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***Cacopsylla pruni* in Poland and its significance as a vector of ‘*Candidatus Phytoplasma prunorum*’**

Wojciech WARABIEDA, Grażyna SOIKA, Mirosława CIEŚLIŃSKA

Research Institute of Horticulture in Skieriewice
Konstytucji 3 Maja 1/3, 96-100 Skieriewice, Poland
E-mail: wojciech.warabieda@inhort.pl

Abstract

The species composition of jumping plant-lice, especially *Cacopsylla pruni* as a vector of ‘*Candidatus Phytoplasma prunorum*’, was researched on stone fruit trees in 53 locations, mainly in Central Poland. Twelve species were found to belong to the families Psyllidae, Aphalaridae and Triozidae. *C. pruni* which made up 59.9% of the collected specimens was noted in eight locations in plum orchards and in two locations in cherry orchards. Only three locations were found to have specimens of *C. pruni* which were infected by ‘*Ca. Ph. prunorum*’, namely on plum trees in the Lesser Poland Province and plum and mirabelle plum trees in the Masoovian Province. In the latter location, specimens of *C. pruni* infected with ‘*Ca. Phytoplasma asteris*’ were also caught in mirabelle plum trees. We can conclude that at this moment *C. pruni* is not a significant threat to Polish stone fruit orchards and its importance in the possible spread of phytoplasma causing European stone fruit yellows is low.

Key words: ‘*Candidatus Phytoplasma asteris*’, European stone fruit yellows.

Introduction

Cacopsylla pruni (Scopoli) is widely distributed in Europe on oligophagous, univoltine species on plants belonging to the genus *Prunus* (Steffek et al., 2012). This species has been found in many locations in Poland: Pomeranian Lakeland, Masurian Lakeland and Małopolska Upland (Klimaszewski, 1963), Kraków-Częstochowa Upland (Smreczyński, 1954), in the Western Beskids, Nowy Targ Basin, in many places in Pieniny and the Tatra Mountains (Smreczyński, 1954; Drohojowska, Głowacka, 2011) and in the Silesian Upland (Kanturski, Drohojowska, 2013).

According to Klimaszewski (1967), in Poland, *C. pruni* commonly occurred on plants of the genus *Prunus*, but due to the relatively small population, this species did not cause economic losses. However, it has been proved that *C. pruni* is a vector of the ‘*Candidatus Phytoplasma prunorum*’ (‘*Ca. Ph. prunorum*’) (Carraro et al., 1998). The presence of this quarantine pathogen, which causes many diseases on *Prunus* trees described as European stone fruit yellows (ESFY), has been reported from most of the countries in the European Union (Steffek et al., 2012). This disease was first described on Japanese plum (*Prunus salicina* Lindley) in Italy (Goidanich, 1933) and has now become a serious problem affecting European producers of apricots (*Prunus armeniaca* L.), Japanese plums and peaches (*P. persica* L.). The highest occurrence of ‘*Ca. Ph. prunorum*’ has been found in the countries of the Mediterranean Basin (Thébaud et al.,

2009; Cieślińska, Morgaś, 2011; Steffek et al., 2012; Sabaté et al., 2015).

The study recently carried out in Polish orchards has pointed to the presence of this pathogen in plum and peach as well as sweet and sour cherry crops (Cieślińska, Morgaś, 2011; Cieślińska, Smolarek, 2015). However, the scale of damage caused by ‘*Ca. Ph. prunorum*’ in Poland is not sufficiently well-known. So far, studies on the occurrence and identification of potential vectors of this phytoplasma in Poland have not been carried out.

The aim of this research was to determine the composition of Psylloidea with particular emphasis on *Cacopsylla pruni* on *Prunus* plants in orchards as well as those growing in the natural environment in selected regions of Poland and to determine the incidence of specimens testing positive for ‘*Candidatus Phytoplasma prunorum*’ in order to assess the risk of this phytoplasma spreading.

Material and methods

The species composition of Psylloidea on plants of the genus Prunus. A study on the occurrence of psyllids was carried out between 2013 and 2016 inclusive in 53 locations in different regions of Poland, mainly in the central area. Besides plum, the monitoring included peach and apricot as well as sweet and sour cherry trees. Additionally, samples were also taken from plants

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growing close to the surveyed orchards, mainly from blackthorn (*Prunus spinosa* L.), and also from coniferous trees such as *Pinus* spp. and *Picea* spp. which are known to be the overwintering places of jumping plant-lice species, including *Cacopsylla pruni*. Samples were taken using the beat-tray method. A sample collection unit was defined as the number of individual specimens obtained from 100 randomly selected branches from a randomly selected test area of approximately 0.5 hectares at each test location.

The collected insect specimens were checked under a stereoscopic microscope and identified to individual species using the entomological key (Ossiannilsson, 1992). The classifications follow that of Burckhardt and Ouvrard (2012). Specimens of *C. pruni* were preserved in 70% ethanol at -20°C until testing for the presence of phytoplasma.

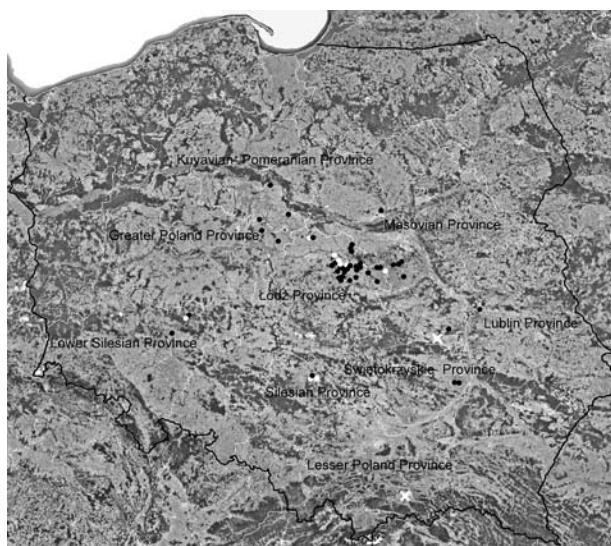
Identification of the phytoplasma in Cacopsylla pruni. The total DNA was extracted from samples of *C. pruni* consisting of 1–3 individuals using a High Pure PCR Template Preparation Kit (Roche). The DNA was subjected to nested polymerase chain reaction (PCR) assay with the universal primer pair P1/P7 (Deng, Hiruki, 1991; Schneider et al., 1995) for amplification of 1.8 kbp product of the 16S ribosomal DNA, the spacer region between the 16S and 23S rDNA genes and the 5'-end of the 23S rDNA of the phytoplasmas. P1/P7 amplicons, diluted 1:29 with sterile water, were used as templates for the second round of PCR with the universal primer pair R16F2n/R16R2 (Gundersen, Lee, 1996). All PCR assays were performed with a thermocycler PTC-200 (MJ Research, USA), and 7 µl of the amplification products was separated in 1% agarose gel in 0.5× TBE (tris-borate-EDTA) buffer, followed by staining in ethidium bromide and visualization of DNA bands using UV transilluminator (Syngen, England). The molecular weight of the PCR products was estimated by comparison with 100 bp DNA ladder (Thermo Scientific Ltd., Lithuania). Five µl of the nested PCR products were separately digested with *Rsa*I and *Ssp*I restriction endonucleases (Thermo Scientific Ltd.). The generated restriction patterns were analysed by electrophoresis in 8% polyacrylamide gels in 1% TBE buffer and compared with the profiles of the following strains, collected by A. Bertaccini (<http://www.ipwgnet.org/collection>); '*Candidatus Phytoplasma prunorum*' (16SrX-B), '*Ca. Ph. mali*' (16SrX-A), '*Ca. Ph. pyri*' (16SrX-C) and '*Ca. Ph. asteris*' (16SrI-B). These strains were also compared with previously published restriction fragment length polymorphism (RFLP) patterns of phytoplasmas (Lee et al., 1998).

Results and discussion

The species composition of jumping plant-lice.

During the study, a total of 178 samples of insects were taken from 53 locations, both from stone fruit trees growing in orchards and plants from surrounding area (Fig. 1).

Twelve jumping plant-lice species were identified in the collected samples, which were classified as belonging to three families: Psyllidae, Aphalaridae and Triozidae. The most represented family was Psyllidae with eight species of psyllids while Aphalaridae and Triozidae were represented only by a small number of species (Table 1).



○ – *C. pruni* found, ● – *C. pruni* not found, X – '*Candidatus Phytoplasma prunorum*' found in *Cacopsylla pruni* bodies

Figure 1. Locations surveyed in order to determine the presence of jumping plant-lice

The percentage of *C. pruni* caught on trees or shrubs differed depending on the plant species, making up 66.9% of all specimens collected from plums (Fig. 2 A), 6.4% of specimens from sweet and sour cherries (Fig. 2 B), 35.0% of specimens on blackthorn (Fig. 2 C) but only 1.5% of the specimens collected from coniferous trees (Fig. 2 D). This may suggest that plum trees are among the preferred host plants of *C. pruni*. The *C. pruni* occurrence was observed in plum orchards primarily in the spring (March–May), but not in the summer (June–July). Similar results were obtained in the studies conducted in other countries, e.g., in France and Spain (Labonne, Lichou, 2004; Thébaud et al., 2009; Sabaté et al., 2015).

Among the psyllids collected from *Prunus* plants, a high proportion of *C. melanoneura* was noted. It should be pointed out that in contrast to males, the females of *C. melanoneura* and *C. affinis* are impossible to distinguish by morphological criteria (Tedeschi et al., 2009). However, given that only two *C. affinis* specimens were found of the dozens of males belonging to these two species, it can be said that *C. melanoneura* was the dominant species in this complex.

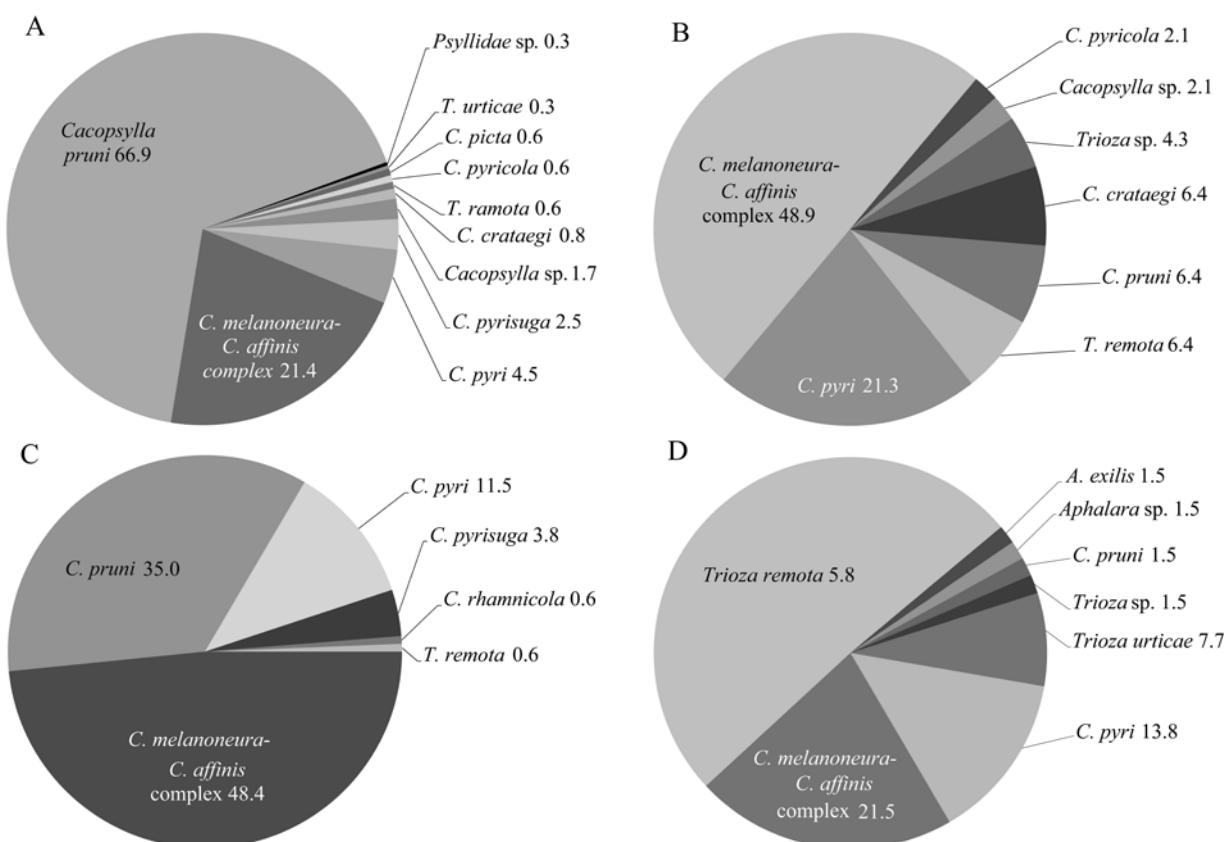
Relatively large number of individuals of this species caught is probably related to the fact that in the spring this species re-migrates from its overwintering sites to hawthorn or apple trees being the most commonly cultivated fruit tree in Poland. Plum trees, as well as other fruit plants, could potentially be only "accidental" feeding or landing places for *C. melanoneura* at this time of year.

It has been shown that among plants in the surrounding orchards, *C. pruni* was found much more often in blackthorn than in coniferous plants in the growing season (Table 2, Fig. 2 C and D). The fact that the *C. pruni* prefers blackthorn has been confirmed and described by other researchers (Labonne, Lichou, 2004). On the other hand, this psyllid readily overwinters on conifer plants, located at some distance from stone fruit tree orchards (Yvon et al., 2004; Čermak, Lauterer, 2008). In our study, however, samples were largely taken from

Table 1. A list of Psylloidea species collected in 2013–2016

Psyllid species	No. of specimens	Plant species
Aphalaridae		
<i>Aphalaera exilis</i> (Weber-Mohr, 1804)	1	C
<i>Aphalaera</i> sp.	1	C
Psyllidae		
<i>Cacopsylla crataegi</i> (Schrank, 1801)	6	SwC, P
<i>Cacopsylla melanoneura</i> (Foerster, 1848)	200	B, C, P, SoC, SwC
<i>Cacopsylla affinis</i> (Low, 1880) complex	3	A, SwC
<i>Cacopsylla picta</i> (Foerster, 1848)	299	B, C, P, SwC
<i>Cacopsylla pruni</i> (Scopoli, 1763)	52	B, C, P, SwC
<i>Cacopsylla pyri</i> (Linnaeus, 1758)	4	P, SoC, SwC
<i>Cacopsylla pyricola</i> (Foerster, 1848)	15	B, P
<i>Cacopsylla pyrisuga</i> (Foerster, 1848)	1	B
<i>Cacopsylla rhamnicola</i> (Scott, 1876)	7	P
<i>Cacopsylla</i> sp.	1	P
Psyllidae		
<i>Trioza remota</i> (Foerster, 1848)	39	C, B, SwC
<i>Trioza urticae</i> (Linnaeus 1758)	6	C, P
<i>Trioza</i> sp.	3	C, SwC
In total	628	—

A – apricot, B – blackthorn, C – conifers, P – European plum, SoC – sour cherry, SwC – sweet cherry

**Figure 2.** Percentages of jumping plant-lice collected from plum trees (A), sweet and sour cherry (B), blackthorn (C) and conifers (D)

conifers growing in the immediate vicinity of the surveyed orchard, which could be the reason why a relatively small number of *C. pruni* were collected. Although *C. pruni* was the most numerous species on *Prunus*, considering its frequency of occurrence in the samples, as well as its occurrence in various locations, it can be concluded that this species usually occurs sporadically and locally. In general, the presence of *C. pruni* was noted in 26 samples, coming from 11 locations (Fig. 1, Table 2).

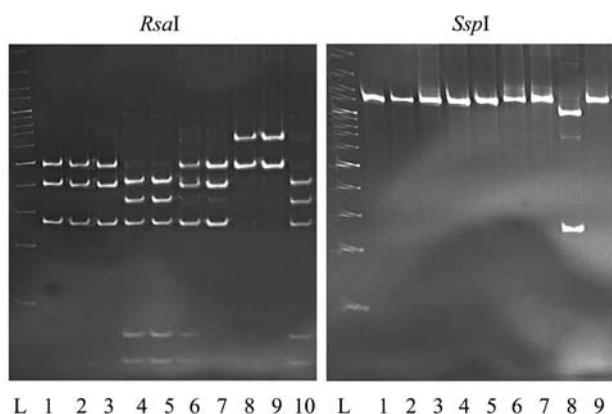
It should be noted that during this study, observations were carried out mainly in protected orchards, in which the number of psyllids could have been reduced as a result of chemical treatments carried out against other pests. Such a possibility is indicated by the results obtained in one of the villages in Masovian Province in 2016. In this area, *C. pruni* was not recorded in the tested plum orchard, which was surrounded by apple trees chemically protected against *C. melanoneura*. However,

Table 2. The frequency of *Cacopsylla pruni* occurrence

Plant species	No of samples with <i>C. pruni</i> / the total number of samples	No of locations with <i>C. pruni</i> / the total number of locations
Plum	16 / 85 (18.8%)	8 / 31 (25.8%)
Peach and apricot	0/6	0/5
Sweet and sour cherry	2 / 56 (3.6%)	2 / 33 (6.1%)
Blackthorn	7 / 20 (35%)	6 / 13 (46.2%)
Conifers	1 / 17 (5.9%)	1 / 12 (8.3%)

this species occurred relatively frequently on unprotected mirabelle plum trees growing at some distance from those protected apple trees. The effect of chemical treatments on *C. pruni* density is indirectly indicated by the fact that, on wild, unprotected blackthorn, this species occurred in 46.2% of the monitored locations and in 35.0% of the samples, while in protected plum orchards *C. pruni* was noted in only 25.8% of the monitored locations and in 18.8% of the samples (Table 2). On the other hand, the preference of blackthorn by *C. pruni* cannot be ruled out (Lauterer, 1999; Labonne, Lichou, 2004).

Occurrence of phytoplasmas. Of the 261 specimens of *C. pruni* which were analysed for phytoplasma infection, 203 were collected from plum trees, 53 from blackthorn, four from cherry, and one from conifers. The presence of '*Ca. Ph. prunorum*' in the *C. pruni* bodies was limited geographically, since the pathogen was detected in only two of the eleven localities where this psyllid species was found. '*Ca. Ph. prunorum*' was identified in three samples of psyllids that came from Masovian Province (one sample from plum trees growing in an orchard and two from the chemically unprotected mirabelle plum trees (*Prunus domestica* subsp. *syriaca*) growing nearby) and one from a plum orchard located in the Lesser Poland Province (Fig. 3).



Lanes: L – molecular marker 100 bp; 1 – *C. pruni* from plum (Masovian Province), 2 – *C. pruni* from plum (Lesser Poland Province), 3–6 – *C. pruni* from mirabelle plum tree (Masovian Province), 7 – K⁺ '*Ca. Ph. prunorum*', 8 – K⁺ '*Ca. P. mali*', 9 – K⁺ '*Ca. P. pyri*', 10 – K⁺ '*Ca. P. asteris*'

Figure 3. Polyacrylamide gel showing the restriction fragment length polymorphism (RFLP) patterns of phytoplasmas ribosomal DNA fragment amplified with R16F2n/R16R2 primers digested with *RsaI* and *SspI* restriction enzymes of *Cacopsylla pruni* samples from *Prunus* sp. trees surveyed in Masovian Province and Lesser Poland Province

Furthermore, it was found that the level of psyllid infection differed between years. For example, in one of the orchards in Lesser Poland Province the level varied between 1.6% and 4.7% (for 64 collected individuals) in 2013, while in 2014 '*Ca. Ph. prunorum*' was not detected in any of the 43 collected specimens. Similarly, in the orchard in the Masovian Province, the infection rate in 2013 for 21 tested specimens of *C. pruni* was between 4.8% and 14.3%, while in 2016 the presence of phytoplasma was not detected in the bodies of insects.

Generally, considering analysis of the total number of *C. pruni* specimens (207) collected from stone fruit trees in all the test locations and tested for the presence of '*Ca. Ph. prunorum*', the degree of infection by this pathogen can be estimated at between 1.9% and 5.3%, or, if we also include specimens collected from blackthorn (53) and coniferous plants (1), the infection rate was between 1.5% and 4.2%. These values were lower than those obtained for *C. pruni* in the apricot orchards in Moravia, or in stone fruit orchards in several regions of Spain, where the rate of infection in the spring period reached tens of percent (Fialová et al., 2004; Sabaté et al., 2015).

Interestingly, two samples of *C. pruni* individuals, caught on mirabelle plum trees in Masovian Province were infected with '*Ca. Ph. asteris*'. On the same plants, *C. pruni* infected with '*Ca. Ph. prunorum*' were found, which confirms that a single psyllid species can carry more than one pathogen. On the other hand, the same phytoplasmas may be transferred between plants by various insects (Weintraub, Beanland, 2006; Bosco et al., 2007). Previous studies indicate that the aster yellows group phytoplasmas are transmitted by different species of leafhoppers. In the literature, there is no information on the transfer of these pathogens by *C. pruni*. Research in this area should be extended and include a greater number of tests to confirm or rule out the relationship between *C. pruni* and '*Ca. Ph. asteris*'.

The threat to stone fruit trees by ESFY depends on many factors. Apart from vector abundance and the level to which they are infected with phytoplasma, differences in the susceptibility of *Prunus* spp. plants to '*Ca. Ph. prunorum*' are also important. Species susceptible to the phytoplasma include Japanese plum, apricot and peach, while plum, sweet cherry, sour cherry, blackthorn and cherry plum are either rarely infected or are at least tolerant to infection (Giunchedi et al., 1982; Jarausch et al., 2000; Carraro et al., 2002). The stone fruit trees grown in Polish orchards are mostly tolerant to phytoplasma. Peach and apricot trees account for only about 6.3% of stone fruit tree plantings, while Japanese plum accounts for no more than 1%.

Due to the relatively large area of host plants and the presence of both the pathogen and its vector in

Poland, under favourable conditions the threat to Polish orchards by ESFA may change. One other factor that is becoming more relevant is the observed climate change taking place in Poland (Tomeczyk et al., 2015). There is evidence that climate warming is having an effect on population size increase of many pests (Krishnareddy, 2013). The increase in air temperature and drought can promote not only the development of psyllids, but also the phytoplasma multiplication in the cells of host plant and in the insect vector. In consequence, this may lead to an increased risk of ESFY occurrence in the orchards. In addition, economic losses in the future may be higher if the planting of thermophilic plants, particularly those sensitive to infection by '*Ca. Ph. prunorum*' such as apricot, peach or Japanese plum increases.

Conclusion

The study showed that at this moment *Cacopsylla pruni* is not a significant threat to Polish stone fruit orchards and its importance in the possible spread of phytoplasma causing European stone fruit yellows is low.

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***Cacopsylla pruni* ir jo kaip '*Candidatus Phytoplasma prunorum*' pernešėjo svarba Lenkijoje**

W. Warabieda, G. Soika, M. Cieślińska

Sodininkystės mokslų institutas Skerneviciėje, Lenkija

Santrauka

Šokinėjančių augalinių blakučių, ypač *Cacopsylla pruni* kaip '*Candidatus Phytoplasma prunorum*' pernešėjo, rūšinė sudėtis buvo tirta kaulavaisių medžiuose 53 vietovėse, daugiausia Centrinėje Lenkijoje. Buvo nustatyta 12 rūsių, priklausančių Psyllidae, Aphalaridae ir Triozidae šeimoms. *C. pruni* sudarė 59,9 % visų surinktų vabzdžių aštuoniuose vietovėse slyvų soduose ir dviejose vietovėse vyšnių soduose. Tik trijose vietovėse buvo rasti *C. pruni* vabzdžiai, užsikrėtę '*Ca. Phytoplasma prunorum*', būtent ant slyvų medžių Mažosios Lenkijos vaivadijoje ir ant mirabelės pomologinės veislių grupės slyvos medžių Mazovijos vaivadijoje. Pastarojoje vietovėje '*Ca. Phytoplasma asteris*' užsikrėtę *C. pruni* vabzdžiai buvo pagauti mirabelės pomologinės veislių grupės slyvos medžiuose. Galima daryti išvadą, kad šiuo metu *C. pruni* nėra didelė grėsmė Lenkijos kaulavaisių sodams, o jo svarba pernešant fitoplazmą, sukeliančią europinę kaulavaisių medžių geltą, nėra didelė.

Reikšminiai žodžiai: '*Candidatus Phytoplasma asteris*', europinė kaulavaisių medžių gelta.