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Impact of insecticides treatment on phytoplasma infection risk in apple orchards

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Abstract

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During 2013–2015, a monitoring study was carried out on the migration, abundance, and infectivity of *Cacopsylla picta* and *C. melanoneura* in apple orchards that were under different types of management. The presence of symptomatic and non-symptomatic apple trees infected by '*Candidatus* Phytoplasma mali' was studied. It was demonstrated that the infectivity of psyllid vectors is the same without regard to the growth management applied in the orchard. The potential risk of phytoplasma spreading in the orchards under an integrated management was lower due to the side-effects of insecticides on psyllids. Their shorter occurrence, lower abundance and the absence of a new vector generation were observed and compared to the orchard grown under organic management.

Keywords: '*Candidatus* Phytoplasma mali'; monitoring; *Cacopsylla picta*; *Cacopsylla melanoneura*

Apple proliferation is the most important phytoplasma disease of pome fruits. It is caused by '*Candidatus* Phytoplasma mali' (apple proliferation phytoplasma, AP). Infected apple trees manifest a wide variability of symptoms: from latent infections (without symptoms); through small leaves, chlorosis, enlargement of stipules, shortening of internodes; to the proliferation of lateral buds, and formation of witches' broom. '*Ca*. P. mali' is spread by infected materials (grafts, buds, and rootstocks), and is transmitted by the psyllid vectors *Cacopsylla picta* and *C. melanoneura* (EPPO 2015). AP infections have a negative impact not only due to direct damage on production, i.e. decreasing of yield and fruit quality as well as quality of seed planting materials, but also indirectly due to their quarantine status and the application of restrictive measures. Apple proliferation disease was first reported at the turn of the 20th century (Németh 1986). In the Czech Republic, its spread was observed from 1953 and by the sixties it was commonly occurring in old apple orchards there (Blattný, Blattný 1960; Blattný et al. 1963). After some decrease, currently, a wide spread of the pathogen and the occurrence of local epidemics was reported throughout European countries (Osler et al. 2001; Jarausch et al. 2004; Paltrinieri et al. 2010; Rumbou et al. 2011; Blystad et al. 2012). This situation is primarily associated with an orchard's management – the transition from conventional to environmentally friendly growth management, as well as with climate changes in general (Seemüller et al. 1998; Lemmetty et al. 2011; Fránová et al. 2013).

The present work is focused on the determination of population dynamics and the percentage of insect vectors infected by '*Ca*. P. mali' in the con-

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text of infection sources within intensive apple orchards, in those maintained under an integrated growth management and those in organic management.

MATERIAL AND METHODS

A comparative study to evaluate the specific risks of the spread of apple proliferation in commercial apple orchards was carried out between 2013–2015 in the central Moravian region within two orchards; one was held under an integrated growth management, and the second under organic growth management in which it was held during last five years.

The population density of psyllids (*Cacopsylla picta* and *C. melanoneura*) was monitored at approx. two weeks intervals (from February to July) using yellow sticky traps; ten sticky traps, 148 × 210 mm in size, were placed diagonally from the orchard's edge in a north-west orientation, each spaced at a distance of 10 m. Individual vectors were determined, numbered, and then stored at –20°C in absolute ethanol for later phytoplasma detection.

The development of symptoms was monitored in both orchards by visual inspection in the late summer period within an area comprised of 5 rows of 50 trees (i.e., a total of 250 trees were evaluated in each orchard). Sampling for phytoplasma detection was done randomly, three two-year-old branches were collected from each 5^{th} tree in a row (i.e., 50 samples per orchard). Total DNA was extracted according to Ahrens and Seemüller (1992) for phytoplasma detection using nested PCR with universal primer pairs P1/P7 followed by R16F2/R2, and phytoplasmas were identified in subsequent RFLP analysis with *Bfm*I, *Mse*I, *Ssp*I, and *Rsa*I restriction enzymes (Fránová et al. 2013).

RESULTS AND DISCUSSION

Cacopsylla picta (Foerster 1848) is generally accepted as one of the most important vectors of '*Candidatus* Phytoplasma mali' in Europe (Jarausch et al. 2003). It is a univoltine species with characteristic migration of new generation to overwintering sites situated on more distant hills, and early spring immigration of overwintered adults to the orchards. The results of the three-year survey in both integrated and organic orchards indicated that the overwintered adults of *C. picta* started colonization of the apple trees in the period from the first half of March to the beginning of April, and the population density culminated in the second half of April (Fig. 1) in both orchards. The presence and the number of vectors differ depending on the management applied in the orchards. Individuals of *C. picta* were noted only until the end of April in the integrated orchard, and only adults of the overwintered generation were noticed there. A different situation was observed in the organic orchard, with a one month longer occurrence of *C. picta* and the presence of individuals of a new generation. Shortening of the vector's presence is clearly correlated with insecticide treatment (Calypso 480 SC, Reldan 22 EC) and its side-effect against *C. picta* in the integrated orchard. This effect also agrees with the observed population density, as the population culminated at only the 31–47% level of population size in the non-treated orchard. The impacts of insecticide application and its side-effect in the effective protection of production orchards under integrated management were also been reported by other authors (Blažek et al. 2005).

C. melanoneura (Foerster 1848) was confirmed as a vector of '*Candidatus* Phytoplasma mali' in Italy, but its vector status in Central Europe is unclear (Tedeschi et al. 2002; Mayer et al. 2009). In our study, the overwintered adults of *C. melanoneura* arrived in both types of orchards during the second half of February, and their population density culminated in second half of April. Individuals were trapped sporadically until the end of April or end of May in the integrated orchard, and until the beginning of June in the organic orchard. Their population size in the integrated orchard reached 47–62% of the maximal level of the organic one, and a new psyllid generation was not found there.

'*Ca*. P. mali' was confirmed in 11.4–20.7% of *C. picta* individuals and in 0.0–7.1% of *C. melanoneura* individuals, both of the overwintering generation. The similar situation was found within individuals of new generation (Table 1). A significantly higher infection rate (χ^2 test at $P = 0.05$) was noted in *C. picta* compared to *C. melanoneura* in both of the studied orchards; however, no significant differences were found in the infestation of each psyllid vector during the 2013–2015 study period, nor between the orchards under the integrated and organic management. The data obtained agree with the earlier observations from the years 2006

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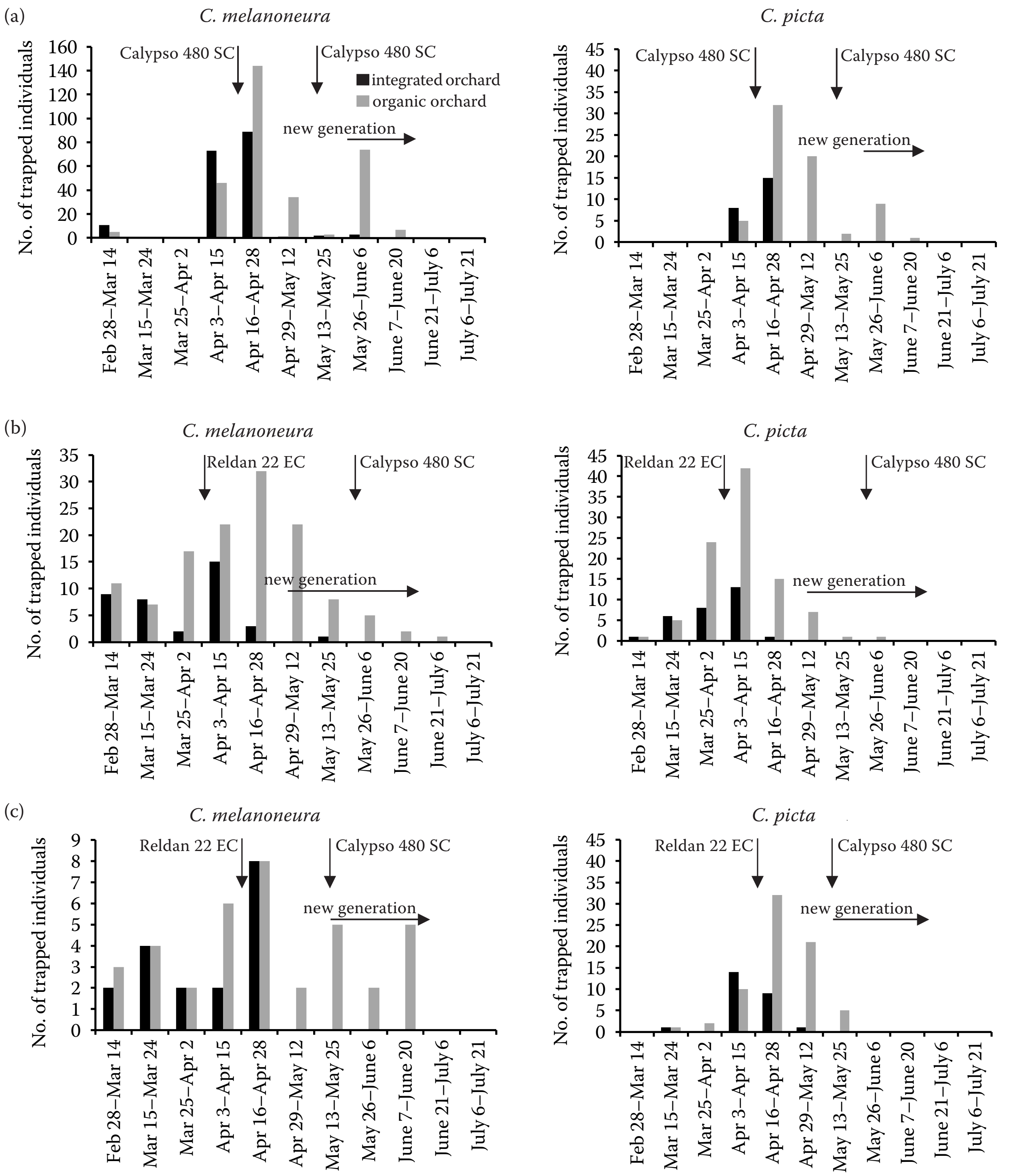

Fig. 1. Population dynamics of '*Ca*. P. mali' vectors in orchards under integrated and organic growth management in (a) 2013, (b) 2014, and (c) 2015

application of insecticides and presence of psyllids of new generation are marked by arrows

and 2007, in which 10–22% phytoplasma positive *C. picta*, and 4–6% *C. melanoneura* individuals were noted, respectively (unpublished data). Observations on the infection rate of *C. picta* is fully in agreement with the situations described in Germany, France, Switzerland, and Italy, and this species is considered as the main vector and an important factor involved in local outbreaks of apple

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Table 1. Psyllids collected by yellow sticky traps during years 2013–2015 in orchards under integrated and organic growth management. The total number of trapped psyllids, number of psyllids of new generation, and distribution of individuals infected by '*Ca*. P. mali' is given

Species	*C. melanoneura*				*C. picta*			
	integrated orchard		organic orchard		integrated orchard		organic orchard	
	Σ	(%) (C/D)	Σ	(%) (C/D)	Σ	(%) (C/D)	Σ	(%) (C/D)
2013 A	176	3.9 (2/51)	232	6.9 (6/87)	23	15.0 (3/20)	59	17.3 (9/52)
2013 B	3	0.0 (0/1)	81	4.5 (1/22)	0	–	10	14.3 (1/7)
2014 A	37	5.6 (1/18)	101	3.2 (3/95)	29	20.7 (6/29)	95	11.4 (11/96)
2014 B	1	nt	26	6.3 (1/16)	0	–	1	nt
2015 A	18	0.0 (0/9)	26	7.1 (2/28)	25	16.0 (4/25)	71	18.0 (11/61)
2015 B	0	–	11	0.0 (0/4)	0	–	1	nt

Σ – total number of trapped individuals; A – overwintering individuals; B – individuals of new generation; C – number of individuals infected by '*Ca*. P. mali'; D – number of PCR analysed individuals; nt – not tested

proliferation (Jarausch et al. 2011; Lemmetty et al. 2011; Jarausch , Jarausch 2014). On the other hand, *C. melanoneura* was only confirmed as a vector in north-eastern Italy (Tedeschi et al. 2002), and its role is still disputed. With regard to their infection rate detected in our study, it is similar to the situation in Italy (Tedeschi et al. 2003). *C. melanoneura* could be considered as a possible vector under our conditions as well.

In addition to infectious vectors, the sources of infection, primarily internal resources '*Ca*. P. mali' infected trees within orchard, play an important role in the epidemiology of '*Ca*. P. mali'. In this context, the incidence of symptomatic and latent infections were studied over a two year period, generally showing a lower occurrence of diseased trees in the integrated orchard (2014: 5.8%; 2015: 7.7%) compared to the organic orchard (2014: 19.6%; 2015: 19.6%). Considering the fact that the orchard under organic management was established in 1979 and the integrated one in 1995, the observed differences in '*Ca*. P. mali' occurrence could be presumed to be a result of different ages of the trees, and with the highest probability are not affected by the management applied during the last five years. The fact that PCR positive trees, except for two, were symptomless during repeated visual inspections is alarming.

Carraro et al. (2004) were not able to detect '*Ca*. P. mali' in shoots and leaves collected from asymptomatic (recovered, never symptomatic) trees, and demonstrated that '*Ca*. P. mali' was not transmittable by grafts in this case. However, in both our earlier (Fialová et al. 2004) and current study, it was possible to detect the presence of AP in the shoots of latently infected non-proliferating trees. Asymptomatic trees should be evaluated as a source of phytoplasma, and could still play an important epidemiological role in spreading of the disease.

It could be concluded that, in general, those orchards without insecticide application are exposed to a potentially higher risk of the spread of '*Ca*. P. mali'. As the main factors involved in could be considered: the significantly longer occurrence of vectors at a locality, their higher abundance, and the presence of a new psyllid generation. The presence of asymptomatic apple trees should be taken into account as an important epidemiological factor too. All of these factors should be monitored, and they are fundamental for successful control of apple proliferation disease.

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