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Evaluation the Significance of '*Candidatus* Phytoplasma Prunorum' Pathogen for Apricot Cultivars

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Abstract In some apricot growing areas in Europe, Asia and Africa, '*Ca*. Phytoplasma prunorum' - the agent of European Stone Fruit Yellows (ESFY) - can cause significant economic losses in the stone fruit orchards. In the present study, '*Ca*. Phytoplasma prunorum' infection of three apricot cultivars was evaluated by molecular methods in Hungary. We found the pathogen to be prevalent in the studied orchard (54%). We investigated the sensitivity of the apricot cultivars to ESFY. Trees were classified into disease categories based on the symptoms, and the number of dead trees was counted separately. From these data, the disease severity index and tree destruction index were calculated, and statistical comparisons of disease incidence were performed. Damage to ESFY can be reduced by using less susceptible cultivars because chemical plant protection is only effective against the vectors. The presence and infection of phytoplasma were mostly tolerated by the cultivar 'Zebra'. The 'Flavorcot' cultivar was moderately affected by ESFY, but the 'Sweetcot' cultivar was very susceptible. '*Ca*. Phytoplasma prunorum' causes significant tree mortality; 44% of the trees molecularly confirmed to be infected had died by the end of the study. 'Zebra' had the lowest tree mortality (15%), while 'Sweetcot' had the highest (65.8%). Examination of the grafts planted as replacements showed that 3.3% of the plants were infected. The spread of diseased material can represent new sources of infection in orchards.

Keywords *Prunus armeniaca* L., ESFY, Apoplexy, Dieback, Propagation Material, Nested-PCR

1. Introduction

'*Candidatus* Phytoplasma prunorum' ('*Ca*. Phytoplasma prunorum') infects stone fruits, causing European stone fruit yellows (ESFY) [1,2]. ESFY is present in many European countries, and in some Asian and African countries [3-7]. In Hungary, the presence of this pathogen was established in the 1990s [8]. The disease is one of the main factors limiting apricot production [2]. International monitoring data indicate that the infection of apricot trees with phytoplasma can be very high in some areas, up to 83% [9-12].

'*Ca*. Phytoplasma prunorum' is a prokaryotic, phloem-limited, pleomorphic, Gram-positive bacterium [2,13,14]. The characteristic symptoms of phytoplasma-infected trees

are chlorotic leaf roll and early defoliation [15-18]. Leaves may be wilted and fragile in texture [15-17,19,20]. Phytoplasma infection is accompanied by phloem necrosis; however, it is important to emphasize that gummosis does not manifest [15,19,20]. Infected trees appear unhealthy and their vigor and vitality are reduced. Branches showing visible symptoms rarely survive for more than a year, whereas attacked trees usually die within 2-4 years [15,17,20]. Some reports indicate that '*Ca*. Phytoplasma prunorum' can cause up to 30-40% mortality of apricot trees [16,19], but interestingly, some apricot trees can recover from symptoms but not from infection by the pathogen. Subsequently, they became tolerant to the ESFY pathogen [21,22].

Phytoplasmas are primarily transmitted by vectors and propagating materials. The principal insect vector of this pathogen is *Cacopsylla pruni* [23]. '*Ca*. Phytoplasma prunorum' can be transmitted via vegetative propagation [24-27], which plays a role in the rapid spread of this pathogen [28].

In the detection of phytoplasmas, it should be noted that they may be present in low concentrations in plants and their distribution is neither constant nor homogeneous [29,30]; thus, ELISA-based methods are not reliable [31]. Several variants of polymerase chain reaction (PCR) (e.g., nested PCR, qPCR) are commonly used to detect phytoplasmas [32]. Both universal and species-specific primers are available for rapid detection and screening [33-36].

Protection of orchards against '*Ca*. Phytoplasma prunorum' is an extremely complex and difficult task, as the authorized pesticides are not effective against the pathogen directly [2,37,38], but the insect vectors can be controlled chemically [39,40]. Furthermore, healthy propagation material plays an important role in phytoplasma disease prevention [2,41]. The best way to prevent ESFY is to grow resistant cultivars; therefore, knowledge of susceptibility is of paramount importance [2,38].

Previous studies have investigated the different susceptibilities of apricot cultivars to ESFY phytoplasma based on visual and/or molecular diagnosis [9,16,38,42,43]. Susceptible cultivars were as follows: 'Bergeron' and 'Veselka', while 'Poyer' and 'Churmai' were tolerant [16,42,44,45]. For some cultivars, the results were inconsistent even within a single study: 'Pinkcot' and 'Aurora' showed low pathogen prevalence in some plots, while in other plots, they were rather highly affected [16].

The objectives of this study were to: i) assess the presence of '*Ca*. Phytoplasma prunorum' in commercial apricot orchards; ii) to investigate the effect of apricot cultivars on the damage caused by ESFY under natural infection; and iii) to test the presence of the pathogen.

2. Materials and Methods

2.1. Characteristics of the Orchard

In this study, we examined the presence of ESFY in a commercial apricot orchard at Érd (Hungary, 47°33'89.53"N, 18°86'88.36"E). The disease severity and tree mortality were investigated on three cultivars: 'Flavorcot', 'Sweetcot', and 'Zebra'. The tested trees were on 'Myrabolan' rootstock. The orchard was established in 2004 in an open-vase training system, without an irrigation system. The distance between rows and plants was 6–7 m and 4 m, respectively. Conventional plant protection methods (such as pesticide application) have also been applied. Note that manual fruit thinning was performed annually.

2.2. Presence and Evaluation the of Symptoms in the Orchard

In 2014, 120-120 approximately 7–10 years old living trees (with 22 ± 3 cm trunk diameter) were randomly selected and marked for comparative analysis in the following years (from 2014 to 2017). The most characteristic symptoms caused by phytoplasma were monitored and evaluated in October each year, when they are most noticeable: malformation of foliage (discoloration, foliage quantity, leaf size and shape, leaf roll); malformation of woody parts (decay of twigs, branches, scaffold limbs, dieback of the whole tree); and growth abnormalities of the tree (shoot formation, intensity of shoot growth, vitality). The trees were ranked on a six-point grading scale according to symptom severity; the ranges were based on our previous study with modifications (Table 1) [46].

We calculated the *disease severity index* (DSI) from grading scale categories (Table 1) by the Townsend-Heuberger formula: $P = \Sigma(n \times v)/(Z \times N) \times 100$, where: P: disease severity index, n: number of plants in specific disease categories, v: disease categories, Z: highest disease category, N: all assessed plants [47].

Sample sizes in nonzero category groups were very low, when compared to category zero, therefore we binarised the six categories and created a *non-diseased* (ND; value: „0”) and a *diseased* (D; value: „≥ 1”) group for calculating disease incidence. These ND and D groups were examined by Chi-square tests followed by Z-test related to the adjusted standardized residuals, using IBM SPSS v25 software [48].

Table 1. Scales used for the assessment of disease severity [46, with modifications]

Grading scale category	Grade description
0	Tree with no symptoms
1	Frequency and severity of symptoms: 1–10% (slight leaf discoloration / leaf roll /decreasing leaf development / decreased shoot development)
2	10–30 % affectedness of the tree (moderate leaf discoloration / leaf roll /decreasing leaf development / decreased shoot development / decreased tree vitality / branch decay)
3	30–50 % affectedness of the tree (high leaf discoloration / leaf roll /decreasing leaf development / decreased shoot development / decreased tree vitality / branch decay, but maximum 1 scaffold limb decay)
4	50–75 % affectedness of the tree (high leaf discoloration / leaf roll /decreasing leaf development / decreased shoot development / decreased tree vitality / branch decay, but maximum 2 scaffold limb decay)
5	75–100 % affectedness of the tree (high leaf discoloration / leaf roll /decreasing leaf development / decreased shoot development / decreased tree vitality / at least 3 scaffold limb decay or total dieback)

The evolution of tree mortality was assessed from 2015 to 2017. We considered 2014 as the baseline year as no prior data for tree mortality was available. The number of dead trees in the orchard was also assessed in each cultivar and compared with the number of living trees in the previous year, resulting in a percentage (*tree mortality index*, TMI). The values were tested by Marascuilo's procedure [49].

2.3. Testing the Presence of '*Ca*. Phytoplasma Prunorum' in Apricot Grafts

Due to the death of apricot trees, the orchards require constant renewal, so pathogen-free propagating material is of paramount importance. Therefore in 151 cases, we tested the presence of '*Ca*. Phytoplasma prunorum' in grafts before planting in 9 cultivars of apricots (namely: 'Bergerouge', 'Bergeval', 'Flavorcot', 'Gönci magyar kajszi', 'Harcot', 'Sweetcot', 'Zebra', 'Kioto', 'Pinkcot'). The scions were grafted on 'Myrabolan', 'Myrabolan 29C', 'St. Julien A' and 'Wavit' rootstocks.

2.4. Molecular Identification of '*Ca*. Phytoplasma Prunorum'

2.4.1. Sampling from the Orchards

In order to establish the presence of ESFY, 50 randomly selected trees (25 asymptomatic and 25 symptomatic) were sampled in October 2014 in each cultivar from the orchard. One to two years old woody parts were sampled for PCR from the scion samples [33].

2.4.2. Sampling from Grafts

We examined 1-year old grafts before replacement of die-backed trees: 151–151 samples were collected for the PCR analyses both from the scions and rootstocks in October 2016. Approximately 10 cm of roots were collected from the rootstocks, and 10 cm of shoot apexes were sampled from scions for DNA isolation.

2.4.3. DNA Isolation and Nested PCR

The surface of the woody parts and roots was disinfected with 70% ethanol, soaked in sodium hypochlorite (NaClO) for 15 minutes, and finally washed 3 times in sterile distilled water. The bark was removed manually.

The deoxyribonucleic acid (DNA) extraction was performed from the 0.5 g vascular tissues, and was carried out according to the protocol described by Daire et al. [50] with some modifications. We added 4 ml extraction buffer per sample, homogenized the plant tissues and incubated for 50 min at 65 ℃. During the incubation, the homogenized tissues were vortexed every 10 minutes to enhance extraction. Extracted DNA was resuspended in 30 μl 1× TE (10 mM TRIS (2-Amino-2-(hydroxymethyl) propane-1,3-diol) pH 7.6; 1 mM EDTA (2,2′,2″,2‴-(Ethane-1,2-diyldinitrilo) tetraacetic acid disodium salt (pH 8)) buffer. The DNA concentration and the quality of the samples were checked with a NanoDrop2000 spectrophotometer (Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania) and stored at -20 ℃ until further processing.

All samples were tested by nested-PCR for '*Ca*. Phytoplasma prunorum'. In the first round of amplification, universal primer pair of Eof (forward 5'–CCAACTTTAATAATAGCAATAGGAA–3') and Eor (reverse 5'–TGATTTATGTTTTCAACTTTTCCA–3') were used [33]. The second round of amplification was carried out with 1.5 μl of the PCR mixture from the first round, using as template and specific primer pair of ACE1

(forward 5'–AATAATCAAGAACAAGAAGT–3') and ACE2 (reverse 5'–GTTTATAAAAATTAATGACTC–3') [18]. The PCR mixture contained: 1.5 µl DNA template, 0.5 µl both forward and reverse primers (10 µM), 12.5 µl Dream Taq Green PCR Master Mix 2x buffer (Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania) and 10 µl sterilised distilled water.

Both PCRs had an initial denaturation at 95 ℃ for 5 min and a final elongation at 72 ℃ for 10 min. In the first PCR, 35 cycles were run as follows: denaturation at 95 ℃ for 60 sec, annealing at 55 ℃ for 60 sec, and elongation at 72 ℃ for 60 sec. The second PCR program had 30 cycles: denaturation at 95 ℃ for 30 sec, annealing at 55 ℃ for 30 sec and elongation at 72 ℃ for 45 sec. The length of the expected PCR product was 237 bp.

3. Results

3.1. The Study of Symptom Types and Cultivar Susceptibility in the Apricot Orchard

As the characteristic symptoms of ESFY, we observed fragile textured leaves, rolling of chlorotic leaves (Figure 1A), wilting leaves (Figure 1B) and early leaf-fall (Figure 1C) on some branches or on the entire tree. Infected scaffold limbs or trees showed reduced vigour: development of short, weak shoots, or complete lack of shoot formation (Figure 1D). More severe cases resulted in the death of twigs, branches, scaffold limbs or the entire trees (Figure 1E–F). We also observed the phloem of highly symptomatic trees that were often necrotic (Figure 1G).

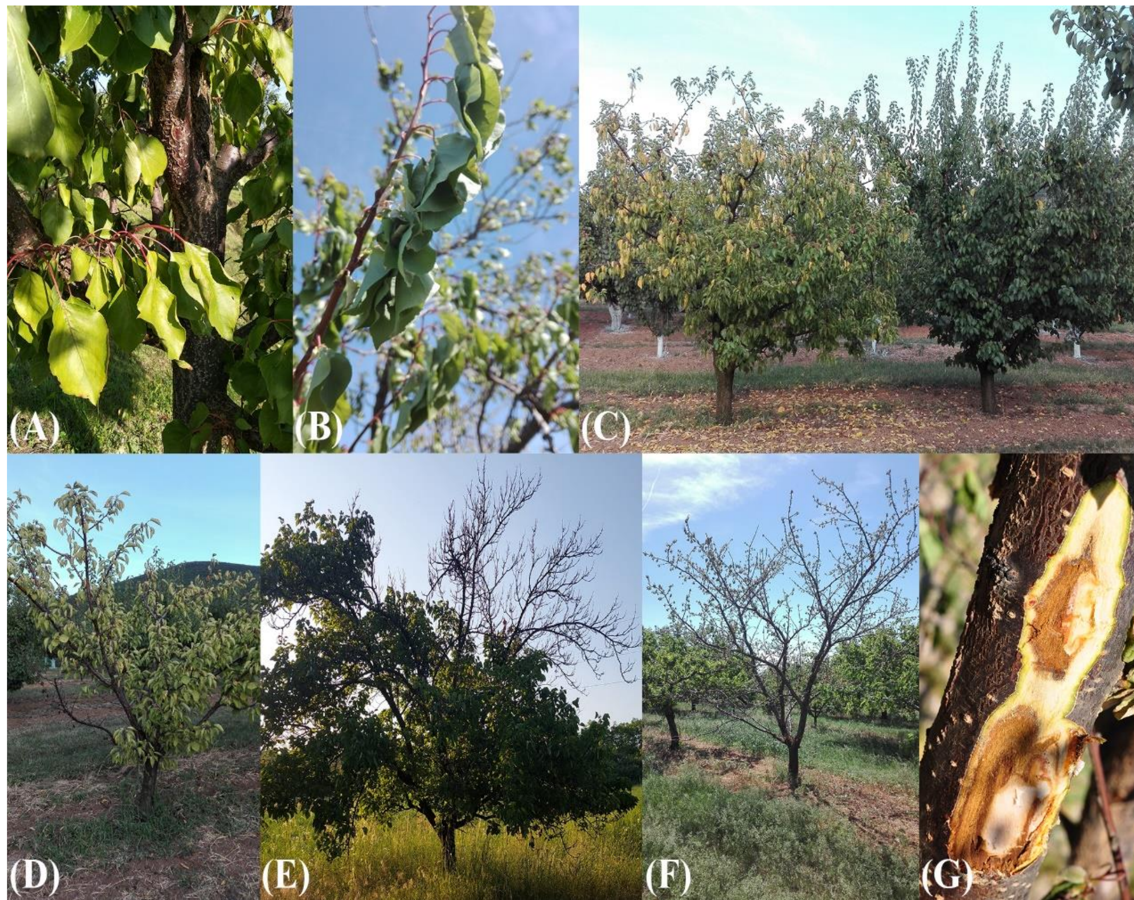

Figure 1. Symptoms of ESYF field surveys: (A) chlorotic leaf roll, (B) wilting of leaves, (C) premature leaf fall, (D) reduced shoot growth, (E) decay of woody parts (partial dieback), (F) complete dieback, (G) phloem necrosis

3.2. Evaluation of Presence of the Pathogen in Apricot Cultivars (2014–2017)

For 2014, the results showed significant differences in the appearance of the disease among cultivars ($\chi^2(2)= 61.29$; $p<0.001$). By assessing the standardized modified residuals, we found that cultivar 'Sweetcot' showed significantly higher relative frequency of disease, than 'Zebra' (Z-test, $p<0.05$). Cultivar 'Sweetcot' had a relatively high disease severity index (25.8%), while those of 'Flavorcot' was moderate (10.2%) and 'Zebra' was low (Table 2). Tree mortality was not examined because 2014 was the baseline year.

In 2015, we found highly significant differences in disease appearance ($\chi^2(2)=87.84$; $p<0.001$) based on Chi-square test. The assessment of standardized modified errors showed a significant difference between 'Zebra' and 'Sweetcot' (Z-test, $p<0.05$). Cultivar 'Sweetcot' had the highest (54.3%) disease severity index, while 'Zebra' showed the lowest (10.2 %). 'Sweetcot' had significantly higher (30%) tree mortality than the other two cultivars (Table 3; Marascuilo's procedure, $p<0.05$).

Table 2. Comparison of disease appearance based on observed and expected appearances with the values of disease severity index (DSI) in 2014

Cultivar	**Comparison of disease appearance**[1]				**DSI (%)**
	Type of data	ND	D	R	
'Flavorcot'	Observed count	94	26	0	10.2
	Expected	92.3	27.7		
'Sweetcot'	Observed count	66	54	+	25.8
	Expected	92.3	27.7		
'Zebra'	Observed count	117	3	-	0.7
	Expected	92.3	27.7		

[1]"ND" = non-diseased trees, "D" = diseased trees, "R" = results of disease incidence comparison of the cultivars: "0" = No significant difference between the expected and observed count values of non-diseased (ND) trees and diseased (D); "+" = observed count value of diseased trees (D) is significantly higher than the expected value; "-" = observed count value of diseased trees (D) significantly lower than the expected value (Z test, $p<0.05$).

Table 3. Comparison of disease appearance based on observed and expected appearances and tree mortality, with the values of disease severity index (DSI) and tree mortality index (TMI) in 2015

Cultivar	**Comparison of disease appearance**[1]				**DSI (%)**	**TMI (%)**[2]	**Comparison of tree mortality**[2]
	Type of data	ND	D	R			
'Flavorcot'	Observed count	64	56	0	31.5	16.7	A
	Expected	66.7	53.3				
'Sweetcot'	Observed count	32	88	+	54.3	30.0	B
	Expected	66.7	53.3				
'Zebra'	Observed count	104	16	-	10.2	10.0	A
	Expected	66.7	53.3				

[1]"ND" = non-diseased trees, "D" = diseased trees, "R" = results of disease incidence comparison of the cultivars: "0" = No significant difference between the expected and observed count values of non-diseased (ND) trees and diseased (D); "+" = observed count value of diseased trees (D) is significantly higher than the expected value; "-" = observed count value of diseased trees (D) significantly lower than the expected value (Z test, $p<0.05$).

[2]Different letters denote significantly different groups (Marascuilo's procedure, $p<0.05$) in 2015.

Table 4. Comparison of disease appearance based on observed and expected appearances and tree mortality, with the values of disease severity index (DSI) and tree mortality index (TMI) in 2016

Cultivar	**Comparison of disease appearance**[1]				**DSI (%)**	**TMI (%)**[2]	**Comparison of tree mortality**[2]
	Type of data	ND	D	R			
'Flavorcot'	Observed count	52	48	0	25.0	10.0	B
	Expected	55.3	44.7				
'Sweetcot'	Observed count	19	65	+	57.9	23.8	C
	Expected	46.4	37.6				
'Zebra'	Observed count	92	19	-	4.7	0.9	A
	Expected	61.3	49.7				

[1]"ND" = non-diseased trees, "D" = diseased trees, "R" = results of disease incidence comparison of the cultivars: "0" = No significant difference between the expected and observed count values of non-diseased (ND) trees and diseased (D); "+" = observed count value of diseased trees (D) is significantly higher than the expected value; "-" = observed count value of diseased trees (D) significantly lower than the expected value (Z test, $p<0.05$).

[2]Different letters denote significantly different groups (Marascuilo's procedure, $p<0.05$) in 2016.

For 2016, we revealed highly significant differences in disease appearance ($\chi^2(2)=70.89$; $p<0.001$). There was a significant difference between cultivars 'Zebra' and 'Sweetcot' (Z-test, $p<0.05$). 'Sweetcot ' had a high disease severity index (57.9%), while that of 'Flavorcot' was medium (25.0%) and 'Zebra' remained still low (4.7%). Tree mortality of 'Zebra ' was significantly lower than those of 'Flavorcot' and 'Sweetcot' by Marascuilo's procedure. Furthermore, there was also a difference between the other two cultivars, 'Sweetcot' had significantly higher tree mortality than 'Flavorcot' (Table 4; $p<0.05$).

In 2017 we found differences in the appearance of the disease among the tested cultivars ($\chi^2(2)=47.72$; $p<0.05$): 'Zebra' and 'Sweetcot' showed significant differences (Z-test, $p<0.05$). This year 'Sweetcot' showed the highest disease severity index (60.6%) in our study. Cultivar 'Flavorcot' and 'Zebra' had medium (30%) and low (14%) disease severity indexes, respectively. Thus 'Sweetcot' mortality index (35.9%) was significantly higher than those of 'Flavorcot' and 'Zebra' (Table 5; Marascuilo's procedure, $p<0.05$).

Table 5. Comparison of disease appearance based on observed and expected appearances and tree mortality, with the values of disease severity index (DSI) and tree mortality index (TMI) in 2017

Cultivar	Comparison of disease appearance[1]				DSI (%)	TMI (%)[2]	Comparison of tree mortality[2]
	Type of data	ND	D	R			
'Flavorcot'	Observed count	44	46	0	30.0	13.3	A
	Expected	45.2	44.8				
'Sweetcot'	Observed count	11	54	+	60.6	35.9	B
	Expected	32.6	32.4				
'Zebra'	Observed count	78	32	-	14.0	4.7	A
	Expected	55.2	54.8				

[1]"ND" = non-diseased trees, "D" = diseased trees, "R" = results of disease incidence comparison of the cultivars: "0" = No significant difference between the expected and observed count values of non-diseased (ND) trees and diseased (D); "+" = observed count value of diseased trees (D) is significantly higher than the expected value; "-" = observed count value of diseased trees (D) significantly lower than the expected value (Z test, $p<0.05$).

[2]Different letters denote significantly different groups (Marascuilo’s procedure, $p<0.05$) in 2017.

3.3. Molecular Detection of '*Ca*. Phytoplasma Prunorum'

3.3.1. Verification from the Orchard

In nested PCR the universal and specific primer sets yielded an approx. 240 base-pair long target sequence (Figure 2). The presence of '*Ca*. Phytoplasma prunorum' was identified by molecular techniques in 54% of the trees tested. Infection was confirmed in 92% of the symptomatic trees and 16% of the asymptomatic trees (Table 6). In two cases, phytoplasma was not detected in symptomatic trees. Among the asymptomatic trees, four were identified as infected, of which three remained asymptomatic until the end of the study. During the survey, 44% of the infected trees died.

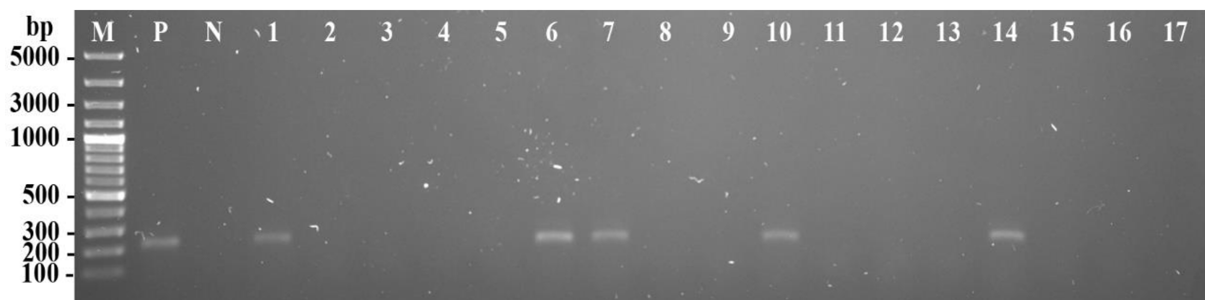

Figure 2. The results of '*Ca*. Phytoplasma prunorum' infection tests on apricot trees of orchard (P: Positive control; N: Negative control; 1–17: examined samples) displayed on agarose gel (1.0%). M: 100 / 1000 bp DNA ladder (Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania)

Table 6. Detection of phytoplasma from the 50 tested trees

Health status of trees	Infected trees	Non-Infected trees
Symptomatic (n=25)	23	2
Asymptomatic (n=25)	4	21

3.3.2. Verification from Apricot Grafts

Despite the fact that the one-year-old plants did not show any abnormalities at the time of the evaluation, the molecular screening of grafts revealed that 2% of the scion and 2% of the rootstock were infected by '*Ca*. Phytoplasma prunorum'. We identified phytoplasma in 2 cases only from the rootstock of 'Myrabolan' cultivar and in 2 cases only from the scion (cv. 'Gönci magyar kajszi', 'Bergerouge'). Phytoplasma was detectable from both rootstock and scion only one case (cv. 'Harcot'-'Myrabolan').

4. Discussion

The emergence of '*Ca*. Phytoplasma prunorum' can cause significant economic losses in apricot orchards [2]. In our study, we found that more than half (54%) of the examined trees were infected with phytoplasma. In previous studies similar results (44,9%-56%) have been obtained [10,16,51,52], but also lower (10,5%-24%) [53-55] and higher (67%-83%) [11,18,36] rates have been identified. Based on these results, a medium level of the pathogen was present in the tested plantation. By the end of the study, 44% of the infected trees had died, suggesting that this pathogen was also responsible for tree mortality [16,20,21,38]. However, the pathogen was not detected in 8% of the symptomatic trees. There are several possible explanations for this observation. The level of phytoplasma in plants may be low and its distribution is not homogeneous [29,30]; therefore, it is possible that the amount of the pathogen in the internal tissues was below the detection level. Another reason may be that in some samples, high levels of enzyme-inhibiting plant polyphenol and polysaccharide compounds can inhibit PCR [32,56].

We noticed that 16% of the infected trees were asymptomatic, which was a known phenomenon in previous studies [12,16,51,57]. Interestingly, in all three cases, the trees remained asymptomatic until the end of the experiment. Some studies have reported that certain plants can recover from symptoms and later become tolerant to the pathogen [21,22,58]. However, to confirm this phenomenon, the trees mentioned above should be further investigated.

While testing the cultivar susceptibility to the '*Ca*. Phytoplasma prunorum', we monitored symptoms that have been described in previous studies, such as: chlorotic leaf roll and early defoliation of yellowing of leaves [15-20,59]. However, the severity of symptoms varied among the tested cultivars, suggesting that the cultivars were affected differently by the phytoplasma, which is in accordance with previous studies [42,43,45,60]. The cultivar 'Zebra' was the least affected of the three tested cultivars, both regarding disease appearance and disease severity index. The latter was only 0,7% per year. Moreover, 'Zebra' has generally been observed to have a low tree mortality index. It would be important to further investigate this remarkably resistant cultivar in the context of the '*Ca*. Phytoplasma prunorum'. 'Zebra' may play an important role as a genetic resource for breeding new ESFY-tolerant cultivars. The results suggest that 'Flavorcot' is moderately susceptible to ESFY, and therefore tree mortality should be expected even with preventive control. The highest disease incidences were found in 'Sweetcot' and the severity of symptoms was also the highest, reaching 60.6% in one year. In addition, high levels of mortality were generally observed in 'Sweetcot' during study years. In total, 65.8% of the trees assessed died, which is much higher than the average tree mortality (30–40%) [16,19]. Planting of 'Sweetcot' in areas infested with phytoplasma can result in high economic losses. Note that at the end of the study, 'Sweetcot' was removed from the surveyed orchard. Our results show that selecting the appropriate cultivars can reduce the damage of ESFY. Furthermore, the disease severity index can be used to assess cultivar susceptibility and allows comparison of the cultivar tested with other cultivars.

In the case of apricot, dieback is one of the most important plant protection problems that can be caused by the phytoplasma as well [15-17,19,20]. Growers usually replace dead trees with new ones, therefore testing the planting material for phytoplasma is of crucial importance. We verified the presence of '*Ca*. Phytoplasma prunorum' in propagation material (scions and/or rootstocks), in contrast to a previous study [61], where phytoplasmas were not detected in asymptomatic apricots (including grafts). This indicates that these grafts could be a new source of infection, and may introduce the phytoplasma into the orchards.

Although we focused our study on the phytoplasma only, it is important to note that other pathogens can also cause apoplexy (e.g. *Cytospora* sp., *Pseudomonas syringae*) [62,63]. In Hungary, there are fewer data available on the importance and prevalence of phytoplasma than for other pathogens mentioned above. To obtain a complete overview of the role of the species in apoplexy, these pathogens need to be investigated in a complex way in the future.

5. Conclusions

'*Ca*. phytoplasma prunorum' caused significant damage in the surveyed apricot orchard and also in other stone fruit plantations. The protection of orchards is difficult, because pesticides are effective only against the vector. In the absence of curative chemical treatments, the tolerance or low susceptibility of cultivars is a key control factor. Our

results indicate that 'Zebra' cultivar can be grown with low risk. Our study highlights that grafted plants used to replace dead trees may be potential sources of infection. Thus, the production and planting of healthy propagating material are cardinal for the subsequent health of the plantation.

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Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

[1] Lorenz K., Dosba F., Poggi-Pollini C., Llacer G., E. Seemüller, "Phytoplasma diseases of *Prunus* species in Europe are caused by genetically similar organisms," Journal of Plant Diseases and Protection, vol. 101, no. 6, pp. 567-575, 1994.

[2] Marcone C., Jarausch B., W. Jarausch, "*Candidatus* Phytoplasma prunorum, the causal agent of European stone fruit yellows: an overview," Journal of Plant Pathology, vol. 92, no. 1, pp. 19-34, 2010.

[3] Al Khazindar M., Salam A. A., "Phytoplasma in stone fruits and date palm in Egypt," Emerging phytoplasma diseases of stone fruits and other crops and their possible impact on EU Countries, Bologna, Italy, Dec., 2011, pp. 23-24.

[4] Siampour M., Izadpanah K., Salehi M., A. Afsharifar, "Occurrence and distribution of phytoplasma diseases in Iran," in Sustainable Management of Phytoplasma Diseases in Crops Grown in the Tropical Belt. Sustainability in Plant and Crop Protection, 1st ed, Springer, 2019, pp. 47-86.

[5] Jarausch B., W. Jarausch, "Psyllid vectors and their control," in Phytoplasmas: Genomes, plant hosts and vectors, 1st ed, CABI, 2009, pp. 250-271.

[6] Tedeschi R., Jarausch B., Delic D., P. Weintraub, "Actual distribution of fruit tree and grapevine phytoplasma diseases and their vectors in Europe and neighboring regions," Phytopathogenic Mollicutes, vol. 3, no. 1, pp. 3-4, 2013. DOI: 10.5958/j.2249-4677.3.1.002.

[7] Ben Khalifa M., H. Fakhfakh, "First report of Candidatus Phytoplasma prunorum infecting almonds in Tunisia," Phytoparasitica, vol. 39, pp. 411-414, 2011. DOI: 10.1007/s12600-011-0166-4.

[8] S. Süle, "Strategies for the control of apricot decline," Mitteilungen Klosterneuburg Rebe und Wein, Obstbau und Früchteverwertung, vol. 49, no. 6, pp. 250-252, 1999.

[9] Suran P., Žďárská I., M. Rejlová, "Sensitivity of apricot cultivars and rootstocks to phytoplasma diseases," Acta Horticulturae, vol. 1290, pp. 99-104, 2020. DOI: 10.17660/ActaHortic.2020.1290.18.

[10] Riedle-Bauer M., Paleskić C., Schwanzer J., Kölber M., Bachinger K., Antonielli L., Schönhuber C., Elek R., Stradinger J., Emberger M., Engel C., Makay M., Zajcsek F., G. Brader, "Epidemiological and molecular study on '*Candidatus* Phytoplasma prunorum' in Austria and Hungary," Annals of Applied Biology, vol. 175, no. 3, pp. 400-414, 2019. DOI: 10.1111/aab.12541.

[11] Jarausch B., Mühlenz I., Beck A., Lampe I., Harzer U., W. Jarausch, "Epidemiology of European Stone Fruit Yellows in Germany," Acta Horticulturae, vol. 781, pp. 417-422, 2008. DOI: 10.17660/ActaHortic.2008.781.59.

[12] Ambrožič Turk B., Mehle N., Brzin J., Škerlavaj V., Seljak G., M. Ravnikar, "High infection pressure of ESFY phytoplasma threatens the cultivation of stone fruit species," Journal of Central European Agriculture, vol. 9, no. 4, pp. 795-801, 2008.

[13] A. Bertaccini, "Phytoplasmas: diversity, taxonomy, and epidemiology," Frontiers in Bioscience, vol. 12, no. 2, pp. 673-689, 2007. DOI: 10.2741/2092.

[14] E. Seemüller, "Apple proliferation: etiology, epidemiology and detection," ATTI Giornate Fitopatologiche, vol. 1, pp. 3-6, 2002. DOI: 10.1400/32240.

[15] G. Morvan, "Apricot Chlorotic Leaf Roll," EPPO Bulletin, vol. 7, no. 1, pp. 37-55, 1977. DOI: 10.1111/j.1365-2338.1977.tb02406.x.

[16] Nečas T., Ondrášek I., B. Krška, "'Candidatus Phytoplasma prunorum' - a pathogen spreading uncontrollably in apricot orchards in the Czech Republic," Acta Horticulturae, vol. 1105, pp. 131–136, 2015. DOI: 10.17660/ActaHortic.2015 .1105.19.

[17] Žežlina I., Rot M., Kač M., S. Trdan, "Causal agents of stone fruit diseases in Slovenia and the potential for diminishing their economic impact–a review," Plant Protection Science, vol. 52, no. 3, pp. 149-157, 2016. DOI: 10.17221/58/2015-PPS.

[18] Jarausch W., Lansac M., Saillard C., Broquaire J., F. Dosba, "PCR assay for specific detection of European stone fruit yellows phytoplasmas and its use for epidemiological studies in France," European Journal of Plant Pathology, vol. 104, no. 1, pp. 17-27, 1998. DOI: 10.1023/A:10086008281 44.

[19] S. Süle, "Phytoplasma diseases on fruits in Hungary," Acta Agraria Debreceniensis, vol. 62, pp. 24-29, 2014. DOI: 10.34101/actaagrar/62/2158.

[20] Kison H., E. Seemüller, "Differences in strain virulence of the European stone fruit yellows phytoplasma and susceptibility of stone fruit trees on various rootstocks to this pathogen," Journal of Phytopathology, vol. 149, no. 9, pp. 533-541, 2001. DOI: 10.1046/j.1439-0434.2001.00671.x.

[21] Osler R., Borselli S., Ermacora P., Loschi A., Martini M., Musetti R., N. Loi, "Acquired tolerance in apricot plants that stably recovered from European stone fruit yellows," Plant Disease, vol. 98, no. 4, pp. 492-496, 2014. DOI: 10.1094/PDIS-03-13-0342-RE.

[22] Osler R., Borselli S., Ermacora P., Ferrini F., Loschi A.,

Martini M., Moruzzi S., Musetti R., Giannini M., S. Serra, "Transmissible tolerance to European stone fruit yellows (ESFY) in apricot: cross-protection or a plant mediated process?," Phytoparasitica, vol. 44, pp. 203-211, 2016. DOI: 10.1007/s12600-016-0509-2.

[23] Carraro L., Osler R., Loi N., Ermacora P., E. Refatti, "Transmission of European stone fruit yellows phytoplasma by Cacopsylla pruni," Journal of Plant Pathology, vol. 80, no. 3, pp. 233-239, 1998.

[24] Riedle-Bauer M., Bachinger K., Stradinger J., Emberger M., Mörtel J., H. Sára, "Transmission of European Stone Fruit Yellows Phytoplasma ('Candidatus Phytoplasma prunorum') during the propagation process," Mitteilungen Klosterneuburg Rebe und Wein, Obstbau und Früchteverwertung, vol. 62, no. 4, pp. 177-181, 2012.

[25] Ermacora P., Loi N., Ferrini F., Loschi A., Martini M., Osler R., L. Carraro, "Hypo-and hyper-virulence in apricot trees infected by European stone fruit yellows," Julius-Kühn-Archiv, vol. 427, pp. 197, 2010.

[26] Seemüller E., Stolz H., H. Kison, "Persistence of the European stone fruit yellows phytoplasma in aerial parts of Prunus taxa during the dormant season," Journal of Phytopathology, vol. 146, no. 8-9, pp. 407-410, 1998. DOI: 10.1111/j.1439-0434.1998.tb04772.x.

[27] Pastore M., Piccirillo P., Tian J., Simeone A. M., Paltrinieri S., A. Bertaccini, "Transmission by patch grafting of ESFY phytoplasma to apricot (Prunus armeniaca L) and japanese plum (Prunus salicina LINDL)," Acta Horticulturae, vol. 550, pp. 339-344, 2001. DOI: 10.17660/ActaHortic.2001.550.49.

[28] Marie-Jeanne V., Bonnot F., Thébaud G., Peccoud J., Labonne G., N. Sauvion, "Multi-scale spatial genetic structure of the vector-borne pathogen '*Candidatus* Phytoplasma prunorum' in orchards and in wild habitats," Scientific Reports, vol. 10, no. 1, pp. 5002, 2020. DOI: 10.1038/s41598-020-61908-0.

[29] Berges R., Rott M., E. Seemüller, "Range of phytoplasma concentrations in various plant hosts as determined by competitive polymerase chain reaction," Phytopathology, vol. 90, no. 10, pp. 1145-1152, 2000. DOI: 10.1094/PHYTO.2000.90.10.1145.

[30] Nečasová J., Nečas T., Eichmeier A., B. Krška, "Use of real-time PCR for the characterization of variable symptoms in ESFY phytoplasma disease," Acta Horticulturae, vol. 1110, pp. 57-62, 2016. DOI: 10.17660/ActaHortic.2016.1110.9.

[31] M. Laimer, "Detection of Phytoplasmas of Temperate Fruit Trees," in Plant Pathology: Techniques and Protocols, Humana Press, 2009, pp. 267-288.

[32] Bertaccini A., Fiore N., Zamorano A., Tiwari A. K., G. P. Rao, "Molecular and serological approaches in detection of phytoplasmas in plants and insects," in Phytoplasmas: Plant Pathogenic Bacteria-III: Genomics, Host Pathogen Interactions and Diagnosis, Springer Nature Singapore, 2019, pp. 105-136. DOI: 10.1007/978-981-13-9632-8_7.

[33] E. Mergenthaler, "Fitoplazmás betegségek Magyarországon: Korszerű diagnosztikai módszerek fejlesztése [Phytoplasma diseases in Hungary: Development of improved diagnostical methods]." Ph.D. thesis, Budapesti Közgazdaságtudományi és Államigazgatási Egyetem, Budapest, 2004.

[34] Danet J. L., Balakishiyeva G., Cimerman A., Sauvion N., Marie-Jeanne V., Labonne G., Laviňa A., Batlle A., Krizanac I., D. Škorić, "Multilocus sequence analysis reveals the genetic diversity of European fruit tree phytoplasmas and supports the existence of inter-species recombination," Microbiology, vol. 157, no. 2, pp. 438-450, 2011. DOI: 10.1099/mic.0.043547-0.

[35] Eichmeier A., Kiss T., Necas T., Penazova E., Tekielska D., Bohunicka M., Valentova L., Cmejla R., Morais D., P. Baldrian, "High-Throughput Sequencing Analysis of the Bacterial Community in Stone Fruit Phloem Tissues Infected by "Candidatus Phytoplasma prunorum"," Microbial Ecology, vol. 77, no. 3, pp. 664-675, 2019. DOI: 10.1007/s00248-018-1250-9.

[36] Jarausch W., Jarausch B., Fritz M., Runne M., Etropolska A., E. Pfeilstetter, "Epidemiology of European stone fruit yellows in Germany: the role of wild Prunus spinosa," European Journal of Plant Pathology, vol. 154, pp. 463-476, 2019. DOI: 10.1007/s10658-019-01669-3.

[37] Fránová J., Přibylová J., Navrátil M., Šafářová D., Ember I., Kölber M., Süle S., Cieślińska M., M. Kamińska, "Phytoplasma diseases and their vectors in Czech Republic, Hungary and Poland," in Phytoplasmas and Phytoplasma Disease Management: How to Reduce Their Economic Impact, IPWG - International Phytoplasmologist Working Group, 2014, pp. 29-35.

[38] S. Richter, "Susceptibility of Austrian apricot and peach cultivars to ESFY," Plant Protection Science, vol. 38, Special Issue: 2, pp. 281-284, 2002. DOI: 10.17221/10467-PPS.

[39] Baldessari M., Trona F., Angeli G., C. Ioriatti, "Effectiveness of five insecticides for the control of adults and young stages of Cacopsylla melanoneura (Förster)(Hemiptera: Psyllidae) in a semi-field trial," Pest Management Science, vol. 66, no. 3, pp. 308-312, 2010. DOI: 10.1002/ps.1876.

[40] Paleskić C., Bachinger K., Brader G., Kickenweiz M., Engel C., Wurm L., Czipin L., M. Riedle-Bauer, "Cage and field experiments as basis for the development of control strategies against Cacopsylla pruni, the vector of European Stone Fruit Yellows," Annals of Applied Biology, vol. 170, no. 3, pp. 357-368, 2017. DOI: 10.1111/aab.12340.

[41] Jarausch W., Danet J., Labonne G., Dosba F., Broquaire J., Saillard C., M. Garnier, "Mapping the spread of apricot chlorotic leaf roll (ACLR) in southern France and implication of Cacopsylla pruni as a vector of European stone fruit yellows (ESFY) phytoplasmas," Plant Pathology, vol. 50, no. 6, pp. 782-790, 2001. DOI: 10.1046/j.1365-3059.2001.00645.x.

[42] Nečas T., Kiss T., Eichmeier A., Nečasová J., I. Ondrášek, "The Effect of Phytoplasma Disease Caused by 'Candidatus Phytoplasma prunorum' on the Phenological and Pomological Traits in Apricot Trees," Notulae Botanicae Horti Agrobotanici Cluj-Napoca, vol. 46, no. 1, pp. 107-114, 2018. DOI: 10.15835/nbha46110879.

[43] Audergon J. M., Castelain C., Morvan G., M. Chastelliere, "Behaviour of 150 apricot varieties after an apricot chlorotic leaf roll inoculation," Acta Horticulturae, vol. 293, pp. 593-

598, 1991. DOI: 10.17660/ActaHortic.1991.293.72.

[44] Brun L., Warlop F., Mercier V., Broquaire J.-M., Clauzel G., Gomez C., Parveaud C.-E., J.-M. Audergon, "Quelle sélection fruitière pour une production durable, à faible niveau d'intrants? Méthodologie pour un réseau de sélection variétale décentralisée," Innovations Agronomiques, vol. 15, pp. 105-115, 2011.

[45] Poggi Pollini C., Forno F., Franchini S., Gobber M., Lanzoni C., Mattedi L., Miorelli P., Profaizer D., Ratti C., "Detection and distribution of European stone fruit yellows (ESFY) in apricot cv. 'Bergeron' and epidemiological studies in the province of Trento (Italy), " 21st International Conference on Virus and other Graft Transmissible Deseases of Fruit Crops, Neustadt, Germany, July, 2009, pp. 383-385.

[46] Koncz L., Petróczy M., Ladányi M., Maitz M., G. Nagy, "Severity of symptoms of European stone fruit yellows on different apricot varieties," Review on Agriculture and Rural Development, vol. 6, no. 1-2, pp. 63-70, 2017. DOI: 10.14232/rard.2017.1-2.63-70

[47] H. Gartner, "Versuche zur Bekampfung von Botrytis cinerea (Grauschimmel) als Traubenfaule. [Experiments to control Botrytis cinerea (grey rot) in the same way as grape rot]." Mitteilungen Klosterneuburg, vol. 21, no. 3, pp. 183–189, 1971.

[48] IBM, "IBM SPSS Statistics for Windows, "Version 25.0., IBM Corporation, Armonk, USA, 2017.

[49] Marascuilo L. A., M. McSweeney, "Nonparametric post hoc comparisons for trend," Psychological Bulletin, vol. 67, no. 6, pp. 401, 1967.

[50] Daire X., Clair D., Reinert W., E. Boudon-Padieu, "Detection and differentiation of grapevine yellows phytoplasmas belonging to the elm yellows group and to the stolbur subgroup by PCR amplification of non-ribosomal DNA," European Journal of Plant Pathology, vol. 103, no. 6, pp. 507-514, 1997. DOI: 10.1023/A:1008641411025.

[51] Mehle N., Ravnikar M., Seljak G., Knapic V., M. Dermastia, "The most widespread phytoplasmas, vectors and measures for disease control in Slovenia," Phytopathogenic mollicutes, vol. 1, no. 2, pp. 65-76, 2011.

[52] Navratil M., Válová P., Fialová R., Petrová K., Fránová J., Nebesárová, J., Poncarová-Voráčková Z., R. Karesová, "Survey for stone fruit phytoplasmas in the Czech Republic," Acta Horticulturae, vol. 550, pp. 377-382. 2001.

[53] Cieślińska M., H. Morgaś, "Detection and identification of '*Candidatus* Phytoplasma prunorum', '*Candidatus* Phytoplasma mali' and '*Candidatus* Phytoplasma pyri' in stone fruit trees in Poland," Journal of Phytopathology, 159. 4. pp. 217-222. 2011.

[54] Ivić D., Plavec J., G. Ivančan, "The occurrence of 'Candidatus Phytoplasma prunorum' in apricot orchards in Baranja," Pomologia Croatica: Glasilo Hrvatskog agronomskog društva, vol. 21, no. 1-2, pp. 101-106. 2017.

[55] Ludvikova H., Franova J., J. Sucha, "Phytoplasmas in apricot, peach and sour cherry orchards in East Bohemia, Czech Republic," Bulletin of insectology, Supplement 64, pp. S67-S68, 2011.

[56] Green M. J., Thompson D. A., D. J. MacKenzie, "Easy and efficient DNA extraction from woody plants for the detection of phytoplasmas by polymerase chain reaction," Plant Disease, vol. 83, no. 5, pp. 482-485, 1999.

[57] Torres E., Martin M., Paltrinieri S., Vila A., Masalles R., A. Bertaccini, "Spreading of ESFY phytoplasmas in stone fruit in Catalonia (Spain)," Journal of Phytopathology, vol. 152, no. 7, pp. 432-437, 2004. DOI: 10.1111/j.1439-0434.2004.00869.x.

[58] Musetti R., di Toppi L. S., Martini M., Ferrini F., Loschi A., Favali M. A., R. Osler, "Hydrogen peroxide localization and antioxidant status in the recovery of apricot plants from European Stone Fruit Yellows," European Journal of Plant Pathology, vol. 112, pp. 53-61, 2005. DOI: 10.1007/s10658-004-8233-z.

[59] Valasevich N., B. Schneider, "Detection, identification and molecular diversity of 'Candidatus Phytoplasma prunorum' in Belarus," Journal of Plant Pathology, vol. 98, no. 3, pp. 625-629, 2016. DOI: 10.4454/JPP.V98I3.031.

[60] Krška B., Gogolková K., Ondrášek I., T. Nečas, "Evaluation of precocious decline of young apricot orchards in the Czech Republic," Acta Horticulturae, vol. 966, pp. 175-182, 2012. DOI: 10.17660/ActaHortic.2012.966.27.

[61] Pignatta D., Forno F., Giunchedi L., Gobber M., Mattedi L., Miorelli P., Poggi Pollini C., Ratti C., Reggiani N., E. Ropelato, "A real time PCR assay for the detection of European Stone Fruit Yellow Phytoplasma (ESFYP) in plant propagation material," Acta Horticulturae, vol. 781, pp. 499-504, 2008.

[62] Z. D. Rozsnyay, "Cytospora canker and dieback of Apricots," EPPO Bulletin, vol. 7, no. 1, pp. 69-80, 1977.

[63] Z. Klement, "Bacterial canker and dieback disease of apricots (Pseudomonas syringae van Hall)," EPPO Bulletin, vol. 7, no. 1, pp. 57-68, 1977.