Epidemiology of European Stone Fruit Yellows in Germany

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Abstract

Since 2000, surveys have been conducted in different stone fruit growing regions in South-western Germany to detect European stone fruit yellows (ESFY) disease in Germany. Each year visual inspections for typical symptoms of ESFY such as early budbreak in late winter and chlorotic leafroll in summer have been done on different *Prunus* species. Branch samples of all trees with typical symptoms as well as randomized samples from trees with doubtful symptoms have been taken in summer and analysed for infection with *Candidatus* Phytoplasma prunorum via PCR using specific primers ECA1/ECA2. The pathogen could be detected in the regions Rheinland, Rheinhessen, Vorder- and Südpfalz and Baden and was present in all cultivated *Prunus* species: *P. armeniaca*, *P. persica*, *P. domestica* and *P. amygdalus*. For apricots, more than 80% of the samples were infected while peach and European plum were less affected. Trees of *P. armeniaca* with typical symptoms showed up to 90% correlation with the presence of the phytoplasma but also a high percentage of trees with doubtful symptoms were highly infected. For *P. persica*, symptom specification was less pronounced. Almost no infection was found in the wild *Prunus* species *P. spinosa* and *P. cerasifera*. In contrast, regular psyllid captures on all different *Prunus* species gave high populations of *Cacopsylla pruni* on *P. spinosa and P. cerasifera* while only few individuals were collected from cultivated orchards. The natural infection rate of field collected *C. pruni* was between 2 and 3%. Transmission trials under controlled conditions showed the capability of *C. pruni* to transmit the phytoplasma to healthy test plants and proved that *C. pruni* is also a vector of *Candidatus* Phytoplasma prunorum in Germany.

INTRODUCTION

All stone fruit species in the southern half of Europe are affected by severe decline diseases associated with phytoplasmas (Jarausch et al., 1998). The European stone fruit yellows (ESFY) phytoplasma, termed now *Candidatus* Phytoplasma prunorum (Seemüller and Schneider, 2004), is the causal agent of apricot chlorotic leaf roll and other decline diseases affecting trees of the genus *Prunus* (Lorenz et al., 1994). Phytoplasmas have also been found on peach and almond (Poggi Pollini et al., 1993; Lederer and Seemüller, 1992) and on wild *Prunus* species (Jarausch et al., 2001; Carraro et al., 2002). Typical symptoms are yellowing and leaf roll in summer and off-season growth in winter, dieback and a more or less rapid decline follow.

Carraro et al. (1998) identified the psyllid species *Cacopsylla pruni* as vector for *Candidatus* Phytoplasma prunorum in Italy and Jarausch et al. (2001) confirmed the vector capacity of this psyllid species in France. Some years later, *C. pruni* was also described as vector for *Candidatus* Phytoplasma prunorum in Spain and Czech Republic (Lavina et. al., 2004; Fialova et al., 2004).

However, after the first detection of *Candidatus* Phytoplasma prunorum in Germany (Lederer and Seemüller, 1992) no further data on this disease, the pathogen or the vector were available for Germany. Since 2000, a new survey was started in order to obtain more information about the pathogen and the presence of the potential vector *Cacopsylla pruni* and finally to estimate the actual spread of this disease in the most

important stone fruit growing regions in South-western Germany.

MATERIALS AND METHODS

Field Surveys

Since 2000, field observations have been conducted in five different regions in South-western Germany. In either spring, autumn or in both seasons, certain regions were surveyed each year: region Mosel, 5 orchards of the *Prunus* species *P. armeniaca* (apricot), *P. persica* (peach) and *P. domestica* (European plum); region Rheinhessen, 14 orchards of *P. armeniaca* and *P. persica*; region Vorderpfalz, 22 orchards of *P. armeniaca*, *P. persica* and *P. amygdalus* (almond); region Südpfalz, 4 orchards of *P. armeniaca* and *P. persica*; and region Ortenau, 10 orchards of *P. domestica*. Plant samples have been taken from symptomatic trees and from a certain number of trees without symptoms.

Insect captures using the beat tray method have been done each year from March until July in the region Rheinhessen in one orchard of *P. armeniaca*, in the region Vorderpfalz in one orchard of each *P. armeniaca* and *P. persica* and on 2 sites with wild *Prunus* species *P. cerasifera* and *P. spinosa* (hawthorn) and in the region Südpfalz in one orchard of *P. armeniaca* and *P. persica*, respectively. All psyllid species were determined from the mixed insect samples and all specimens of *Cacopsylla pruni* were separated for DNA extraction and PCR analysis.

Nucleic Acid Extraction and Phytoplasma Detection by PCR

Total DNA from plant material was extracted from leaf petioles or branch phloem. Total DNA from insects was extracted from single individuals of *C. pruni*. For plant as well as for insect material a modified CTAB-based protocol as published by Maixner et al. (1995) was employed. PCR detection was done with *Candidatus* Phytoplasma prunorum-specific primers ECA1/ECA2 as published by Jarausch et al. (1998).

Transmission Trials

Transmission trials were carried out with overwintering adults of *C. pruni* from field captures in closed glass vessels under controlled conditions in the greenhouse (16 h light at 20°C, 8 h dark at 15°C) using micropropagated GF-8.1 (*P. marianna*) (Jarausch et al., 1994) as healthy test plants.

Acquisition/transmission procedures were conducted with young adults (springtime generation) from rearing cages in the greenhouse. The young individuals were transferred from the healthy rearing plants (GF-8.1) to plants infected with *Candidatus* Phytoplasma prunorum for acquisition feeding for a variable number of days. After a defined acquisition period, groups of 10 individuals were transferred to healthy GF-8.1 plants in glass vessels and kept there under controlled conditions until their natural death. All dead psyllids were collected from the vessels and analysed for infection with *Candidatus* Phytoplasma prunorum via PCR. The test plants were cultivated in the greenhouse under insect-proof conditions. The analysis for infection with the phytoplasma was done after 6, 12 and 18 months by PCR detection.

RESULTS AND DISCUSSION

From 2000 until 2005, a total of 55 orchards with different *Prunus* species have been surveyed in 5 regions in South-western Germany in order to demonstrate the spread of ESFY disease in the most important stone fruit growing regions in Germany. The study showed that *Candidatus* Phytoplasma prunorum was present in all regions with various infection rates in the different orchards and on the different *Prunus* species. Summarizing all PCR-positive samples from all regions, 64% of all samples were infected with the phytoplasma. Table 1 demonstrates the host range for *Candidatus* Phytoplasma prunorum in Germany. Thus, the most affected species in all investigated regions was *P. armeniaca* (apricot) with more than 80% of infected samples, while *P. domestica* (European plum)

and *P. persica* (peach) were less affected. This observation agrees with reports from other European countries (Desvignes and Cornaggia, 1982; Carraro et al., 1998; Jarausch et al., 2000).

The analysis of the symptom specificity revealed a high correlation between typical symptoms in the late winter period (February) such as early bud break (Table 2). For this symptom we found a high correlation with phytoplasma detection by PCR for *P. armeniaca* but also for *Prunus* species that in general show less or no symptoms such as *P. amygdalus*, *P. domestica* or *P. cerasifera*. The 100% correlation between early bud break and positive PCR result in the case of *P. cerasifera* (Table 2) must be considered cautiously as the sample number is very low. Surprisingly, early bud break was not a specific symptom for *P. persica* in Germany.

As it is already reported by other authors (Jarausch et al., 2001; Carraro et al., 2002; Labonne and Lichou, 2004), we can confirm that the wild *Prunus* species *P. spinosa* and *P. cerasifera* do not show obvious symptoms and the infection rate with *Candidatus* Phytoplasma prunorum is very low. Regarding summer symptoms (August), we distinguished between specific symptoms such as chlorotic leaf roll or complete dieback of the trees and rather unspecific symptoms such as chlorosis without leaf roll or non-chlorotic leaf roll. For *P. persica* reddened, longitudinally rolled leaves were considered as specific symptom. The analysis of the samples due to this classification is shown in Table 3. The results confirm that *P. armeniaca* is the most affected *Prunus* species with a high correlation for specific symptoms but as well as for non-specific symptoms. Interestingly, more than 20% of the samples from other, less susceptible *Prunus* species with non-specific symptoms were infected with phytoplasma. This observation signifies that the latent infection in an orchard can be estimated much higher than it is expressed by visible symptoms. Again, the symptomatology of *P. persica* remained unclear and visual symptoms were not reliable enough for monitoring the disease in peach orchards in Germany.

Those latently infected trees represent an enlarged source of inoculum for insect vectors which acquire the pathogen and transmit it across the orchard. As *Cacopsylla pruni* has been identified as vector for *Candidatus* Phytoplasma prunorum in many European countries (Carraro et al., 1998; Jarausch et al., 2001), we concentrated our research for the vector in Germany on this psyllid species. *C. pruni* was found in all ESFY-infected orchards by the regular psyllid captures during 3 years in 3 different fruit growing regions in South-western Germany. Wild *Prunus* species such as *P. cerasifera* or *P. spinosa* were the main host plants for *C. pruni* in Germany while population densities on cultivated *Prunus* species were always lower (data not shown). This result fits with observations from other European countries (Carraro et al., 2002; Yvon et al., 2004). In some regions (Rheinhessen, Südpfalz), we also found important populations on *P. armeniaca* depending on the climatic conditions in the year of investigation. From the PCR analysis of each single *C. pruni*, we calculated a mean natural infection rate of about 2–3% during all 3 years of investigation.

In order to verify that *C. pruni* is a vector for *Candidatus* Phytoplasma prunorum in Germany, we conducted transmission trials under controlled conditions in the greenhouse with field collected overwintering adults and springtime generation adults from rearing. During 3 years of study we obtained a successful transmission with overwintering adults of *C. pruni* on 21% of the test plants (6 plants PCR positive out of 28 plants tested). In contrast, in acquisition/transmission trials with springtime generation adults, only 1 test plant out of 32 became infected thus corresponding to a transmission rate of 3%. Carraro et al. (2001) also found better transmission efficiency with overwintering adults but also obtained better results for the springtime generation. The transmission trials under our conditions have still to be improved and the specific transmission parameters have to be characterized in detail; however, from our results we can conclude that *C. pruni* is also a vector of *Candidatus* Phytoplasma prunorum in Germany.

CONCLUSIONS

Our survey for several years showed that the ESFY pathogen is widely spread in all important stone fruit growing regions in South-western Germany. All cultivated stone fruit species were found infected but *P. armeniaca* is the most affected species. Infected trees may die off rapidly from one year to the other and thus cause a complete economic loss for the grower.

The vector *Cacopsylla pruni* is present in all investigated stone fruit growing regions with wild *Prunus* species as preferred host plant. The overwintering generation appears to be the most efficient vector. At present, no commercial insecticides are available for an efficient control.

We hypothesize that a high percentage of latent infected trees together with an important number of potential insect vectors that acquire the pathogen from these inoculum sources is responsible for the high presence of ESFY in Germany. In order to control a further spread of the disease we propose a complete uprooting of all infected trees and an efficient vector control focused on the most dangerous developmental stages.

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Tables

Table 1. Host plants for *Candidatus* Phytoplasmas prunorum in Germany and percentage of PCR positive samples from different *Prunus* species.

	Total nb. tested	nb. PCR positive	% positive
Cultivated species			
P. amygdalus (almond)	12	8	67
P. armeniaca (apricot)	290	242	83
P. cerasifera (Myrobalan)	3	3	100
P. domestica (European plum)	24	16	67
P. persica (peach)	131	28	21
Wild species			
P. cerasifera	6	1	17
P. spinosa	5	0	0
Total	471	298	

Table 2. Correlation between symptoms and PCR results for late winter symptoms.

Species	Total early bud break	PCR + early bud break	% PCR+
P. amygdalus	10	8	80
P. armeniaca	55	52	95
P. cerasifera	3	3	100
P. domestica	17	14	82
P. persica	35	5	14
Total	120	82	

Table 3. Correlation between symptoms and PCR results for summer symptoms.

Species	Chlorotic leaf roll*		Apoplexie/ die-back		Unspecific symptoms	
	Total	PCR+	Total	PCR+	Total	PCR+
P. amygdalus	nt	nt	nt	nt	2	0
P. armeniaca	100	89 (89%)	31	29 (94%)	105	70 (67%)
P. domestica	0	0	2	1 (50%)	5	1 (20%)
P. persica	25	5 (20%)	36	9 (25%)	35	9 (26%)
Total	125	94	69	39	147	80

*For *P. persica* reddened, longitudinally rolled leaves were considered as specific symptom.