpfalz	NE1	IP	2007	10	14/20	10–20
2002–2005	Vorderpfalz	NW1	IP	2005	59	11/12	> 50
2006–2008	Vorderpfalz	NW1a	IP	2008	75	26/26	> 75
2006–2008	Vorderpfalz	NW1b	IP	2008	5	12/19	1–5
2002–2005	Vorderpfalz	NW2	IP	2005	1	2/2	1–5
2003–2008	Vorderpfalz	NW5	IP	2006	16	81/107	10–20
2003–2005	Vorderpfalz	NW6	IP	2005	3	39/40	1–5
2004	Vorderpfalz	NW7	IP	2003	5	2/2	1–5
2003	Vorderpfalz	NW8	IP	2003	5	2/2	1–5
2002–2004	Vorderpfalz	WaS1	Scattered	2002	40	2/5	25–50
2002	Vorderpfalz	WaS3	Scattered	2002	25	1/1	25–50
2002–2003	Vorderpfalz	WaS4	Scattered	2002	75	1/1	50–75
2002–2003	Vorderpfalz	WaS5	Scattered	2002	75	4/4	50–75
2002	Vorderpfalz	WaS6	Scattered	2002	50	2/2	50–75
2002–2003	Vorderpfalz	WaS7	Scattered	2002	66	2/2	50–75
2005–2007	Südpfalz	Inter1	IP	2005	10	96/100	10–20
2005–2007	Südpfalz	Inter2	IP	2005	3	41/46	1–5
2005–2007	Südpfalz	Inter3	IP	2005	0	nt	<1
2006–2007	Südpfalz	Inter3/H	Scattered	2006	40	2/5	25–50
2005–2007	Südpfalz	Inter4	IP	2005	17	144/152	10–20
2002–2007	Nordbaden	KA1	IP	2006	24	13/13	20–30
2002–2008	Nordbaden	KA2	IP	2006	5	nt	1–5
2002–2003	Nordbaden	KA3	Scattered	2003	50	nt	25–50
2003	Nordbaden	KA4	Scattered	2003	50	nt	25–50
2003–2005	Mittelbaden	OG1	IP	2005	11	5/5	10–20
2005	Mittelbaden	OG2	IP	2005	2	nt	1–5
2005–2006	Südbaden	FR	IP	2005	12	nt	10–20
2003–2005	Weinsberg	LPPS	IP	2005	24	nt	20–30
2003–2006	Bodensee	MKD1	IP	nt	nt	nt	na
2003–2004	Bodensee	MKD2	IP	nt	nt	nt	na
2003–2004	Bodensee	MKD3	Organic	nt	nt	nt	na
2003–2006	Bodensee	MKD4	IP	2005	5	nt	1–5
2004–2006	Bodensee	MKD5	Scattered	2005	25	nt	25–50
2005–2007	Alsace	Ver1	IP	2006	20	10/10	20–30
2005–2007	Alsace	Ver2	Scattered	2006	25	12/12	25–50
2005–2007	Alsace	Ver3	IP	2006	50	14/14	30–50
2005–2007	Aargau	CH1	IP	2006	5	nt	1–5
2005–2007	Solothurn	CH2	IP	2006	50	9/11	30–50

^a IP = integrated production, nt = not tested, and na = not analyzed because no *Cacopsylla picta* were captured in this orchard.

^b Results of polymerase chain reaction (PCR) testing.

subsequent specific identification using the key of Ossiannilsson (32). During 2002 to 2007, regular insect captures were done from February to September each week or every 2 weeks. Because the insect captures started before the remigration of *C. picta* from overwintering hosts to the orchards, the first appearance of the remigrants in the orchard could be determined. From this time point, the orchard colonization period of the remigrants was calculated and weekly captured psyllids were grouped together for further data analysis. In this way, psyllid colonization classes of 2 days and 1 to 8 weeks were formed. In 2008 and 2009, psyllid captures for rearings and specific trials were concentrated in a particular orchard only in the known period of the population peak.

The natural infection rate with '*Ca. P. mali*' was determined in all individuals of *C. picta* collected during the regular collections at the different sites during their whole life cycle on apple. This mainly included overwintered adults (remigrants) and a small portion of new imagines just after hatching (emigrants) before their migration to the overwintering hosts.

Transmission trials with field-collected *C. picta*. Remigrant individuals for transmission trials were collected mainly at four different sites (commercial orchards NW1 and NW1a and untreated orchards HA1 and MeStr) (Table 1) with infection rates >50%. Because new adults of *C. picta* were difficult to capture in sufficient quantities in the orchards, newly hatched adults from breedings on healthy apple plants were transferred prior to the transmission trial on micropropagated AP-infected apple plants for a 7-day acquisition feeding as described by Jarausch et al. (16). Groups of 5 to 20 specimens of both generations were caged on micropropagated healthy apple plants ('Golden Delicious' or 'Royal Gala') in glass vessels or cages for 2 to 4 weeks as described by Jarausch et al. (16). The vessels were closed with an anti-aphid mesh. Dead individuals were regularly picked up and immediately frozen at -20°C until DNA extraction. Alive individuals were recollected from vessels after an incubation period of 2 weeks and from cages after an incubation period of 4 weeks and frozen at -20°C until DNA extraction.

After the experiment, all test plants were treated with insecticides (Confidor at 0.16 g/liter or Calypso at 0.2 ml/liter) and maintained in the greenhouse under insect-proof conditions. The test plants were regularly checked for symptom expression and analyzed by PCR for infection with '*Ca. P. mali*' 6 and 18 months after the end of the insect incubation period.

'*Ca. P. mali*' detection in insect and plant material. A simplified cetyltrimethylammonium bromide (CTAB) method for DNA extraction based on a protocol of Maixner et al. (24) was used as follows for nucleic acid extraction from both insect and plant material.

Insect material. Single insects collected from apple and frozen at -20°C were homogenized with a conical pestle (PP, 1.5 ml; Eppendorf, Hamburg, Germany) in 1.5-ml centrifugation tubes containing 100 μl of extraction buffer (2% CTAB, 1.4 M NaCl, 20 mM EDTA [pH 8.0], 100 mM Tris-HCl [pH 8.0], and 2% PVP 25) as grinding additive. After incubation at 60°C for 30 min, the homogenate was extracted with chloroform and the nucleic acids were precipitated with isopropanol from the supernatant. The resulting pellet was washed with 70% ethanol and was finally resuspended in 25 μl of sterile water and stored at -20°C .

Plant material. Total nucleic acids from field samples of apple as well as from test plants at the second test (18 months post-inoculation) were extracted from 0.5 g of phloem of roots or shoots. Only if test plants were still too small (e.g., at the first test 6 months postinoculation) were 0.3 g of leaf midribs used for extraction. All plant material was extracted according to the protocol specified for insect material with the following modifications: phloem tissue or leaf midribs were ground in extraction buffer in enzyme-linked immunosorbent bags with a homogenizator (Bioreba, Switzerland) and the homogenate was transferred

to 2-ml centrifugation tubes. Plant debris were precipitated by a centrifugation at low speed (4 min, $2,200 \times g$) and 1 ml of the supernatant was further processed as mentioned above. The purified DNA was resuspended in 100 μl of sterile water and stored at -20°C .

PCR analysis. PCR amplification of phytoplasma 16S rDNA was routinely carried out using universal ribosomal primers fU5/P7 (23,35) on DNA samples from psyllids and universal ribosomal primers fU5/rU4 (1,23) for plant material. All samples which gave a positive signal with universal primers were subjected to a new PCR with the nonribosomal-specific primers AP3/AP4 following the protocol for a '*Ca. P. mali*'-specific assay described by Jarausch et al. (19). Each PCR product (8 μl) was analyzed by agarose gel electrophoresis and visualized under UV after ethidium bromide staining.

Quantitative real-time PCR. The phytoplasma concentrations in infected individuals of *C. picta* were determined by quantitative real-time PCR (qPCR) using the assay based on SYBR Green technology described by Jarausch et al. (18). This assay amplifies a specific gene fragment of '*Ca. P. mali*' with the primers AP3/AP4. A 10-fold dilution series from $1.0\text{e} + 08$ to $1.0\text{e} + 01$ of a plasmid carrying the target gene fragment of '*Ca. P. mali*' was carried out in PCR-negative DNA extracts of *C. picta* for absolute quantification by the standard curve method. Naturally infected individuals of *C. picta* were used as a positive control. The qPCR assay was carried out on a Chromo4 Real-Time PCR detector (BioRad, München, Germany). The phytoplasma concentration determined in the sample was referred to as the phytoplasma concentration in one single individual.

Statistical analysis. All statistical analysis was performed using the biostatistics program R (R development core team, 2.9.0). The specific analysis of these empiric data obtained during this study was based on the initial discrimination between normal or nonparametrical distribution of values by the Saphiro-Wilk normality test. The following calculations were adapted to the particular data sets.

Data obtained for the infection rate of *C. picta* in different orchards as well as data obtained for the phytoplasma concentration in remigrant *C. picta* gave a nonparametrical distribution; the Bartlett test of homogeneity of variances showed that variances were not homogeneous. A Kruskal-Wallis rank sum test was adopted to determine differences among means. If the Kruskal-Wallis rank sum test showed differences among means, a Duncan test (confidence level = 95%) was adopted to rank these differences.

RESULTS

Population dynamics, abundance, and natural infection rate of *C. picta*. In order to study the incidence of AP related to the presence of psyllid populations, a wide spectrum of apple orchards with various degrees of infection with '*Ca. P. mali*' have been monitored over several years in different apple-growing regions of Germany, northern Switzerland, and eastern France. The AP infection status of each psyllid sampling site has been evaluated by visual diagnosis and molecular confirmation by PCR for those years where the insect captures have been performed (Table 1). The combined information of both data sets, visual inspection, and PCR testing was used to group the orchards into different infection classes (Table 1). In 51 of 52 sites, regular insect captures were carried out during 2002 to 2007 (Table 1). Due to various reasons such as uprooting of the orchard, modification of the agricultural practice, or unavailability of advisors, insect captures could not be done each year in every orchard.

Data about the different psyllid species captured in apple orchards of southwest Germany, northern Switzerland, and eastern France have already been published (13). Apart from *C. mali* and *C. melanoneura*, *C. picta* was the most important *Cacopsylla*

sp. found on apple. This was also the case for the other orchards investigated in this study. The absolute number of individuals collected in the various areas during all years of study is summarized in Table 2. Despite a varying number of sites and frequencies among the different areas of this monitoring, an obvious concentration of this species occurred in a zone around southwest Germany, eastern France, and northern Switzerland whereas it was rarely present in the far northern and southeastern orchards in Germany. These variations in vector population densities coincide with geographic differences in the incidence of the disease (Table 1). The AP monitoring of the investigated orchards confirmed the estimations of plant health inspectors and previous data (37) that the incidence of the disease is very low in the north of Germany (Schleswig-Holstein and Rheinland) and highest in the southwestern part, including the Alsace region in France.

The results presented in Table 2 also reveal that the presence and abundance of *C. picta* underlay important variations among the years in all investigated areas. Hence, similarly for all regions, population densities were always much higher in 2003 and 2005 compared with all other years. The population dynamics of this monophagous psyllid on apple was representatively illustrated for the year 2005 for those zones with the highest abundance of insect population and incidence of the disease (Fig. 1). The first overwintered adults remigrating from their overwintering habitats arrive in apple orchards by mid-March (week 11), reach a maximum in the first half of April (week 13 to 15), and complete their life cycle on apple during the following 5 weeks (Fig. 1). The first imagines of the new generation hatch by mid-June, whirling within orchards for a further 2 weeks before they migrate to their overwintering sites.

TABLE 2. Number of ‘*Candidatus Phytoplasma mali*’-infected per total number of captured individuals of *Cacopsylla picta* collected on *Malus domestica* in different apple-growing regions of Germany, eastern France (Alsace), and Northern Switzerland (Aargau, Solothurn) during 2002 to 2007^a

Region	Number of sites (Sites) and numbers of infected individuals/captured individuals (Ratio)												Total ^b
	2002		2003		2004		2005		2006		2007		
	Sites	Ratio	Sites	Ratio	Sites	Ratio	Sites	Ratio	Sites	Ratio	Sites	Ratio	
Schleswig-Holstein	nt	...	2	0/0	2	0/0	2	0/1	nt	...	nt	...	0/1
Rheinland	1	0/3	1	0/9	1	0/3	1	0/5	nt	...	nt	...	0/20
Rheinessen	3	0/10	5	5/73	2	0/1	2	0/13	nt	...	nt	...	5/97
Vorderpfalz	10	20/187	12	67/574	8	13/89	6	32/269	4	2/17	5	4/17	138/1,153
Südpfalz	nt	...	nt	...	nt	...	4	3/31	5	2/27	5	2/26	7/84
Nordbaden	3	4/21	4	17/149	2	3/27	2	14/140	2	0/2	2	0/1	38/340
Mittelbaden	1	0/0	1	0/26	nt	...	2	21/263	nt	...	nt	...	21/289
Südbaden	nt	...	nt	...	nt	...	1	3/20	1	0/1	nt	...	3/21
Weinsberg	nt	...	1	0/4	1	0/1	1	0/1	nt	...	nt	...	0/6
Bodensee	nt	...	4	0/0	5	0/4	3	1/6	3	0/2	nt	...	1/12
Alsace	nt	...	nt	...	nt	...	3	22/156	3	0/1	3	0/0	22/157
Aargau	nt	...	nt	...	nt	...	1	5/55	1	0/4	1	1/3	6/62
Solothurn	nt	...	nt	...	nt	...	1	0/9	1	0/2	1	0/0	0/11
Total	...	24/221	...	89/835	...	16/125	...	101/969	...	4/56	...	7/47	241/2,253

^a Values in this table comprise the totality of all captures including regular captures for population dynamics in selected orchards as well as additional occasional captures for trials on abandoned sites; nt = not tested.
^b Total numbers of infected/captured individuals.

Fig. 1. Population dynamics of *Cacopsylla picta* in different apple-growing regions in southwest Germany, northern France (Alsace), and northern Switzerland (Aargau, Solothurn) 2005.

A reliable estimation of the natural infection rate of *C. picta* could only be achieved if a substantial number of individuals could be tested. Whereas no infected individual was captured in the north of Germany, the highest infection rates occurred in those regions with more important populations of *C. picta*, reaching percentages of 11 to 15% for Vorderpfalz, 11% for Nordbaden, and 14% for the Alsace region. Over the years, a rather stable percentage of $\approx 10\%$ of infected individuals was found in these regions. Therefore, these data indicate a correlation between the incidence of AP in a given region and the infection rate of the *C. picta* population in this region.

Relationship of the infection rate of overwintered *C. picta* and the infection status of the orchard. A more detailed analysis of the relationship between the AP infection rate of the orchard and the corresponding portion of infected *C. picta* is shown in Table 3. The total spectrum of investigated sites included commercial orchards with integrated production or organic farming as well as field sites of abandoned or scattered orchards without any treatment. The infection rates in orchards with integrated production varied from <1 to $>50\%$. The investigated organically cultivated orchards showed lower infection rates of only 5%. In contrast, all abandoned or scattered sites were highly AP infected in all different areas. Thus, based on visual monitoring and PCR detection, all investigated sites could be assigned to at least seven classes of infection variously dispersed among the different types of orchards (Table 1). Although the estimation of the infection rate of overwintered *C. picta* captured in the orchards of the different infection classes could only be regarded as reliable for those where a substantial number of individuals could be tested, the infection rates were 5 to 14% yielding a mean infection rate of 10.6%. The statistical analysis of the data revealed no relationship between infection rate of *C. picta* and infection of the orchard by '*Ca. P. mali*' (Kruskal-Wallis $\chi^2 = 16.2185$, $df = 11$, $P = 0.1332$). The general comparison between the type of orchards (abandoned, integrated production, or organic farming) showed a tendency of higher infection rates of *C. picta* in abandoned orchards which, however, were not statistically significant (Kruskal-Wallis $\chi^2 = 4.7151$, $df = 2$, $P = 0.09465$) (Fig. 2).

Gender balance of captured and infected *C. picta*. The separation of all captured and tested *C. picta* with respect to their sex allowed us to follow the evolution of the gender balance of collected overwintered individuals by time and to analyze the gender balance of infected remigrants. Evolution of the gender balance of all overwintered *C. picta* by time for the years 2003 and 2005, where a substantial number of data were available for this analysis, is demonstrated in Figure 3. A similar tendency appears for both years: an equal gender balance at the beginning of the remigration period followed by a clear predominance of

captured females with advanced colonization time (Fig. 3). A detailed analysis focusing on the ratio of captured and infected males and females and differentiating among cultivated and uncultivated sites is presented in Table 4. The recapitulation for all years resulted in a similar percentage of 10% infected males compared with 11% infected females for both culture practices. A Kruskal-Wallis test did not reveal significant differences between the infection rate of males in cultivated or uncultivated orchards and the infection rate of females, respectively (Kruskal-Wallis $\chi^2 = 5.2439$, $df = 3$, $P = 0.1548$). A higher number of females was constantly captured, yielding an overall male/female ratio of 1:1.4 in cultivated orchards and 1:1.5 in uncultivated orchards. Although the same gender balance also was observed in cultivated orchards for infected individuals, a slightly higher portion of females was infected in uncultivated orchards (ratio 1:2).

Transmission trials with overwintered adults of *C. picta*. In order to elucidate the potential vector capacity of field-collected remigrants of *C. picta*, transmission trials were conducted under controlled conditions. A summary of these trials of a 6-year

Fig. 2. Distribution of infected remigrants of *Cacopsylla picta* in different types of orchards. Means of the percentage of infected *C. picta* (males and females) were compared by a Kruskal-Wallis rank sum test (Kruskal-Wallis $\chi^2 = 4.7151$, $df = 2$, P value = 0.09465). No significant difference was observed.

TABLE 3. Relationship between infection status of the orchard and number of phytoplasma-infected overwintered individuals of *Cacopsylla picta* based on captures made during 2002 to 2008^a

Orchard type	Number of sites	Years of psyllid captures	AP infection class of the orchard	Number of <i>C. picta</i> individuals tested	Number of PCR-positive <i>C. picta</i>	Infection of <i>C. picta</i> (%)
Organic farming	1	2004–2005	<1	1	0	0
Organic farming	4	2002–2005	1–5	112	8	7.1
Integrated production	1	2005–2006	<1	17	0	0
Integrated production	12	2002–2008	1–5	268	34	12.7
Integrated production	8	2002–2008	10–20	361	28	7.8
Integrated production	3	2002–2005	20–30	135	7	5.2
Integrated production	2	2005–2006	30–50	28	2	7.1
Integrated production	1	2002–2005	>50	119	15	12.6
Integrated production	1	2007–2008	>75	21	3	14.3
Abandoned (untreated)	9	2002–2008	25–50	688	81	11.8
Abandoned (untreated)	5	2002–2003	>50	76	7	9.2
Abandoned (untreated)	1	2002–2006	>75	441	56	12.7
Total	48	...	...	2,267	241	10.6

^a AP = apple proliferation and PCR = polymerase chain reaction.

repetition period is shown in Table 5. The results of 2002 have already been published (16,17). The data clearly show that successful transmission was not only an occasional event but also could be repeated each year. Plant material as well as experimental conditions were kept as homogeneous as possible; thus, the differences in transmission success must be attributed to the heterogeneity of field-collected specimens. However, despite these variations, successful transmission occurred each year, yielding rates of 8 to 45%. Although not all phytoplasma-infected individuals of *C. picta* were able to transmit ‘Ca. P. mali’ under the applied experimental conditions, the presence of infected *C. picta*

in a trial corresponded generally well to the transmission events. Thus, the results demonstrate the high vector capacity of overwintered *C. picta*.
Acquisition and transmission capacity of new imagines of *C. picta*. Likewise, the acquisition of ‘Ca. P. mali’ and its subsequent transmission to healthy test plants was studied with new individuals from breedings of *C. picta* in the greenhouse. As for overwintered adults, successful transmission occurred in each year of repetition (Table 6). Despite the more or less homogeneous insect material from breedings, transmission rates varied between 2 and 20% among the years of investigation. In these

Fig. 3. Trend of gender balance changes by time of remigrant adults of *Cacopsylla picta* in two different years of high population densities. Only data from orchards in which *C. picta* regularly was caught were analyzed; absolute numbers are given inside the columns.

trials, a much higher portion of PCR-positive individuals was not able to transmit compared with naturally infected overwintered adults. However, transmission events were likewise correlated to the presence of infected *C. picta*.

Concentration of ‘*Ca. P. mali*’ in infected remigrants of *C. picta*. The phytoplasma titer was measured by qPCR in infected individuals of *C. picta* at different periods after the first remigrants were found on apple. The first infected specimens of *C. picta* were already found 2 days after the first appearance in the orchard. All infected individuals captured during the subsequent weekly collections were grouped for data analysis in classes of 1 to 8 weeks of orchard colonization. By this means, the development of the phytoplasma titer could be followed for a period of at least 8 weeks. The mean concentrations deviated between $2.0e + 08$ and $6.0e + 08$ copies per individual, except for a considerably high value of circa $9.0e + 08$ copies after 8 weeks (Fig. 4). However, the graph did not show a clear rise of the phytoplasma load with increasing orchard colonization time. The mean phytoplasma concentration varied consistently among the weeks (e.g., the statistical analysis revealed differences in phytoplasma load between individuals captured after 8 weeks with those captured after 2 days, 1 week, and 6 weeks, respectively, but not with those caught after 2 weeks) (Fig. 4). Thus, the phytoplasma titer measured in individuals already collected 2 days after the first appearance was as high as for those specimens tested after 6 weeks, indicating that overwintered *C. picta* arrive already highly phytoplasma loaded in the orchards when remigrating from their overwintering sites in early spring (Table 7).

DISCUSSION

The epidemiology of a disease underlies multiple biotic and abiotic factors which interact in manifold variable conditions. An epidemiological cycle is a complex system which requires an extensive survey of its different components during several years. Hence, the main objective of this study was to collect precise and repetitive data over several years in order to provide a comprehensive description of the epidemiology of ‘*Ca. P. mali*’ and its main vector in Germany and neighboring regions, *C. picta*. By the selection of many multifaceted sites and surroundings, we intended to disclose differences and common aspects of the epidemiology and etiology of AP and to work out general conclusions for the spread of this disease and its management. Until now, only a few reports exist about the epidemiology of AP disease and the search for its vectors, and all these studies are limited to the description of a particular situation in one defined, local area (5,9,10,26,40–42). The spectrum of sites investigated during this study included orchards in various areas with specific biogeographical and climatic properties. As published previously, *C. picta* and *C. melanoneura* are actually the most abundant psyllid species in apple orchards of the studied regions (13). However, *C. melanoneura* was not found to be infected with ‘*Ca. P. mali*’ and, therefore, the population of *C. melanoneura* in Germany and neighboring regions is regarded as being unable to vector the disease (27). In contrast, the overall survey showed a clear coherence between the incidence of ‘*Ca. P. mali*’ and the presence of *C. picta*: a low incidence and absence or very few *C.*

TABLE 4. Ratio of captured and infected males and females of overwintered *Cacopsylla picta* collected in cultivated and uncultivated apple orchards during 2002 to 2009

Year	Orchard type	Male infected/total (%)	Female infected/total (%)	Male/female captured	Male/female infected
2002	Cultivated	0/17 (0)	5/43 (11.6)	1:2.5	0:5
	Uncultivated	1/40 (0.03)	16/85 (18.8)	1:2.1	1:16
2003	Cultivated	12/115 (10.4)	9/133 (6.8)	1:1.2	1:0.75
	Uncultivated	24/236 (10.2)	44/349 (12.6)	1:1.5	1:1.8
2004	Cultivated	4/16 (25)	4/38 (10.5)	1:2.4	1:1
	Uncultivated	2/25 (8)	6/45 (13.3)	1:1.8	1:3
2005	Cultivated	27/279 (9.7)	32/360 (8.9)	1:1.3	1:1.2
	Uncultivated	19/145 (13.1)	23/188 (12.2)	1:1.3	1:1.2
2006	Cultivated	0/9 (0)	1/15 (6.7)	1:1.7	0:1
	Uncultivated	0/7 (0)	3/25 (12)	1:3.6	0:3
2007	Cultivated	0/2 (0)	2/10 (20)	1:5	0:2
	Uncultivated	2/14 (14.3)	3/21 (14.3)	1:1.5	1:1.5
2008	Cultivated	0/20 (0)	4/27 (14.8)	1:1.4	0:4
	Uncultivated	1/4 (25)	1/4 (25)	1:1	1:1
2009	Cultivated	3/16 (18.8)	1/42 (2.4)	1:2.6	1:0.33
Total	Cultivated	46/474 (9.7)	58/668 (8.7)	1:1.4	1:1.3
Total	Uncultivated	49/471 (10.4)	96/717 (13.4)	1:1.5	1:2
Total	...	95/945 (10.1)	154/1,385 (11.1)	1:1.5	1:1.6

TABLE 5. Summary of transmission trials with overwintered adults of *Cacopsylla picta* conducted in climatic chambers in the greenhouse during 2002 to 2007

Overwintered <i>C. picta</i> ^a	2002	2003	2004	2005	2006	2007
PCR + insects/total recollected insects tested	11/92	32/255	8/62	22/256	3/34	6/32
PCR + plants/plants tested	5/11	12/36	1/13	4/32	2/24	4/12
Transmission (%)	45	33	8	13	8	33

^a PCR = polymerase chain reaction.

TABLE 6. Summary of the acquisition and transmission trials with new adults of *Cacopsylla picta* from breedings in the greenhouse conducted during 2002 to 2007

New adults of <i>C. picta</i> ^a	2002	2003	2004	2005	2006	2007
PCR + insects/total recollected insects tested ^b	1/56	26/135	5/58	28/261	9/150	146/365
PCR + plants/plants tested	1/5	4/28	1/21	1/23	4/25	1/49
Transmission (%)	20	14	5	4	16	2

^a PCR = polymerase chain reaction.

^b All individuals tested after acquisition feeding and subsequent latency period during transmission.

picta in the far northern part and in southeastern Germany compared with a concentration of the disease and the vector along a southwestern axis comprising the southwestern parts of Germany and adjacent areas of France (Alsace) and Switzerland (Aargau and Solothurn). Regardless of the highly unequal population densities, the population dynamics were similar in the different regions and were in general agreement with the life cycle of *C. picta* described for other regions (26). However, we consistently captured the first remigrants in the second half of March, which is at least 2 weeks earlier than in other reports (26,30). This might be due to the more extensive captures carried out in the present study or to climatic differences between the studied regions (e.g., Trentino in northern Italy).

Although a good correlation between the incidence of the disease and the proportion of infected individuals was observed on a regional scale, yielding a relative constant mean percentage $\approx 10\%$ of naturally infected overwintered *C. picta* every year, a more detailed analysis of the correlation between the infection status of a particular orchard and the abundance of infected *C. picta* revealed a more complex and heterogeneous situation. No clear relationship occurred in terms of orchard type, infection rate of the orchard with ‘*Ca. P. mali*’, and abundance and infection rate of *C. picta*. Thus, in IP orchards with low AP infection, we found an important population of *C. picta* and a high number of infected individuals and, vice versa, at some highly infected abandoned sites only few specimens were collected. We conclude that those infected *C. picta* we found in a particular orchard already acquired the agent in the previous year elsewhere and they did not necessarily go back to the orchard where they were born or where they acquired the phytoplasma. On the other hand, the orchard type does not really influence the frequency of the vector; only when intensive spraying starts due to cultural practices in some orchards is the population reduced. However, when overwintered adults of *C. picta* arrive on apple in early spring, spraying has not yet started in our regions and, thus, the psyllid can move around unimpaired. Although the gender

balance was equal at the beginning of the remigration period, females showed an increased life span and stayed up to 2 months in the orchards. Thus, the total number of females was higher regardless of the orchard type. Interestingly, the ratio of infected males to infected females was similar to the ratio of captured males to females, resulting in a similar infection rate of both genders. This indicates that both genders acquire the phytoplasma equally well. Furthermore, the analysis of the concentration of ‘*Ca. P. mali*’ in overwintered *C. picta* from the first arrival until the last collected specimens showed that the first remigrants already arrived with a high phytoplasma load and the titer did not significantly rise in individuals collected later. This leads to the conclusion that overwintered adults have already acquired the phytoplasma during larval development or as emigrant adults in the previous year. The phytoplasma multiplies in the insect during the whole overwintering period, also confirming for *C. picta* the hypothesis of winter retention of the pathogen which has already been described for *C. melanoneura* (42) and for *C. pruni*, the vector of ‘*Ca. P. prunorum*’ (6,43). The hypothesis of primary pathogen ingestion by new adults of *C. picta* is also supported by data recently published by Mayer and co-workers (28–30), who found that emigrants of *C. picta* were particularly attracted by infected apple trees before migrating to their overwintering hosts.

Comparative data in terms of the natural infection status of *C. picta* are rare. Only Carraro et al. (5) determined the proportion of phytoplasma-positive and inoculative *C. picta* in groups of overwintering adults and of the springtime generation. Their cumulative data showed that 45% of remigrants in spring carried the phytoplasma compared with 14% infection in groups of the springtime generation. However, these data, based on calculations in groups of psyllids, cannot be directly compared with our quantification in individuals of *C. picta*. Furthermore, the vector situation in the area investigated by Carraro and colleagues (5) seems to be quite different because the population densities in our regions were much lower and the portion of new adults among the total numbers of insects tested was negligible, which was also reported by other authors (10). However, the detection of an agent in the insect’s body by molecular means does not yet prove that it is a real vector (25,44). Therefore, in addition to the direct determination of the natural infection status in overwintered adults, we proved the vector capacity of both generations of *C. picta* within specific transmission trials under experimental conditions. We could clearly show that both generations were able to transmit the phytoplasma repeatedly to healthy test plants but with a higher efficiency by overwintered adults. Oppositional results were reported by Mattedi et al. (26) and Carraro et al. (5), who found higher transmission rates with specimens of the new generation of *C. picta*. However, this obvious contradiction can be based on two assumptions: (i) the acquisition and transmission conditions in our trials were not ideal, at least for new adults, or (ii) the popu-

Fig. 4. Mean phytoplasma concentration in overwintered individuals of *Cacopsylla picta* captured in the orchards at different periods after remigration in spring (psyllid colonization time in the orchard = 2 days to 8 weeks). Boxplot of means of phytoplasma concentration at different times compared by a Kruskal-Wallis rank sum test (Kruskal-Wallis $\chi^2 = 20.6716$, $df = 8$, P value = 0.008073). Letters above each column indicate the differences evidenced by a subsequent Duncan test; confidence level = 0.95, variation coefficient = 125.35%.

TABLE 7. Quantification of ‘*Candidatus Phytoplasma mali*’ in remigrants of *Cacopsylla picta* at their first appearance in the orchards in different apple-growing regions

Mean phytoplasma copies/individual	Region	Date of first appearance
4.07e + 08	Südpfalz	20 March 2005
1.38e + 08	Nordbaden	24 March 2005
1.69e + 08	Nordbaden	24 March 2005
7.05e + 07	Nordbaden	24 March 2005
2.32e + 08	Nordbaden	24 March 2005
5.08e + 08	Nordbaden	24 March 2005
7.23e + 08	Nordbaden	24 March 2005
3.25e + 08	Südbaden	20 March 2005
4.39e + 08	Switzerland	21 March 2005
1.34e + 08	Alsace	24 March 2005
2.91e + 08	Alsace	24 March 2005
4.21e + 08	Alsace	24 March 2005

lations of *C. picta* in areas located north and south of the Alps are different. However, all three studies with new adults of *C. picta* demonstrate that the latent period or incubation time necessary from initial acquisition to the ability to transmit the phytoplasma can be very short (e.g., a few weeks), whereas it has been reported for another psyllid vector, *C. pruni*, that the transmission of 'Ca. P. prunorum' takes place after the overwintering period of several months (43). Data reported by Pedrazzoli et al. (33) further demonstrated that new adults of *C. picta* can efficiently acquire the phytoplasma from test plants but phytoplasma multiplication to high titers happens only in few individuals within 2 weeks post-inoculation. Although acquisition efficiencies of new adults were much lower in our trials, the data confirm the low transmission efficiency of new adults, probably due to the low number of individuals which were sufficiently highly phytoplasma loaded. We suppose that the physiology of the individual determines its vector capacity. However, the simple fact that successful transmission occurred with new adults of *C. picta* and that, for some individuals, a rather short latency period is sufficient to obtain an important phytoplasma titer for transmission indicate that emigrant as well as remigrant adults of *C. picta* are vectors for 'Ca. P. mali'. In agreement with Carraro et al. (5), we conclude that *C. picta* is able to transmit the pathogen during the entire period where it resides on apple and, in contrast to *C. pruni* (43), we propose a polycyclic vector model for *C. picta*: the overwintered individuals are more numerous vectors because they already acquired the phytoplasma in the previous year and the agent had a long latency period to multiply in the insect's body but a certain portion of the new generation already becomes infective and transmits the pathogen in the same season before migrating to overwintering hosts.

It is still unclear which is the most critical and most efficient period and habitat for *C. picta* to acquire the phytoplasma and what happens during the overwintering period; therefore, it is difficult to give general control recommendations. However, our study demonstrated that the situation in a fruit crop area can be rather variable with regard to infection status, population density, wild inoculum sources in the surroundings, agricultural practices, and so on, and that the infection of an orchard is not an immediate result of 1 year but reflects the sum of pathogen-related infection events over a longer, multiyear period. Consequently, control strategies on a single orchard level are not sufficient. We recommend comprehensive control management on a regional level, including control of the remigrants, prevention of the larval development, and impairment of the motility of new adults. Considering this aspect, previous studies have shown that newly hatched adults of *C. picta* were more attracted by the odor of infected trees compared with healthy ones (28,30). In this period, classical control measures are critical; therefore, alternative control strategies such as use of biological traps based on the release of infochemicals (28) could be envisaged. A further advantage of such control is that it could be used regardless of the different orchard types and particular cultural practices of neighboring areas and country-specific plant-protection guidelines.

In conclusion, this study provided important data for a better understanding of the epidemiology of AP, not only in Germany but also in general. *C. picta* proved to be a very efficient vector and has to be regarded as main vector in all regions where it is present. Although the disease is spread in a polycyclic manner, the main spread takes place by highly infected overwintered adults on a regional scale. In consequence, control strategies against the spread of AP also have to be applied on a regional scale and include consequent uprooting of infected trees as well as vector control.

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LITERATURE CITED

- Ahrens, U., and Seemüller, E. 1992. Detection of DNA of plant pathogenic mycoplasma-like organisms by a polymerase chain-reaction that amplifies a sequence of the 16S RNA gene. *Phytopathology* 82:828-832.
- Anonymous. 1991. European guidelines for organic production 2092/91.
- Anonymous. 2006. Richtlinie für den kontrollierten, Integrierten Anbau von Obst und Gemüse in der Bundesrepublik Deutschland. II. überarbeitete und erweiterte Richtlinie, Stand. Fachgruppe Obstbau/Gemüsebau. www.obstbau.org
- Canik, D., and Ertunc, F. 2007. Distribution and molecular characterization of apple proliferation phytoplasma in Turkey. *Bull. Insectol.* 60:335-336.
- Carraro, L., Ferrini, F., Ermacora, P., Loi, N., and Labonne, G. 2008. Infectivity of *Cacopsylla picta* (Syn. *C. costalis*), vector of 'Candidatus Phytoplasma mali' in North East Italy. *Acta Hortic.* 781:403-408.
- Carraro, L., Ferrini, F., Labonne, G., Ermacora, P., and Loi, N. 2004. Seasonal infectivity of *Cacopsylla pruni*, vector of European stone fruit yellows phytoplasma. *Ann. Appl. Biol.* 144:191-195.
- Carraro, L., Loi, N., and Ermacora, P. 2001. Transmission characteristics of the European stone fruit yellows phytoplasma and its vector *Cacopsylla pruni*. *Eur. J. Plant Pathol.* 107:695-700.
- Čermák, V., and Lauterer, P. 2008. Overwintering of psyllids in South Moravia (Czech Republic) with respect of the vectors of the Apple Proliferation cluster phytoplasmas. *Bull. Insectol.* 61:147-148.
- Delic, D., Martini, M., Ermacora, P., Carraro, L., and Myrta, A. 2005. first report of fruit tree phytoplasmas and their psyllid vectors in Bosnia and Herzegovina. *J. Plant Pathol.* 41:195-203.
- Fialova, R., Navratil, M., Valova, P., and Lauterer, P. 2008. Molecular tests to determine apple proliferation phytoplasma presence in psyllid vectors from apple tree orchards in the Czech Republic. *Acta Hortic.* 781:471-475.
- Frisinghelli, C., Delaiti, L., Grando, M. S., Forti, D., and Vindimian, M. E. 2000. *Cacopsylla costalis* (Flor 1861), as a vector of apple proliferation in Trentino. *J. Phytopathol.* 148:425-431.
- Jarausch, B. 2003. Welche Rolle spielen Blattsaugerarten bei der Übertragung von Apfelfeuerkrankung-Phytoplasmen in deutschen Apfelanlagen? *Obstbau* 4:205-206.
- Jarausch, B., Burckhardt, D., Lauterer, P., and Jarausch, W. 2009. Psyllids (Hemiptera, Psylliidea) captured in commercial apple and stone fruit orchards in southwest Germany, eastern France and northwest Switzerland. *Mitt. Schweiz. Entomol. Ges.* 82:205-215.
- Jarausch, B., Fuchs, A., Schwind, N., Krczal, G., and Jarausch, W. 2007. *Cacopsylla picta* as most important vector for 'Candidatus Phytoplasma mali' in Germany and neighbouring regions. *Bull. Insectol.* 60:189-190.
- Jarausch, B., and Jarausch, W. 2010. Psyllid vectors and their control. Pages 250-271 in: *Phytoplasmas—Genomes, Plant Hosts and Vectors*. P. G. Weintraub and P. Jones, eds. CABI, England.
- Jarausch, B., Schwind, N., Jarausch, W., and Krczal, G. 2004. Overwintering adults and springtime generation of *Cacopsylla picta* (synonym *C. costalis*) can transmit apple proliferation phytoplasmas. *Acta Hortic.* 657:409-413.
- Jarausch, B., Schwind, N., Jarausch, W., Krczal, G., Dickler, E., and Seemüller, E. 2003. First report of *Cacopsylla picta* as a vector of apple proliferation phytoplasma in Germany. *Plant Dis.* 87:101.
- Jarausch, W., Peccerella, T., Schwind, N., Jarausch, B., and Krczal, G. 2004. Establishment of a quantitative real-time PCR assay for the quantification of apple proliferation phytoplasmas in plants and insects. *Acta Hortic.* 657:415-420.
- Jarausch, W., Saillard, C., Dosba, F., Bové, J. M. 1994. Differentiation of mycoplasma-like organisms (MLOs) in European fruit trees by PCR using specific primers derived from the sequence of a chromosomal fragment of the apple proliferation MLO. *Appl. Environ. Microbiol.* 60:2916-2923.
- Krczal, G., Krczal, G., Krczal H., and Kunze, L. 1989. *Fiebiella florii* (Stal)—a vector of the apple proliferation agent. *Acta Hortic.* 235:99-104.
- Kunze, L. 1989. Apple proliferation. Pages 99-113 in: *Virus and Viruslike Diseases of Pome Fruits and Simulating Noninfectious Disorders*. P. R. Fridlund, ed. Cooperative Extension College of Agriculture and Home Economics, Washington State University, Pullmann.
- Lauterer, P. 1999. Results of the investigations on Hemiptera in Moravia, made by the Moravian museum (Psylliidea 2). *Acta Mus. Morav. Sci. Nat.* 84:71-151.

23. Lorenz, K. H., Schneider, B., Ahrens, U., and Seemüller, E. 1995. Detection of the apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and nonribosomal DNA. *Phytopathology* 85:771-776.
24. Maixner, M., Ahrens, U., and Seemüller, E. 1995. Detection of the German grapevine yellows (Vergilbungskrankheit) MLO in grapevine, alternative hosts and a vector by a specific PCR procedure. *Eur. J. Plant Pathol.* 101:241-250.
25. Marzachi, C., Milne, R. G., and Bosco, D. 2004. Phytoplasma-plant-vector relationship. Pages 211-241 in: *Recent Research and Development in Plant Pathology*. S. G. Pandalai and A. Gayathri, eds. Research Signpost, Kerala, India.
26. Mattedi, L., Forno, F., Cainelli, C., Grando, M. S., and Jarausch, W. 2008. Research on '*Candidatus* Phytoplasma mali' transmission by insect vectors in Trentino. *Acta Hort.* 781:369-374.
27. Mayer, C. J., Jarausch, B., Jarausch, W., Vilcinskas, A., and Gross, J. 2009. *Cacopsylla melanoneura* has no relevance as vector of apple proliferation in Germany. *Phytopathology* 99:729-738.
28. Mayer, C. J., Vilcinskas, A., and Gross, J. 2008. Pathogen-induced release of plant allomone manipulates vector insect behaviour. *J. Chem. Ecol.* 34:1518-1522.
29. Mayer, C. J., Vilcinskas, A., and Gross, J. 2008. Phytopathogen lures its insect vector by altering host plant odor. *J. Chem. Ecol.* 34:1045-1049.
30. Mayer, C. J., Vilcinskas, A., and Gross, J. 2010. Chemically mediated multitrophic interactions in a plant-insect vector-phytoplasma system compared with a partially nonvector species. *Agric. For. Entomol.* Online publication. doi:10.1111/j:1461-9563.
31. Mütter, J., and Vogt, H. 2003. Sampling methods in orchard trials: A comparison between beating and inventory sampling. *IOBC/WPRS Bull.* 26:67-72.
32. Ossiannilsson, F. 1992. The Psylloidea (Homoptera) of Fennoscandia and Denmark. *Fauna Entomologica Scandinavica* 26. E. J. Brill, Leiden.
33. Pedrazzoli, F., Gualandri, V., Forno, F., Mattedi, L., Malagnini, V., Salvadori, G., and Ioriatti, C. 2007. Acquisition capacities of the overwintering adults of the psyllid vectors of '*Candidatus* Phytoplasma mali'. *Bull. Insectol.* 60:195-196.
34. Rui, D., Ciferri, R., and Refatti, E. 1950. La virosi degli "scopazzi del melo" nel Veronese. *Not. Mal. Pianta* 13:7-11.
35. Schneider, B., Seemüller, E., Smart, C. D., and Kirkpatrick, B. C. 1995. Phylogenetic classification of plant pathogenic mycoplasma-like organism or phytoplasmas. Pages 369-380 in: *Molecular and Diagnostic Procedures in Mycoplasmaology*. S. Razin and J. G. Tully, eds. Academic Press, San Diego, CA.
36. Seemüller, E. 1990. Apple proliferation. Pages 67-68 in: *Compendium of Apple and Pear Diseases*. A. L. Jones and H. S. Aldwinkle, eds. American Phytopathological Society, St. Paul, MN.
37. Seemüller, E., Kison, H., and Lorenz, K. H. 1998. On the geographic distribution and prevalence of the apple proliferation phytoplasma in low-intensity orchards in Germany. *J. Plant Dis. Prot.* 105:404-410.
38. Seemüller, E., and 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. *Int. J. Syst. Evol. Microbiol.* 54:1217-1226.
39. Tedeschi, R., and Alma, A. 2006. *Fieberiella florii* (Homoptera: Auchenorrhyncha) as a vector of '*Candidatus* Phytoplasma mali' Plant Dis. 90:284-290.
40. Tedeschi, R., and Alma, A. 2007. '*Candidatus* Phytoplasma mali': The current situation of insect vectors in northwestern Italy. *Bull. Insectol.* 60:187-188.
41. Tedeschi, R., Bosco, D., and Alma, A. 2002. Population dynamics of *Cacopsylla melanoneura* (Homoptera: Psyllidae), a vector of apple proliferation phytoplasma in Northwestern Italy. *J. Econ. Entomol.* 95:544-551.
42. Tedeschi, R., Visentin, C., Alma, A., and 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). *Ann. Appl. Biol.* 142:285-290.
43. Thébaud G., Yvon M., Alary R., Sauvion N., and Labonne G., 2009. Efficient transmission of '*Candidatus* Phytoplasma prunorum' is delayed by eight months due to a long latency in its host-alternating vector. *Phytopathology* 99:265-273.
44. Weintraub, P. G., and Beanland, L. 2006. Insect vectors of phytoplasmas. *Annu. Rev. Entomol.* 51:91-111.