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Occurrence of different *Cacopsylla* species in apple orchards in South Tyrol (Italy) and detection of apple proliferation phytoplasma in *Cacopsylla melanoneura* and *Cacopsylla picta*

(Hemiptera: Psylloidea)

Stefanie Fischnaller[1], Martin Parth, Manuel Messner, Robert Stocker, Christine Kerschbamer, Yazmid Reyes-Dominguez und Katrin Janik

Summary: Preventing the diffusion of phytoplasma associated diseases until now is based mainly on indirect control measurements against the transmitting insect vectors. Apple proliferation, one of the economically most important pests in European apple cultivation is caused by the apple proliferation (AP) phytoplasma ('*Candidatus* Phytoplasma mali'), which is spread by the psyllids *Cacopsylla* (*C.*) *picta* (Foerster, 1848) and *C. melanoneura* (Foerster, 1848). Current control measures primarily comprise treatments against these AP phytoplasma transmitting vectors. The surveillance of *C. picta* and *C. melanoneura* population dynamics, as well as the determination of their infection rate in the field are crucial prerequisites to develop suitable and appropriate strategies to limit further spread of AP phytoplasma. Furthermore, the analysis of the species composition of the genus *Cacopsylla* present in apple orchards provides important information about the presence of other insect vectors potentially involved in spreading AP or other diseases. During an intensive monitoring program realized in the valleys of Val Venosta and Burggraviato (South Tyrol, Italy), the hotspots of apple proliferation epidemics, over 13,000 *Cacopsylla* individuals were captured and the occurrence of 16 species of the genus *Cacopsylla* was confirmed. The presence of *C. picta* was recorded in more than 50% of the investigated apple orchards and the natural infection rate of this vector was about 21% in a three-year average. Conversely, *C. melanoneura* was confirmed in more than 90% of the investigated sites but its low infection rate of about 1% further supports that it plays a rather secondary role in spreading AP phytoplasma in South Tyrol.

Keywords: apple proliferation, insect vectors, *Cacopsylla*, population dynamics

1. Introduction

Apple proliferation (AP) is one of the major economical important diseases in European apple growing regions (Mattedi et al. 2007; Minarro et al. 2016). The causative agent of AP is '*Candidatus* Phytoplasma mali' ('*Ca.* Phytoplasma mali'; AP phytoplasma), cell wall deficient bacteria which belong to the 16SrX-phytoplasma group (Lee et al. 2000). Manipulating the physiology of the infected plant, linked to profound disturbance in the normal balance of growth regulators, the disease causes symptoms on leaves, roots, shoots and fruits (Rui 1950; Lepka 1999; Aldaghi et al. 2012; Kartte & Seemüller 1988; Giorno et al. 2013; Zimmermann et al. 2015; Janik et al. 2017). The impaired quality and quantity of the yield implies severe economic losses on the production site (Strauss 2009). In South Tyrol (Alto Adige, Italy), the largest continuous apple producing area in Europe covering more than 18.000 ha (Dalla Via 2013), severe outbreaks were documented in 2001, 2006 and 2011/2012 that led to high economic losses in the region (Österreicher & Unterthurner 2013).

[1] Corresponding author, e-mail: steffi.fischnaller@laimburg.it Published online 30 December 2017

Phytoplasma are mainly transmitted by phloem-sucking insects (Lee et al. 2000; Weintraub & Beanland 2006), but transmission via root grafts (Baric et al. 2008; Ciccotti et al. 2008) or by grafting infected propagation material to healthy plants (Seemüller et al.1984) has also been described. The interaction between phytoplasma and their insect vectors is very specific: After the ingestion of the phytoplasma during feeding of the insect on an infected plant, the bacteria must circumvent the immune defence system of the insect and replicates inside the salivary glands. After this acquisition and replication phase, the bacteria can be transmitted with the saliva by the infected insect vector by feeding on other, healthy host plants (Weintraub & Beanland 2006).

Insect vectors of phytoplasma diseases are found in the order Hemiptera, especially in the family of Cicadellidae (Wilson & Weintraub, 2007). Until now, confirmed vectors in the family of Psyllidae are described mainly for phytoplasma diseases belonging to the 16SrX group, comprising also AP phytoplasma (Seemüller et al. 2004). *Cacopsylla picta* (Foerster, 1848) and *C. melanoneura* (Foerster, 1848) are two confirmed transmitting vectors of AP phytoplasma (Frisinghelli et al. 2000; Tedeschi et al. 2002; Jarausch et al. 2003; Tedeschi 2004). The latter seems to have no impact on the AP transmission in Germany (Jarausch et al. 2007; Mayer et al. 2009) and seems to play only a secondary role in South Tyrol considering low infection rates in the field (Baric et al. 2010).

Psyllid species are predominantly associated with perennial dicotyledonous angiosperma and are narrowly host-specific (Hodkinson 1974; Hodkinson 2009). *C. melanoneura* is described as widely oligophagous on hawthorn (*Crataegus* spp.) and on apple (*Malus* spp.) (Ossiannilsson 1992; Tedeschi et al. 2008). The dietary behaviour of *C. picta* is not fully clear, yet. It is assumed that *C. picta* is narrowly oligophagous on *Malus* spp. (Lauterer 1999) or strictly monophagous on *M. domestica* (Ossiannilsson 1992). Both insect vector species are monovoltine and, as most temperate psyllid species, they disperse and overwinter as adults on evergreen shelter plant (Burckhard et al. 2014). During winter, they undergo a reproductive diapause (Hodkinson 2009) and the hibernation of emigrant *C. picta* usually takes place on conifers, whereas it is still unknown, if they feed on those shelter plants. From the winter shelter plants *C. picta* ("remigrants") migrates into the apple orchards.

Beside the obligate planting of certified pathogen-free stocks and the instant eradication of infected plants, management of AP is primarily based on insecticide applications during vector presence (Weintraub & Beanland 2006). Thus, vector monitoring is fundamental to obtain reliable data on their diffusion, abundances and population dynamics for the development of suitable and appropriate phytosanitary control strategies. Furthermore, elucidating the species community of the genus *Cacopsylla* in the agroecosystem at a regional level is of great interest, to provide data on the occurrence of other vectors possibly involved in insect-mediated disease spread (Tedeschi & Alma 2007; Mattedi et al. 2008; Baric et al. 2010; Peusens et al 2014; Minarro et al. 2016). The aim of this study was (i) to identify the species community of *Cacopsylla* present in the agroecosystem "apple orchard", (ii) examine occurrence, population dynamics and natural infection rates of the two confirmed AP vectors *C. picta* and *C. melanoneura* and (iii) elucidate the presence of *Cacopsylla*-species mentioned previously in the context of AP and possibly involved in the spreading of the disease.

2. Study sites

The monitoring was performed from 2014 until 2016 in "Burgraviato" and "Val Venosta", the main areas of AP occurrence in South Tyrol. The sample sites were chosen regarding the incidence of infected trees in previous years (from 0.0% up to 20.1% infected trees/site; data provided by "Südtiroler Beratungsring für Obst- und Weinbau"), environmental surrounding (woodland, fallow, other crops) and coverage of different absolute altitudes, ranging from 200 to 900m a.s.l. The cultivars were restricted to the main apple varieties in the respective area 'Golden Delicious' and 'Gala'. From 44 (sampling year: 2014) to 50 (2015, 2016) sample sites were investigated during the monitoring program (Fig. 1, Table 2). In 2015, two orchards had to be replaced because of the eradication of all apple trees in these sites. Thus, a total of 52 different orchards was examined during the whole vector monitoring program: 46 orchards were intensively managed according to the national and regional guidelines of integrated fruit production (Agrios 2017), four apple orchards were under organic-biological cultivation. To obtain important information on dynamics of the natural population development, a non-insecticide treated site and an abandoned apple orchard were included in the monitoring program.

3. Material and Methods

3.1 Field monitoring

Beat tray sampling was carried out every 7-14 days from end of February until end of October and 100 to 200 randomly chosen branches (one branch per tree) per site and day were sampled. Sampling was conducted under dry and mostly wind-still weather conditions (Horton 1999). Additionally, yellow sticky traps (150x80 mm Rebell®giallo, Andermatt Biocontrol AG, Switzerland) were placed at a height of approximately 1.5-2 m from end of February until mid-July and replaced every 7-14 days (Kaloostian 1961; Jenser et al. 2010).

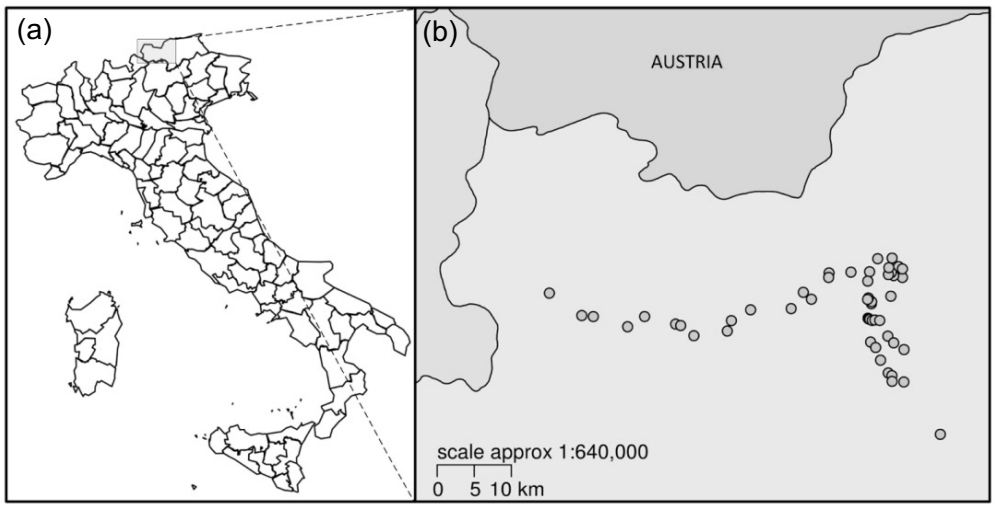

Fig. 1. (a) Location of South Tyrol, Italy, (b) sample sites surveilled from 2014 to 2016 in "Val Venosta" (white dots) and "Burgraviato" (grey dots).

On each sampling date the phenology parameters of the apple trees in the orchard were recorded, following the scoring system of Hack (1992). Psyllidae caught by yellow sticky traps were removed, cleaned from glue using n-Hexan (≥95%) and Aceton (≥99%) and stored in Ethanol (75% v/v). Beat tray samples were dry-stored at -80°C. Specimens belonging to *Cacopsylla* were sexed, and, if possible, identified up to species level (Ossiannilsson 1992; Hodkinson & White 1979; Conci & Tamanini 1990, Burckhardt & Lauterer 2009; Burckhardt 2010). Dominance (Engelmann 1978) and frequency of every *Cacopsylla* species was determined in the sampled area.

3.2 Molecular Analysis (Infection rate)

DNA from collected *C. picta* and *C. melanoneura* individuals was extracted using the DNeasy Blood & tissue kit following the kit instruction for DNA extraction from insects (Qiagen, Hilden, Germany). The presence of AP phytoplasma specific DNA in the samples was determined using AP-specific PCR primers from Monti et al. (2013) and the SYBR-based PCR-protocol described in Mittelberger et al. (2017).

4. Results

A total of 13,000 adults belonging to the genus *Cacopsylla* was sampled during field monitoring from 2014 to 2016, 88.4 % of which were captured by the beat tray method. Only 11.6 % were captured by yellow sticky traps.

As females of the genus *Cacopsylla* could not always be unambiguously identified at species level, for some species only adult males were considered in the faunistic analysis; this applies to *C. brunneipennis*, *C. elegantula*, *C. iteophila*, *C. peregrina*, *C. pulchra*, *C. pyrisuga*, *C. rhamnicola* and *C. saliceti*. Furthermore, the correct identification for *C. cf. ambigua*, *C. cf. propinqua and C. cf. pyricola* is not ascertained as only females had been recorded. Due to the resemblance of some species, a molecular tool for identification of ten *Cacopsylla* species has been developed recently (Oettl et al. 2015).

In total 94% *Cacopsylla* individuals could be determined at species level and the presence of 16 different *Cacopsylla* species could be confirmed in the investigated area of "Burgraviato" and "Val Venosta". Some species were rare and have been registered in less than one third of the investigated sites. Frequent species in the agroecosystem "apple orchard" (Table 1) were *C. melanoneura* (98.1%), *C. pruni* (65.4%), *C. pulchra* (63.5%), *C. pyri* (59.6%), *C. picta* (55.8%), *C. pulchella* (51.9%), *and C. affinis* (46.2%).

According to the classification of Engelmann (1978) *C. mali*, *C. melanoneura* and *C. pulchra* can be considered as the main species (index >3.2: main species) in the area. Eight species were less abundant and only sporadically detected in the investigated orchards (index < 0.32). *C. mali* was the most abundant species (79.1% of total catches), but records were restricted mostly to an abandoned apple orchard in "Val Venosta" (91.6% of total catches). Considering only male catches, 3.8% of total captures were identified as *C. pulchra* and their presence was confirmed in more than 60% of all sampled sites. More than 16% of *C. pulchra* adults were detected on apple by beat tray sampling.

Table 1: *Cacopsylla* species collected in 52 sites investigated (2014- 2016), considering for each species the total number of captured individuals per species (n), the relative abundance and the percentage of reports within the investigated region (frequency), as well as the relative numbers of individuals captured by beat tray sampling[*: only male-catches considered].

	Total [n]	Abundance [%]	Frequency [%]	Beat Tray [%]
Cacopsylla affinis (Löw, 1880)	50	0.40	46.15	72.00
*Cacopsylla brunneipennis** (Edwards, 1896)	11	0.09	13.46	0.00
Cacopsylla crataegi (Schrank, 1801)	3	0.02	5.77	33.33
*Cacopsylla elegantula** (Zetterstedt, 1840)	2	0.02	3.85	0.00
*Cacopsylla iteophila** (Löw, 1876)	25	0.20	9.62	12.00
Cacopsylla mali (Schmidberger, 1836)	10,600	84.12	21.15	99.71
***Cacopsylla melanoneura* (Förster, 1848)**	**840**	**6.67**	**98.08**	**84.64**
*Cacopsylla peregrina** (Förster, 1848)	1	0.01	1.92	0.00
***Cacopsylla picta* (Förster, 1848)**	**147**	**1.17**	**55.77**	**75.51**
Cacopsylla pruni (Scopoli, 1763)	67	0.53	65.38	22.39
Cacopsylla pulchella (Löw, 1877)	262	2.08	51.92	0.38
*Cacopsylla pulchra** (Zetterstedt, 1838)	506	4.02	63.46	16.60
Cacopsylla pyri (Linnaeus, 1758)	78	0.62	59.62	16.67
*Cacopsylla pyrisuga** (Förster, 1848)	2	0.02	3.85	0.00
*Cacopsylla rhamnicola** (Scott, 1876)	4	0.03	3.85	100.00
*Cacopsylla saliceti** (Förster, 1848)	3	0.02	5.77	33.33
***Cacopsylla* [species level]**	**12,601**			
***Cacopsylla* [genus level]**	**13,400**			

The presence of the confirmed psyllid AP vectors was verified in almost all sites investigated (Table 2): With over 800 individuals caught, *C. melanoneura* was one of the most abundant *Cacopsylla* species in the apple orchards. Its frequency varied from 52.3% in 2014 to 92% in 2016. In contrast, *C. picta* occurred in 56% of the orchards, with 147 adults captured in the 3 years. More than 90% of all *C. picta* adults were sampled in 2014, but contrary to other investigations in previous years, no more than 0.08 individuals per branch at one sample day were registered. In 2015 and 2016 *C. picta* remigrants were rarely found, and their presence was recorded only in 8 and 5 orchards, respectively. In these two years, no filial generation (emigrants) of this species was caught.

Table 2: AP vector presence and species record for each site investigated from 2014-2016. Alt. = Altitude, Lat., = Latitude, Long. = Longitude, *C. mel.* = *Cacopsylla melanoneura*.

Site	District	Farming	Cultivar	Alt. [m.a.s.l	Lat./Long [WGS84]	Years of investigation	*C. picta* [n]	*C. mel.* [n]	Species [n]
SCH	Val Venosta	insecticide-free	'Golden Delicious'	900	46.657659, 10.577335	2014, 2015, 2016	44	382	8
EY1	Val Venosta	conventional	'Golden Delicious'	900	46.630536, 10.634565	2014, 2015, 2016	7	17	10
EY2	Val Venosta	organic	'Golden Delicious'	900	46.629361, 10.654882	2014, 2015, 2016	6	26	7
LAA	Val Venosta	conventional	'Golden Delicious'	900	46.617113, 10.71488	2014, 2015, 2016	0	3	3
KOR	Val Venosta	conventional	'Golden Delicious'	900	46.629334, 10.745025	2014, 2015, 2016	2	13	6
SCL	Val Venosta	conventional	'Gala'	700	46.620124, 10.79928	2014, 2015, 2016	0	5	4
GOL	Val Venosta	conventional	'Golden Delicious'	700	46.618487, 10.808258	2014, 2015, 2016	0	2	3
LAT	Val Venosta	conventional	'Golden Delicious'	650	46.606275, 10.831569	2014, 2015, 2016	3	13	6
TAR	Val Venosta	abandoned	'Golden Delicious'	800	46.611963, 10.89027	2014, 2015, 2016	30	10	7
KAS	Val Venosta	conventional	'Golden Delicious'	650	46.6243, 10.897308	2014, 2015, 2016	1	6	7
TSC	Val Venosta	conventional	'Golden Delicious'	600	46.637501, 10.931318	2014, 2015, 2016	0	10	7
NAT	Val Venosta	conventional	'Gala'	600	46.63879, 11.002255	2014, 2015, 2016	0	1	6
NA1	Val Venosta	conventional	'Golden Delicious'	600	46.65852, 11.0239	2014 (eradicated)	0	2	1
NA2	Val Venosta	conventional	'Golden Delicious'	600	46.658178, 11.024274	2015, 2016	0	8	4
PLA	Val Venosta	conventional	'Golden Delicious'	550	46.650257, 11.037999	2014, 2015, 2016	0	0	1
PAR	Val Venosta	conventional	'Golden Delicious'	650	46.681952, 11.069166	2014, 2015, 2016	2	6	5
TOL	Val Venosta	conventional	'Golden Delicious'	650	46.676477, 11.068381	2014, 2015, 2016	0	4	5
MIT	Burgraviato	conventional	'Gala'	450	46.682994, 11.107427	2014, 2015, 2016	1	1	6
ALG	Burgraviato	conventional	'Gala'	350	46.672175, 11.137174	2014, 2015, 2016	2	1	4
GRA	Burgraviato	conventional	'Gala'	350	46.683255, 11.140264	2014, 2015, 2016	0	2	6
DTI	Burgraviato	conventional	'Gala'	700	46.699164, 11.15446	2014, 2015, 2016	3	4	8
RIF	Burgraviato	conventional	'Golden Delicious'	500	46.699772, 11.179915	2014, 2015, 2016	1	8	7
TIA	Burgraviato	conventional	'Gala'	400	46.688249, 11.17352	2014, 2015, 2016	2	2	6
TIK	Burgraviato	conventional	'Golden Delicious'	400	46.679853, 11.17352	2015, 2016	0	2	5
OB1	Burgraviato	conventional	'Golden Delicious'	400	46.682323, 11.178261	2015, 2016	0	6	3
OB2	Burgraviato	conventional	'Golden Delicious'	400	46.67859, 11.182734	2014, 2015, 2016	0	2	4
MER	Burgraviato	conventional	'Golden Delicious'	300	46.653851, 11.177777	2014, 2015, 2016	2	5	6
SC1	Burgraviato	conventional	'Gala'	600	46.689395, 11.191232	2014, 2015, 2016	1	6	7
SC2	Burgraviato	conventional	'Golden Delicious'	650	46.676538, 11.197909	2014, 2015, 2016	2	17	6
SC3	Burgraviato	conventional	'Golden Delicious'	650	46.686921, 11.198136	2014, 2015, 2016	6	4	6
MA1	Burgraviato	conventional	'Golden Delicious'	300	46.644695, 11.143432	2015, 2016	0	2	4
MA2	Burgraviato	conventional	'Golden Delicious'	350	46.647199, 11.143679	2015, 2016	0	4	3
MA3	Burgraviato	conventional	'Golden Delicious'	400	46.651712, 11.138071	2015, 2016	0	2	4
MA4	Burgraviato	conventional	'Golden Delicious'	450	46.650054, 11.136713	2015, 2016	0	6	2
TS1	Burgraviato	conventional	'Gala'	450	46.627427, 11.137516	2014, 2015, 2016	2	13	6
TS2	Burgraviato	conventional	'Golden Delicious'	400	46.626491, 11.138599	2014, 2015, 2016	1	24	6
TS3	Burgraviato	conventional	'Golden Delicious'	350	46.625895, 11.140672	2014, 2015, 2016	2	1	6
TS4	Burgraviato	conventional	'Golden Delicious'	300	46.624665, 11.145035	2014, 2015, 2016	0	0	7
TS5	Burgraviato	organic	'Gala'	300	46.625071, 11.149663	2014, 2015, 2016	5	2	5
LA1	Burgraviato	conventional	'Golden Delicious'	300	46.624566, 11.158074	2014, 2015, 2016	2	4	5
LA2	Burgraviato	conventional	'Gala'	300	46.60566, 11.172286	2014, 2015, 2016	0	1	4
NON	Burgraviato	conventional	'Gala'	250	46.597541, 11.182721	2014, 2015, 2016	2	1	6
VO1	Burgraviato	conventional	'Gala'	600	46.598598, 11.142103	2014, 2015, 2016	1	12	5
VO2	Burgraviato	conventional	'Golden Delicious'	700	46.591898, 11.151089	2014, 2015, 2016	0	7	5
NAR	Burgraviato	conventional	'Golden Delicious'	700	46.576625, 11.159448	2014, 2015, 2016	1	9	8
TIR	Burgraviato	conventional	'Golden Delicious'	600	46.561112, 11.173073	2014, 2015, 2016	5	2	5
PR1	Burgraviato	organic	'Gala'	600	46.558427, 11.180567	2014 (eradicated)	7	1	4
PR2	Burgraviato	organic	'Golden Delicious'	600	46.558234, 11.177133	2015, 2016	0	1	5
PRI	Burgraviato	conventional	'Golden Delicious'	600	46.551228, 11.179777	2014, 2015, 2016	2	7	8
NAL	Burgraviato	conventional	'Gala'	300	46.550467, 11.200426	2014, 2015, 2016	0	4	4
GAR	Burgraviato	conventional	'Golden Delicious'	300	46.589388, 11.200886	2014, 2015, 2016	2	154	8
UNT	Burgraviato	conventional	'Gala'	250	46.487279, 11.264795	2014, 2015, 2016	0	2	5

Table 3: Absolute numbers of remigrant and emigrant AP vectors (*C. melanoneura* and *C. picta*) based on beat tray sampling and yellow stick traps.

Species	**Beat Tray**		**Yellow Sticky Traps**	
	Remigrants	Emigrants	Remigrants	Emigrants
***Cacopsylla melanoneura* [n]**	674	335	205	14
***Cacopsylla picta* [n]**	80	32	37	0

Absolute numbers of beat tray captured individuals were much higher for both, *C. melanoneura* and *C. picta,* compared to catches by yellow sticky traps (Table 3). Furthermore, only 4% of emigrant *C. melanoneura* and no emigrants of *C. picta* had been captured by yellow sticky traps.

Flight periods varied between the years for both, *C. melanoneura* and *C. picta* (Fig. 2). In general, remigrants of *C. melanoneura* were captured from February until the end of April. First emigrants appeared at the end of April, last individuals in the middle of June. Adults of *C. picta,* returning from their overwintering shelter plants, were not detected before mid-March in the orchards. In 2014 the last remigrants were recorded in the beginning of June, whereas the presence of the emigrant generation was captured from mid-June until the end of July, in some cases until the begin of August (Table 2: site SCH, TAR).

Flight periods of the AP vectors were correlated with the physiological state of the apple trees in the respective orchards (Fig. 3): Highest densities of *C. melanoneura* remigrants were recorded during the end of the dormant status (A), but decreased drastically during first stages of the development of flowering buds (B= silver tip, C=half inch green). The presence of *C. melanoneura* was recorded until bloom (F=full bloom, G= petal fall). First adults of the filial generation appeared during bloom, whereas highest densities were recorded during the end of blooming (G, H) and beginning of fruit setting (I). Due to low densities of *C. picta* in 2015 and 2016, flight periods could be only determined in one year: Highest densities were recorded during bloom (F-G), whereas first emigrants appeared at the beginning of fruit setting (I).

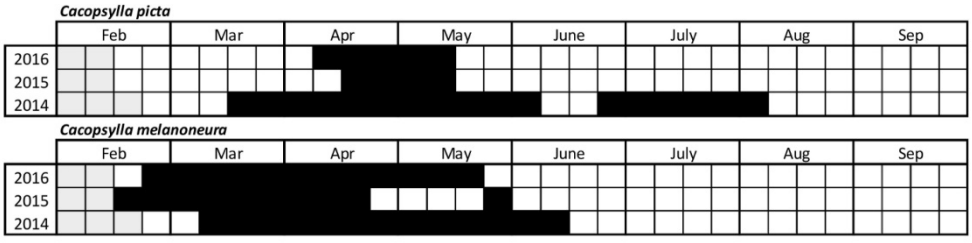

Fig. 2: Flight periods of the confirmed AP vectors *Cacopsylla picta* and *C. melanoneura* in 2014 to 2016, in the surveyed area of "Burgraviato" and "Val Venosta", Italy. [2014: 44 sample sites, 2015, 2016: 50 sample sites; grey boxes: no sampling; black boxes: presence confirmed].

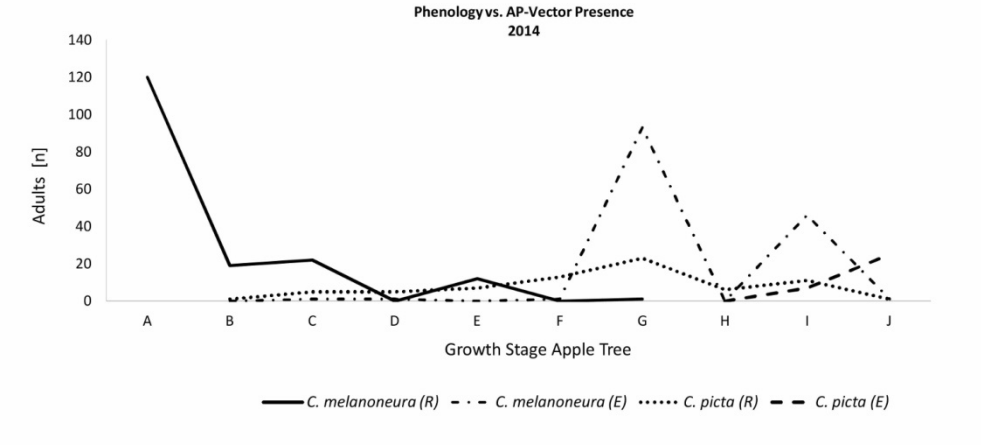

Fig. 3: Beat tray captured specimens of *Cacopsylla melanoneura* and *C. picta* in 2014 correlated with the phenology of apple tree registered during sampling. [Growth stage apple Tree = A) dormant status, B) Silver Tip, C) Half Inch Green, D) Tight Cluster, E) Pink, F) First Bloom, G) Petal Fall, H) Post Bloom, I) Fruit Set I, J) Fruit Set II; R= remigrants; E= emigrants].

The results of phytoplasma detection from field-captured individuals of the two confirmed AP vectors are shown in Table 4. In the three years *C. picta* showed an average infection rate of 21 %. AP-positive specimens were captured in 12 different sites. In contrast, 1 % of analysed individuals of *C. melanoneura* were infected with the causal agent of AP captured in 3 different sites.

Table 4: Relative Infection Rate for *Cacopsylla melanoneura* and *Cacopsylla picta* captured during field monitoring from 2014 to 2016 [AP+ = phytoplasma detected].

C. picta	**Remigrants (AP+/analysed)**	**Remigrants (AP+/analysed)**	**Sampled (n)**	**Analysed (n)**	**Analysed (%)**	**AP+ (%)**
2014	18/90	8/31	130	121	93.08	21.49
2015	3/11	0/0	11	11	100.00	27.27
2016	0/6	0/0	6	6	100.00	0.00
Total	**21/107**	**8/31**	**147**	**138**	**93.88**	**21.01**
C. melan.	**Remigrants (AP+/analysed)**	**Remigrants (AP+/analysed)**	**Sampled (n)**	**Analysed (n)**	**Analysed (%)**	**AP+ (%)**
2014	1/188	0/96	346	284	82.08	0.35
2015	0/98	0/23	127	121	95.28	0.00
2016	3/137	2/54	368	191	51.90	2.62
Total	**4/423**	**2/173**	**841**	**596**	**71**	**1.01**

5. Discussion

The field monitoring performed in 52 apple orchards from 2014 to 2016 confirmed the presence of 16 different *Cacopsylla* species (Table 1). The occurrence of these insects was proven by beat tray sampling and the use of yellow sticky traps. Most species were only occasionally recorded in the sampling sites; thus, they can be categorized as sporadical or subrecedent species in the apple orchards of "Val Venosta" and "Burgraviato" (Engelmann 1978).

A previous monitoring surveillance conducted 2006 in apple orchards located in South Tyrol confirmed the presence of 10 *Cacopsylla* species (Walch 2006). Considering further two species (*C. pyricola* and *C. nigrita*) captured by Walch (2006), the occurrence of 18 different *Cacopsylla* species can be confirmed in the apple growing region of South Tyrol.

Our results revealed that *C. mali*, *C. melanoneura* and *C. pulchra* were the most abundant species (Table 1). These three species represented 94 % of all *Cacopsylla* catches, similar to the results obtained in apple orchards located in southwest Germany, where these species accounted to 85 % of the total *Cacopsylla* fauna (Jarausch et al. 2009). In contrast to Lauterer (1999) describing *C. mali* as one of the most abundant psyllid species in some European areas, we confirmed its presence in only 21% of the sample sites. In total, 99 % of the *C. mali* individuals were caught in an abandoned apple orchard (Table 2: site TRS), indicating that this species is prone to insecticide treatment. Our survey showed a relatively high presence of *C. pulchra*, since this species was detected in more than 60 % of the sample sites. This palearctic species hibernates on conifers and is associated with various Salicaceae (Ossiannilsson 1992, Lauterer 1999). Over 16 % of *C. pulchra* individuals (both remigrants and emigrants) were caught using the beat tray sampling method (Table 1), suggesting that this species completes its developmental cycle in the apple orchards. Nevertheless, a potential host plant should be evaluated regarding its suitability for the insect to complete its life cycle on this plant. Thus, feeding, oviposition, larval development and survival rates must be determined to judge whether a certain plant can be considered a host plant for a respective insect (Hodkinson 2009). Interestingly, during the field study conducted in 2006 *C. pulchra* was not determined in the sample sites (Walch 2006).

Both confirmed AP vectors, *C. melanoneura* and *C. picta*, are present in the investigated region (Table 2). *C. melanoneura* was considered as the main vector, until Frisinghelli et al. (2000) and Jarausch et al. (2003) revealed the importance of *C. picta* during disease transmission. The presence of *C. picta* in the South Tyrolean apple growing region was confirmed in 2004 by Wolf & Zelger (2006). Despite its status as an extremely rare species in Italy (Conci et al. 1992), subsequently conducted surveys in 2006 revealed relatively high densities of *C. picta* in the region of "Burgraviato" (max 1.8 adults/branch) (Walch 2006; Wolf 2013). In 2014 abundances were relatively low and drastically decreased in the following two years (Table 1, Table 4). Our results regarding the population dynamics for both, *C. melanoneura* and *C. picta*, are comparable to those obtained by previous investigations in Italy and Germany (Tedeschi et al. 2002; Tedeschi et al. 2003; Jarausch et al. 2003; Wolf & Zelger 2006; Mattedi et al. 2008; Tedeschi et al. 2012). In 2015 and 2016 the population densities of *C. picta* were mostly under the detection limit. However, caution must be taken in interpreting the results, as employed monitoring tools could underestimate the actual insect population in the field: Beat tray captures are instantaneous, but are influenced by temperature and daytime, whereas sticky traps are cumulative, but there catching efficacy is affected by the position in the orchard and thus hamper the acquisition of correct results (Adams et al. 1983; Adams &

Los 1998). Krysan & Horton (1991) showed a poor correlation between trap and beating tray catches of pear psyllid, as well as sex, seasonal and morphotype-biased differences between the results achieved with the two different methods. We showed that the numbers of *C. melanoneura* and *C. picta* caught with the beat tray method were much higher than with yellow sticky traps (Table 1, Table 3). Interestingly, emigrants of both, *C. melanoneura* and *C. picta* were captured only sporadically with sticky traps (Table 3). Host plant finding by herbivorous insects includes chemical and visual clues (Bernays & Chapman 2007) and remigrants show other orientation behaviour than emigrants due to their differential host and shelter plant preferences (Sutton 1984, Horton 1994, Hodkinson 2001). This differential behaviour may be the reason why emigrants of *C. picta* and *C. melanoneura* were only captured in low numbers on yellow sticky traps but in higher numbers using the beat tray method (Table 3). Until now, no selective and efficient trap systems are available for psyllid insect vectors. However, they would be of great benefit to get more reliable data about psyllid abundances.

Determining first appearance and population peaks of insect vectors returning from shelter plants are crucial for the development of an adequate phytosanitary strategy. This is particularly important to impede the transmission of AP phytoplasma to healthy plants by insects. According to Malagnini et al. (2010) *C. melanoneura* starts to emigrate when the average maximum temperature in the orchard lies above 9.5°C for 7 days. Based on these findings, Tedeschi et al. (2012) developed a prediction model for the remigration of overwintering adults of *C. melanoneura* for "Valsugana", Italy. For *C. picta* no validated prediction model exists. Furthermore, the knowledge of hibernation sites to identify triggers inducing the migration to the apple orchards seems crucial for the development of suitable prediction models.

Despite the efforts to identify overwintering sites of the AP vectors, the exact triggers (photoperiod, temperature) that lead the insects to leave the overwintering sites remain unknown. In South Moravia, Čermak & Lauterer (2008) recorded overwintering adults of *C. melanoneura* on *Picea* spp. and *Pinus* spp., whereas *C. picta* was only present on *Picea* spp. In South Tyrol, the overwintering plants neither of *C. picta* nor of *C. melanoneura* could be identified yet, impeded by the evidence that psyllids effectively disperse over long and short distances (Hodkinson 1972). This is supported by the observation that emigrants of *C. picta* can be found on conifers at high altitudes in Germany (Jarausch & Jarausch 2014).

Obviously, flight periods varied between years and altitude and are biased for example due to insecticide treatments as well as weather and climate conditions (Table 3). Host plant growth and psyllid development are essentially linked to each other and the host plant is indispensable for the insects′ successful life cycle completion (Hodkinson 2009). The investigated area covers different altitudes, from 200 to 900 m a.s.l. and this affects the phenological stages of apple trees. Consequently, correlating flight periods of AP vectors with the growing stage of apple trees seems appropriate to elucidate their population dynamics (Fig. 3): High densities of *C. melanoneura* were observed at the end of the dormant stadium and in the beginning of flower bud development, whereas the population peak of *C. picta* was highest during bloom. In 2013 the vector control strategy was extended and an application of insecticides of the (non bee-harming) tau-fluvalinate group during bloom was recommended to reduce *C. picta* in the orchards. In the following years upon this measure *C. picta*

densities decreased, indicating that the insecticide treatment during bloom efficiently reduced the *C. picta* population.

C. picta infection rates were much higher than the infection rates of *C. melanoneura* (see Table 4). These findings differ from results obtained in north-eastern and north-western Italy where the infection rates of *C. melanoneura* are higher and this insect is supposed to be the main AP-transmitting vector (Tedeschi et al. 2002; Tedeschi et al. 2003; Tedeschi et al. 2012). In contrast, Mattedi et al. (2006) showed only low transmission capability of *C. melanoneura*. Mayer et al. (2009) found, that *C. melanoneura* has no impact on spreading the disease in Germany, due to low infection rates in the field. These results were comparable to data obtained by Baric et al. (2010) in South Tyrol: *C. picta* specimens captured in 2006 in South Tyrolean apple orchards showed an average natural infection rate about 11.1 %. *C. melanoneura* sampled in the same year demonstrated an infection rate of 0.6 % (Baric et al. 2010), although occurring in higher numbers than *C. picta* (Walch 2006). The involvement of other Hemiptera species in spreading AP phytoplasma other than *C. picta* and *C. melanoneura* is not clear. The present study confirmed the occurrence of *C. affinis, C. crataegi, C. mali* and *C. peregrina* previously reported to carry *'Ca.* phytoplasma mali*'* (Tedeschi & Alma 2007; Tedeschi et al. 2009; Mattedi et al. 2008; Baric et al. 2010; Peusens et al. 2014; Minarro, et al. 2016). However, a successful transmission of AP phytoplasma by these species has never been shown.

The monitoring program conducted from 2014 to 2016 revealed low population densities of *C. picta* in the surveyed area of South Tyrol. Nevertheless, high percentages of about 21 % of infected adults of *C. picta* in the field, as well as the laboratory-proven ability of females to transmit AP phytoplasma to its progenies (Mittelberger et al. 2017), indicates the high potential risk even at low population densities. Additionally, the survey of *Cacopsylla* species present in the region of South Tyrol confirmed the presence of other putative vectors possibly involved in the spreading of AP. Further investigations are necessary to elucidate their potential role in AP transmission in Europe and at local scale, to prevent a further spread of the disease.

7. Zusammenfassung

Die Eindämmung der Ausbreitung von Phytoplasma-induzierten Krankheiten an Kulturpflanzen basiert derzeit hauptsächlich auf indirekten Maßnahmen, welche v. a. auf die Überträger dieser Krankheiten selbst gerichtet sind. Apfeltriebsucht (AP) ist eine der wichtigsten Krankheiten im europäischen Apfelanbau. Sie wird von dem zellwandlosen Bakterium '*Candidatus* Phytoplasma mali' verursacht, welches von *Cacopsylla picta* (Foerster, 1848) und *Cacopsylla melanoneura* (Foerster, 1848) (Hemiptera: Psylloidea) auf Apfelbäume übertragen wird. Kenntnisse über deren Populationsdynamiken in betroffenen Gebieten sowie die Bestimmung ihrer Infektionsraten im Feld sind wichtige Bausteine, um effiziente und angepasste Strategien zu entwickeln, die eine weitere Ausbreitung der Krankheit verhindern. Auch ist die Analyse des in Apfelanlagen vorkommenden Artenkomplexes der Gattung *Cacopsylla* von Bedeutung, um die Präsenz weiterer möglicher Vektoren zu ermitteln, die bei der Verbreitung von AP oder anderen Krankheiten eine Rolle spielen könnten. Im Zuge eines dreijährig angelegten Monitoring-Programms in Vinschgau und Burgrafenamt (Südtirol, Italien), den Kernzonen der AP-Epidemiologie, wurden über 13.000 *Cacopsylla*-Individuen gesammelt und die Präsenz von 16 Arten dieser Gattung bestätigt. *C. picta* wur-

de in über 50% der untersuchten Apfelanlagen nachgewiesen, wobei über 20% der im Feld gefangenen Tiere nachweislich mit AP infiziert waren. Im Vergleich dazu wurde die Präsenz von *C. melanoneura* in 90% der untersuchten Anlagen nachgewiesen. Die niedrige Infektionsrate von ca. 1% bestätigt jedoch die untergeordnete Rolle von *C. melanoneura* bei der Verbreitung der AP-Phytoplasmose in Südtirol.

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Addresses of authors

Stefanie Fischnaller, Martin Parth, Manuel Messner, Robert Stocker, Christine Kerschbamer, Yazmid Reyes-Dominguez und Katrin Janik: Research Centre Laimburg, Laimburg 6, Pfatten, 39040 Auer, Alto Adige (Italy)