OCCURENCE OF *CA.* PHYTOPLASMA PRUNORUM IN THE CARPATHIAN-BASIN

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Abstract

Phytoplasmas are serious pathogens of several important plants throughout the world. Apricot phytoplasma disease ("Ca. Phytoplasma prunorum") was first reported from France in Europe, in 1924 from. In 1992, the disease was identified in Hungary. Our goals were to diagnose the occurrence of Ca. Phytoplasma prunorum on stone fruits in North-Hungarian growing areas by visual diagnostics and to confirm data by laboratory PCR-based examinations. Plant samples were tested in laboratory trials, and the presence of phytoplasma were confirmed from apricot, peach, cherry, sour cherry and wild plum Field investigation were also done in a Western-Romanian apricot plantation, and the presence of phytoplasma disease was also identified. It was the first time that symptoms of Ca. Phytoplama prunorum had been observed in the western part of Romania. On the basis of these observations it seems evident that the notable losses caused by Ca. Phytoplasma prunorum is a new plant hygiene problem for fruit growers to manage, especially for apricot producers in Hungary and other Cental-European countries as well.

Key words: *Ca.* Phytoplasma prunorum, European stone fruit yellows pytoplasma, ESFY, *Ca.* Phytoplasma vitis, stone fruits, apricot, peach.

INTRODUCTION

Phytoplasmas are serious pathogens of several important plants throughout the world, causing a wide variety of symptoms that range from mild yellowing to the death of infected plants. Stolbur disease of potato and tomato (*Candidatus* Phytoplasma solani) is one of the most common plant disease caused by phytoplasmas. Apple and pear have also phytoplasma originated diseases (*Ca.* Phytoplasma mali, *Ca.* Phytoplasma pyri), but grape and maize are also endangered by phytoplasmas. Coconut, sugarcane, rice, sandal wood in tropical and sub-tropical regions of the world also have serious diseases caused by phytoplasmas. Phytoplasma diseases have increasing importance for fruit growers. These agents reside in the phloem tissues of the plants, and they require a vector to be transmitted from plant to plant, and this normally takes the form of sap sucking insects such as leaf hoppers in which they are also able to replicate. Phytoplasmas cannot be transmitted mechanically.

Until 1967, plant diseases known as „yellows diseases" were thought to be caused by viruses. In 1967, Japanese researchers (Doi Y. et al., 1967) found microorganism by electron microscope in yellows diseased plants. This new class of plant disease agents was named a "mycoplasma-like organism" (Welliver, 1999). In 1992, characterization of the organisms

associated with yellows diseases had progressed to a point where it became clear they were unique and should be given their own name: PHYTOPLASMA (Gundersen et al., 1994).

Apricot phytoplasma disease was reported first in Europe in 1924, in France (Chabrolin, 1924). „*Candidatus* Phytoplasma prunorum (Seemüller, Schneider, 2004) [(syn: European Stone Fruit Yellows Phytoplasma; ESFY (Kövics, 2009)] is one of the most important diseases of apricot in Hungary. In Hungary and in the neighbouring Central-European countries it is a relatively new and serious pathogen for the apricot cultivation. The disease was observed first on apricot in Hungary in 1992 (Süle, unpublished). Later on occurrence of the ESFY was confirmed by molecular biological examinations in Hungary (Viczián et al., 1997; Süle et al., 1997). After that, similar symptoms were also observed on other stone fruits (Mergenthaler E., 2004). Occurrence of phytoplasma disease was observed in peach (Németh et al., 2001), *Prunus serrulata* (Lorenz K. H. et al., 1994), *Prunus mahaleb* cv. Cemany (Varga et al., 2001) and *Prunus spinosa* (Jarausch et al., 2000) as well. The disease on cherry was reported in France as "Molieres-disease", but several experts thought that cherry is resistant to phytoplasma infection (Jarausch W. et al., 2000). European plums have been determined to be tolerant to *Ca.* Phytoplasma prunorum, whereas Japanese plums are highly susceptible (Mona G. et al, 2008). The psyllid *Cacopsylla pruni* Scopoli was described as the main vector of *Candidatus* Phytoplasma prunorum.

Another importand phytoplasma disease is the Flavescence Dorée Phytoplasma on grape. It was first observed in France in 1949. Then in a few decades the disease appeared in many countries of southern Europe, such as Croatia, Austria and Slovenia. The disease agent in the new nomenclature called *Candidatus* Phytoplasma vitis. The pathogen is on the list of quarantine pests in the European Union. The introduction and dissemination of the pathogen is prohibited. The disease was identified in Hungary in Zala county in the August of this year (Kriston et al., 2013). Vector of the disease is the *Scaphoides titanus*, which was found in 2006 in Hungary. Since then it spread throughout the country.

MATERIAL AND METHOD

Objectives of our work were to examine stone fruit plantations in the Carpathian-Basin, and to identify infections of *Candidatus* Phytoplasma prunorum. Field examinations were done on several stone fruit gardens in Borsod-Abaúj-Zemplén county (on Gönc Apricot Growing Area), in Hajdú-Bihar county (Hajdúdorog) and in West-Romania, near Oradea city (Biharpüspöki) between 2009-2012 (12 apricot cultivars, 1 peach population, 4 sour cherry and 1 cherry plantation). Our first goal was to visually identify *Ca.* Phytoplasma prunorum infection on stone fruits

(especially on apricot) on the examined Northern-Hungarian an Romanian growing areas.

During the field examinations infection ratio (I%) and infection index (Ii) [according to a classification system (Table 1)] were calculated in the various stone fruit plantations based on the visible symptoms of the disease caused by *Ca.* Phytoplasma prunorum. The classification system contains 5 infection degrees, and the symptoms are getting more and more serious from the I. degree towards the V. degree. Infection degrees were classified on the basis of the following symptoms:

-on leaves: yellow colour change and rolling of leaves to its abaxial surface,
-on branches: general yellowing or „scalding-like" drying,
-in the trunk: having striped the bark of tree, orange or light brown colour change is visible in the phloem
-on trees: general yellowing on several branches or general drying similar to the destruction of the apricot die-back (apoplexy) but there is no secretion of resin; withered, dead or felled tree,
-at the plantation: infections and destruction of trees starting in a circular direction around the infected tree.

Usually 100 trees were examined on a site (except the smaller fruit gardens). 10 fruit trees of a circle were randomly selected for examination from 10 circles, that is 100 trees in total.

Table 1

Scale of infection index (Fi) classification system (Tarcali, Kövics , 2009)

Infection degrees	Symptoms
I	Healthy tree
II	Symptoms on leaves, on 1 branch
III	General yellowing or drying, symptoms on several branches
IV	1 dead branch
V	Dead or felled tree

Plant samples were collected from the supposedly infected trees, based on the visible symptoms (living leaves, pieces of branches and pieces of roots) by a sharp sampling knife for further laboratory examinations. The identification of phytoplasma is only possible from living plant parts. Identification is not possible from dead plant samples because of the life of the pathogen twit to the living phloem of the plant (Mergenthaler, 2004).

In the laboratory, molecular biological examinations were applied (PCR) to identify first the phytoplasma, and then the kind of the phytoplasma. The primers, sequences and programs which were applied on the PCR examination in order to identify the phytoplasma are shown in the Table 2.

Table 2

Used sequences and programs on laboratory examinations (name of primer (1), sequences (2), position (3), programme (4)

Name of primer (1)	Sequences (5'->3') (2)	Position (bp) (3)	Programme
P1	AAGAGTTTGATCCTGGCTCAGGATT	6-28	94°C-5min; 94°C-1min **55**°C-1min
P7	TTCTCGGCTACTTCCTGC	1818-1836	72°C-2min (35 cycles); 72°C-10min
fU5	CGGCAATGGAGGAAACT	370-387	95°C-3min; 95°C-1min **55**°C-1min
rU3	TTCAGCTACTCTTTGTAACA	1230-1250	72°C-1min (35 cycles); 72°C-5min
ECA1	AATAATCAAGAACAAGAAGT		95°C-1min; 95°C-30sec **55**°C-30sec
ECA2	GTTTATAAAAATTAATGACTC		72°C-30sec (35 cycles); 72°C-3min
fO1	CGGAAACTTTTAGTTTCAGT	61-81	94°C-3min; 94°C-1min **55**°C-1min
rO1	AAGTGCCCAACTAAATGAT	1115-1135	72°C-1min (35 cycles); 72°C-7min

RESULTS AND DISCUSSION

Results of the field examinations

Field examinations on the research of *Ca* Phytoplasma prunorum was begun on 02 October, 2009 in the village of Bekecs (near Szerencs city). Four apricot plantations, one peach, one cherry, and three sour cherry plantations were examined on that year. The visual experience was the view of a very depressing situation, the apricot plantations were heavily destructed by phytoplasma disease, and a great number of apricot trees were dead or felled in the fruit gardens. Yellowing and rolling leaves on the apricot branches and several drying branches were found on the apricot trees. A similar situation was visible on peach, and the same was experienced on cherry and sour cherry trees. Very serious destruction of 85% was experienced in the Bekecs-Téglaszín apricot garden on 3 hectares. It was the most heavily infested and destructed apricot population among the examined spots. Very serious infection ratio was measured; out of the one hundred examined trees as many as, 85 were infected, and according to the classification system (Table 1) 65 were dead or felled, as it is visible in the column V. of Table 3, showing the degree of infection. Other stone fruit kinds were also examined during the field investigations. A more moderate infection was experienced in a 12-13-year-old peach cultivar where phytoplasma infection with a rate of 21% was experienced. The destruction rate on peach was not so high as it was on apricot, but the problem with *Ca.* Phytoplasma prunorum seemed to be evident there. Three sour cherry and one cherry plantations were examined as well. Destruction (of various rates) caused by phytoplasma were found. On the first examined sour cherry plantation the infection rate was very high (62%) and there were several withered or felled trees. It was easily observable that sour cherry and cherry are also endangered by *Ca.* Phytoplasma prunorum infection. A

comparatively new, only 4-year-old apricot plantation was examined first. Most trees were healthy, but there were a few trees (2%) infected by Ca. Phytoplasma prunorum (Table 3). According to the description by Süle et al. (2003), on the apricot trees the first symptoms of the pathogen can be observed from the age of 3 or 4, and this thesis was justified in the visited apricot garden. The cultivars (cv.) of apricot grown on the plantations were the following ones: Ceglédi Óriás (Cegléd Giant), Ceglédi Arany (Cegléd Gold) and Magyar Kajszi (Hungarian Apricot). The cultivar Cegléd (new local varieties in Hungary) are more susceptible to phytoplasma disease than the old one, the Hungarian Apricot.

In 2010, further field research work were done in 6 fruit gardens in Borsod-Abaúj-Zemplén County, and 1 apricot plantation was visited and examined in West-Romania, in the village of Biharpüspöki, near the city of Oradea. Very high infection rates were measured in Bükkaranyos on 2 apricot gardens (I%: 77-84) and 1 sour cherry plantation (I%: 59) as it is shown in Table 3. No high infection rates were measured in the examined Romanian apricot garden, but the presence of apricot phytoplasma disease was evident, and it was the first time that *Ca* Phytoplama prunorum had been identified in the western part of Romania.

In 2011, field investigations were done repeated in Bekecs area, and in Biharpüspöki. The infection data were higher on every examined plots compared to the data of the previous years. A new field investigation spot, a sour cherry plantation in Hajdúdorog (Hajdú-Bihar County) was also examined. According to warnings of local growers there were several suspicious sour cherry trees, but there were not found any phytoplasma infected trees on our field investigation.

According to the results of the field experiences and the data of infection in the last 3 years, we can conclude on the plant health conditions of stone fruit plantations on the examined areas are rather bad. It was confirmed on apricot, peach and sour cherry in Bekecs area by the results of field investigations this year (illustrated by the photos on Figure 1-3) Besides, the data of the latest field examinations show that phytoplasma infection rate on peach (Bekecs-Majos plantation) has been increased significantly, as we can see the date of 22 September investigation spot (I%: 19) and 27 September investigation spot (I%: 38) in the Table 3. The latest data of field examination in Biharpüspöki (Romania) shows that infection of phytoplasma disease on apricot also increased (illustrated by photo on Figure 4).

Fig. 1. Drying apricot trees in Bekecs
(Photo: G. Tarcali)

Fig. 2. Dryed leaves on apricot
(Photo: G. Tarcali)

Fig. 3. General yellowing on peach
in Bekecs
(Photo: G. Tarcali)

Fig. 4. Yellowing and rolling
leaves on apricot in Biharpüspöki,
(Photo: G. Tarcali)

Results of the laboratory examinations

As many as 44 plant samples were collected on the field areas, which were examined in laboratory by PCR in the last 4 years (2009-2012). The presence of "*Ca.* Phytoplasma prunorum" was detected on 24 examined samples (Table 4). Phytoplasma infection was detected from the collected plant samples on all examined stone fruit varieties (apricot, peach, cherry, sour cherry, wild plum) respectively. The results of phytoplasma identifications are shown on Figure 5 and 6. The presence of the pathogen without any typical symptoms in tolerant wild plum was also detected, and it is obvious that wild plum may have an important part in the spreading of the pathogen.

Plant samples were also collected and examined in 2012 on the examined plots (Bekecs - apricot, peach, sour cherry, Biharpüspöki – apricot).

Table 3

Phytoplasma infection data on the examined fields

Spots of the field examinations	Time	Tree kind	Age (year)	Area (ha)	Number of trees	Degree of infection					Ii	I%
						I	II	III	IV	V		
1.Bekecs	02.10.2009.	Apricot	4	20	100	98	1	1	-	-	1,03	2
2.Bekecs-Majos	02.10.2009.	Apricot	8-9	5	100	45	4	6	5	40	2,91	55
3.Bekecs-Téglaszín	02.10.2009.	Apricot	8	3	100	15	7	7	6	65	3,99	85
4.Bekecs	02.10.2009.	Apricot	12-13	10	100	30	6	4	35	25	3,21	70
5.Bekecs-Majos	02.10.2009.	Peach	8	6	100	79	7	2	2	10	1,57	21
6.Bekecs-Mélyárok	02.10.2009.	Cherry	10	22	100	70	9	4	6	11	1,79	30
7.Bekecs-Téglaszín	02.10.2009.	Sour cherry	8-9	5	100	38	14	10	8	30	2,78	62
8.Bekecs	02.10.2009.	Sour cherry	7	5	100	91	3	1	1	4	1,24	9
9.Bekecs-Mélyárok	02.10.2009.	Sour cherry	30	8	100	64	6	9	13	8	1,95	36
10.Bükkaranyos	07.09.2010.	Apricot	13	22,6	70	11	12	2	10	35	3,66	84
11.Bükkaranyos	07.09.2010.	Apricot	13	22,6	78	17	6	3	11	41	3,68	78
12.Bükkaranyos	07.09.2010.	Sour cherry	7	5	104	43	7	12	12	30	2,78	59
13.Bükkaranyos	07.09.2010.	Wild plum	13	-	20	20	-	-	-	-	-	-
14.Rátka	07.10.2010.	Apricot	21	50	100	41	10	9	11	28	2,72	59
15.Göncruszka	07.10.2010.	Apricot	4	5	54	34	4	4	3	9	2,06	37
16.Vizsoly	07.10.2010.	Apricot	~12	6	50	46	1	2	1	-	1,16	8
17.Boldogkőváralja	07.10.2010.	Apricot	~25	15	100	23	24	12	21	26	3,21	77
18.Abaújkér	07.10.2010.	Apricot	~15	10	50	45	3	1	1	-	1,16	10
19.Biharpüspöki(RO)	14.10.2010.	Apricot	25	6	100	97	2	1	-	-	1,04	3
20.Biharpüspöki(RO)	06.08.2011.	Apricot	26	6	100	87	6	3	3	1	1,25	13
21.Bekecs-Majos	05.06.2011.	Apricot	10-11	5	100	35	7	8	7	43	3,16	65
22.Bekecs-Majos	05.06.2011.	Peach	10	6	100	81	6	-	1	12	1,57	19
23.Bekecs-Mélyárok	05.06.2011.	Cherry	12	22	100	64	11	6	4	15	1,95	36
24.Bekecs-Mélyárok	05.06.2011.	Sour cherry	32	8	100	55	11	10	15	9	2,22	45
25.Hajdúdorog	25.05.2011.	Sour cherry	12	11	100	100	-	-	-	-	-	-
26.Bekecs-Majos	28.08.2012.	Apricot	11-12	5	100	29	8	9	8	46	3,34	71
27.Bekecs-Majos	28.08.2012.	Peach	11	6	100	62	8	8	7	15	2,05	38
28.Bekecs-Mélyárok	28.08.2012.	Sour cherry	33	8	100	63	5	11	13	8	1,98	37
29.Biharpüspöki(RO)	11.09.2012.	Apricot	27	6	100	81	8	5	2	4	1,40	19

Table 4

Rates of examined and DNA-isolated samples of different fruit trees and the results of phytoplasma detection in 2009-2012

Fruit tree species	Number of examined samples	Number of positive samples	Identified phytoplasma
apricot (*Prunus armeniaca*)	21	12	ESFY
peach (*Prunus persica*)	6	2	ESFY
cherry (*Prunus avium)*	2	2	ESFY
sour cherry (*Prunus cerasus*)	10	5	ESFY
wild plum (*Prunus cerasifera*)	1	1	ESFY
apricot (*Prunus armeniaca*) in Biharpüspöki, Romania	4	2	ESFY

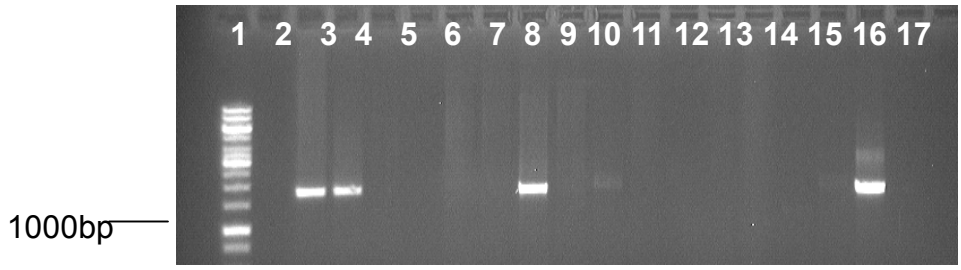

Fig. 5. DNA fragments amplified by P1/P7 primers in 1% agarose gel

1: DNA ladder; 2,5,6,8,10,12,13,14,15,16: negative samples; 18: positive ESFY control; 19: negative control; 3, 4, 7: apricot samples infected by phytoplasma; 9: infected wild plum sample; 11, 17: infected sour cherry and cherry samples

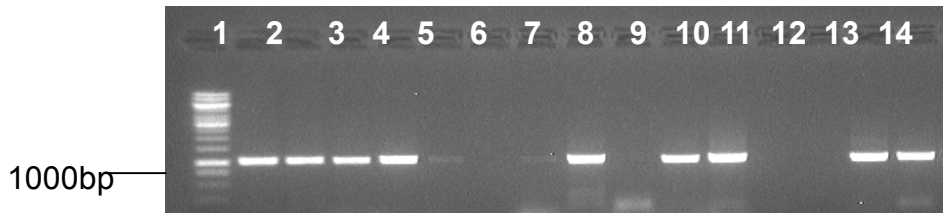

Fig. 6. DNA fragments amplified by FO1/rO1 group-specific primers in 1% agarose gel

1: DNA ladder; 2,9: direct PCR; 2, 3, 4: infected apricot samples; 5: infected wild plum sample; 6, 7, 8: negative sour cherry and peach samples; 9: positive ESFY control; 10-16: nested PCR: 10: negative control; 16: positive ESFY control; 11: infected apricot sample; 12, 15: infected sour cherry samples; 13,14: negative sour cherry and peach sample

CONCLUSIONS

The disease caused by *Ca.* Phytoplasma prunorum is continously increasing and as a relatively new problem for fruit growers in Hungary, it seriously threatens the most important Hungarian apricot cultivations, and other stone fruits areas. After the experiences of the field examinations it was confirmed by the laboratory results that *Ca.* Phytoplasma prunorum is a rather serious danger for stone fruits in Hungary and Central-Europe.

Our experiences resulting from our investigation show that we have to pay more attention to the increasing phytoplasma problem in stone fruits, and have to develop new and effective management strategies.

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