



HUNGARIAN UNIVERSITY OF AGRICULTURE AND
LIFE SCIENCES

The role of pathogens in apricot apoplexy

DOI: 10.54598/005250

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Budapest
2024

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1. RESEARCH BACKGROUND AND OBJECTIVES

The cultivation of apricot in Hungary has centuries-old traditions (Surányi, 2003). Over time, it has become a defining horticultural culture, undergoing gradual developments in cultivation technology (Pedryc, 2003). However, the cultivation and plant protection of apricot are burdened with numerous challenges, requiring a high level of expertise. Among these, apricot apoplexy stands out, which is by no means a new problem in our country, and is possibly as old as apricot cultivation itself (Nyujtó and Surányi, 1981; Szalay, 2003). Based on previous works, in Hungary, the apoplexy disease is mainly caused by *Pseudomonas syringae* van Hall, '*Candidatus* Phytoplasma prunorum' Seemüller & Schneider, and *Cytospora cincta* Sacc. pathogens (Klement, 1977a; Rozsnyay, 1977; Rozsnyay and Klement, 1977; Süle, 2014). The presence of these pathogens may remain latent for a while, but after the first symptoms are observed, partial or complete tree decay can occur in an extremely short time (Husz, 1941). The mentioned pathogens can also infect trees collectively. Due to the similarity of symptoms, in certain cases, identifying the responsible pathogen for the symptoms may be difficult or impossible through visual inspection (Rozsnyay and Klement, 1973; Rejlová *et al.*, 2021). Each of the mentioned pathogens can cause wilting and premature shedding of leaves, phloem necrosis, as well as partial or complete die-back. However, certain symptoms can only be attributed to specific pathogens (Klement, 1977a; Morvan, 1977; Rozsnyay, 1977; Biggs and Grove, 2005; Kennelly *et al.*, 2007; Lamichhane *et al.*, 2014; Žežlina *et al.*, 2016). For example, infections caused by *P. syringae* and *C. cincta* can lead to gumming cankers on woody parts (Klement, 1977a; Rozsnyay, 1977), while the characteristic symptom caused by '*Ca. Phytoplasma prunorum*' is leaf yellowing (Morvan, 1977; Necas *et al.*, 2015; Žežlina *et al.*, 2016).

Apoplexy has long been a challenge for experts in cultivation and plant protection. Understanding the significance and spread of pathogens, as well as examining integrated approaches to prevention and plant protection methods, is essential for maintaining the profitability of cultivation.

Our main objectives of the study are summarized as follows:

- Testing apricot trees showing symptoms of apoplexy and asymptomatic apricot trees for the main pathogens of the disease: '*Candidatus Phytoplasma prunorum*', *Pseudomonas syringae*, and *Cytospora* species;
- Understanding the relationship between the appearance of symptoms and infection in sampled trees, and the detected pathogens;
- Collection of potential psyllid vectors in apricot orchards and their immediate surroundings, species, gender identification, and detection of the '*Ca. Phytoplasma prunorum*' pathogen from individuals and their host plants;
- Collection of *Cytospora* isolates from designated orchards and their determination using molecular methods;
- Understanding the effects of *Trichoderma asperellum* 'T34' strain and contact-based fungicides on *C. sorbicola* species under *in vitro* conditions;
- Assessment of the susceptibility of apricot scion and rootstock to apoplexy in commercial orchards.

2. MATERIALS AND METHODS

Mapping the pathogens of apoplexy in apricot orchards

We collected 40 samples each from four apricot orchards (Érd, Pomáz, Soroksár, Sós-kút) over a period of three years (2014–2016): 20 samples from trees showing symptoms of apoplexy and 20 samples from asymptomatic trees in each orchard. We collected 2–3 year old branches from the trees for total nucleic acid (TNA) extraction, using the protocol by Daire *et al.* (1997) with some modifications. The detection of pathogens was performed using polymerase chain reaction (PCR) or nested-PCR. For *P. syringae*, we targeted the *syrB* (Sorensen, 1998) and *psy* genes (Guilbaud *et al.*, 2016), for *Cytospora* spp., we targeted the β -tubulin gene (Luo *et al.*, 2017), and for 'Ca. Phytoplasma prunorum', we targeted the ORF2 - putative nitroreductase gene - ORF3 region (Jarausch *et al.*, 1998; Mergenthaler, 2004). The relationship between symptom appearance and infection, as well as between symptom appearance and detected pathogens in the sampled trees, was analyzed using the Generalized Linear Mixed Model (GLMM) with binomial error correction in the R statistical program (R Core Team, 2023).

Studies on psyllids

Psyllids were collected using the beat tray method from March to June during the growing seasons of 2015 and 2016. Regular collections were conducted in the apricot orchard in Pomáz, with supplementary collections carried out in orchards in Soroksár and Sós-kút. The psyllid specimens were collected from apricot, apricot tree rootstock sucker, plum, blackthorn and hawthorn.

The identity species and sex of the collected psyllids were determined based on their morphological characteristics using a dichotomous key (Ossiannilsson, 1992; Burckhardt and Jarausch, 2007a, b). Subsequently, the genetic material of the psyllids was extracted using the Doyle and Doyle purification method (Doyle, 1990). The morphological identification of *Cacopsylla pruni* and *C. crataegi* was further confirmed using molecular methods (PCR-RFLP, Oetl and Schlink, 2015; Sanger sequencing, Sanger and Coulson, 1975). The biotype of *C. pruni* specimens was determined using PCR assays described by Peccoud *et al.* (2013) with primer sets labeled as 2 and 3.

The occurrence of *C. pruni* and *C. crataegi* specimens per plant species was compared using Z-tests, the 'Ca. Phytoplasma prunorum' infectivity of the two

species was tested using Fisher's exact test, and the ratios of phytoplasma-positive males and females within each psyllid species were also compared using Fisher's exact test with the R statistical program (R Core Team, 2023).

The 'Ca. *Phytoplasma prunorum*' infection was investigated in several plant species from which psyllids were collected. During this investigation, samples were taken from the rootstock suckers ('Myrobalan'), blackthorn (*Prunus spinosa*), and hawthorn (*Crataegus monogyna*).

Isolation and identification of *Cytospora* species, and investigations on plant protection against *C. sorbicola*

Isolation and identification of *Cytospora* species

In 2015 and 2016, we searched for symptomatic trees with pseudopycnidia characteristic of *Cytospora* species and cankers on branches and twigs in the orchards of Érd, Pomáz, Soroksár, and Sósút (Willison, 1936; Biggs and Grove, 2005; Fan *et al.*, 2015). The woody parts with pseudopycnidia were incubated in humidity chamber (at 10 °C, in the dark). Conidial mass emerging from the fruiting bodies was used to start cultures on malt extract agar (MEA) medium. In the case of cankers, tissue samples were taken from the border between diseased and healthy phloem tissues (living tissues), inoculated onto MEA medium, and pure cultures were established.

Based on previous studies (Willison, 1936; Surve-Iyer *et al.*, 1995; Lawrence *et al.*, 2018; Fan *et al.*, 2020), we selected cultures that exhibited morphological characteristics typical of *Cytospora* spp. For the molecular identification of the selected cultures, DNA extraction was performed using the CTAB method (Sambrook and Russell, 2001), followed by determination of the base sequences of the ITS2 region and β -tubulin gene amplified by PCR using Sanger sequencing (Sanger and Coulson, 1975). Subsequently, BLAST analysis was used to identify the sequences with the highest similarity to our obtained sequences in the GenBank database (NCBI, 2023).

Examination of the effect of fungicidal active agents against *Cytospora sorbicola*

For the test, four isolates of *C. sorbicola* were selected, and their susceptibility to fungicides was assessed by *in vitro* conditions using a poisoned agar plate method. The tested the active substances were authorised in apricot

orchards at the time the study was carried out and were among the most commonly applied contact fungicides: tribasic copper sulfate-„1” (Bordóilé Neo SC), copper + mancozeb (Cupertine M), tribasic copper sulfate-„2” (Cuproxtat FW), mancozeb (Dithane M 45), captan (Merpan 80 WDG), and copper hydroxide (Vitra rézhidroxid). For the two tribasic copper sulfate compounds, we aimed to assess the differences in effectiveness caused by different adjuvants. Three concentrations were tested during their application (calculated with a spray volume of 1000 l/ha): the authorised maximum application doses (100% concentration), half of the maximum application dose (50% concentration), and one-tenth of the maximum application dose (10% concentration). The control plates used for each isolate did not contain any fungicide. Ten repetitions were conducted for each isolate and treatment.

Competitive and parasitic ability of *Trichoderma asperellum* 'T34' for its against the pathogen *Cytospora sorbicola*

The mycoparasitic strain of *Trichoderma asperellum* 'T34' necessary for the study was isolated from the Trifender WP formulation. Two isolates of *C. sorbicola* were selected for the experiment. The biocontrol ability of *T. asperellum* 'T34' against the pathogen was examined using a confrontation test (Sivan and Chet, 1989). Two methods were employed for inoculating the mycoparasitic fungus onto MEA agar:

1. The hyperparasite was placed onto the agar simultaneously with the pathogen.
2. The hyperparasite was inoculated onto the agar on the second day after pathogen inoculation.

The experiment was conducted with 4 replicates per inoculation method and isolate, using 10 Petri dishes (85 mm) each. The growth inhibition ability of *Trichoderma* species against the pathogen was calculated using the following formula (El-Naggar *et al.*, 2008):

$$I = \frac{C - T}{C} \times 100$$

where:

I= Percentage of growth inhibition of *C. sorbicola*

C= growth of non-treated control *C. sorbicola* mycelium

T= growth of *C. sorbicola* in double culture.

Evaluation of the susceptibility to apoplexy of apricot cultivars and rootstocks

Between 2014 and 2017, the incidence of apoplexy in apricot trees was assessed in orchards located in Érd and Sósút every October. Fourteen cultivar-rootstock combinations were included in the survey (**Table 1.**).

Table 1. Rootstock-scion combinations tested in orchards and their quantity

| Orchard | Apricot cultivar | Rootstock type | | |
|---------|-----------------------|----------------|------------------------------------|------------------|
| | | Myrobalan (N) | plum inter-stem on 'Myrobalan' (N) | wild apricot (N) |
| Érd | 'Flavorcot' | x (140) | - | - |
| | 'Sweetcot' | x (140) | - | - |
| | 'Zebra' | x (125) | - | - |
| Sósút | 'Bergeron' | x (120) | x (65) | x (100) |
| | 'Gönci magyar kajszi' | x (120) | x (120) | x (120) |
| | 'Magyar kajszi C.235' | x (100) | - | - |
| | 'Mandulakajszi' | x (120) | x (120) | x (120) |
| | 'Tomcot' | x (120) | - | - |

Legend: N=number of trees

The observed symptoms on the trees were evaluated on a six-grade scale (0–5). Data were categorized and compared using three methods during the study: (1) by cultivar across different rootstock variants, (2) by rootstock variants irrespective of the cultivar, and (3) by rootstock variants across different cultivars. The datasets from the four years were summarized and analyzed considering the locations. Symptom appearance data in apricot trees were evaluated using two methods. In the first method, a symptom severity index was calculated from symptom categories using the Townsend-Heuberger formula (Gartner, 1971).

In the second method, the data were binarized, creating a symptom-free (SF; value: "0") and a symptomatic (S; value: "1") group. Marascuilo's test ($p < 0.05$) was used to compare the SF and S groups, examining the frequency of the disease.

The quantity of dead trees was compared to the number of trees assessed in 2014. The proportion of dead trees was compared using Marascuilo's test ($p < 0.05$).

Statistical analyses were conducted by the R environment (R Core Team, 2023).

3. RESULTS

Detection of apoplexy pathogens in apricot orchards and their role in symptom appearance

In the examined samples, 28.1% tested positive for '*Ca. Phytoplasma prunorum*', 17.5% for bacteria belonging to the *P. syringae* species complex, and 23.8% for *Cytospora* species. However, considering the types of mixed pathogen presence, the proportions of individual infections decreased (**Table 2.**). The presence of the pathogens tested differed between orchards in several cases.

Table 2. Percentages of apricot trees not confirmed for the presence of examined pathogens and apricot trees infected individually or in combination by examined pathogens

| Type of infection | | Detection rate (%) | Total Detection rate (%) | | 42,5 |
|--------------------------------------|----------------|--------------------|--------------------------|----|------|
| Individual infection | CPp | 15,6% | 37,5 | | |
| | Ps | 9,4 | | | |
| | Cyt | 12,5 | | | |
| Mixed infection with two pathogens | Ps + Cyt | 2,5 | 13,1 | 15 | |
| | CPp + Ps | 3,8 | | | |
| | CPp + Cyt | 6,9 | | | |
| Mixed infection with three pathogens | CPp + Ps + Cyt | 1,9 | 1,9 | | |

Legends: CPp: '*Ca. Phytoplasma prunorum*'; Ps: *Pseudomonas syringae*; Cyt: *Cytospora* sp.

The GLMM model revealed that the manifestation of symptoms is significantly influenced by infection. Among the trees where no pathogen was detected, there were more symptom-free ones than those showing symptoms. Conversely, among the trees with confirmed infections, there were more showing symptoms than symptom-free ones ($p=0.0006$).

Analyzing the relationship between symptom manifestation and detected pathogens, among the examined trees, from individual infections of '*Ca. Phytoplasma prunorum*' and from mixed infections of '*Ca. Phytoplasma prunorum*' + *Cytospora* sp. were most frequently confirmed. Based on symptom manifestation, more than half of the trees were symptom-free in cases where infections of *P. syringae* (66.7%) and *P. syringae* + *Cytospora* sp. (75%) were detected, or when no identifiable pathogen was found (63.2%). However, in the majority of other individual and mixed infections, trees predominantly exhibited

symptoms of apoplexy (GLMM model; $p=0.0004$). The distribution of symptomatic and symptom-free trees infected with the examined pathogens differed in several instances across the orchards.

Studies on psyllids

Morphological identification of *Cacopsylla* individuals and their distribution by plant species and gender

Based on morphological identification, the vast majority of collected psyllids belonged to the *Cacopsylla pruni* and *C. crataegi* species. Most *C. pruni* psyllids were collected from apricot, while most of the *C. crataegi* individuals originated from both apricot and hawthorn. The presence of *C. pruni* was significantly higher on apricot than that of *C. crataegi* (Z-test, $p=0.004$). The proportion of female *C. pruni* (Z-test, $p<0.001$) and *C. crataegi* (Z-test, $p<0.056$) collected from apricot was higher than that of males. More *C. crataegi* were collected from hawthorn compared to *C. pruni* (Z-test, $p<0.001$). Among the former species, there were more females than males (Z-test, $p<0.063$). Only a few psyllid individuals were collected from the other investigated plant species.

Detection of 'Candidatus Phytoplasma prunorum' in *Cacopsylla* psyllids

Among all collected *C. pruni* psyllids from the surveyed plants, 6.6% were infected by the 'Ca. Phytoplasma prunorum' pathogen, while 2.6% of *C. crataegi* individuals were infected. There was no significant difference in the quantity of infected females and males within each species (Fisher's exact test, $p_{C. pruni}=0.47$; $p_{C. crataegi}=0.69$). The prevalence of phytoplasma infection was higher among *C. pruni* individuals compared to those of *C. crataegi*, although we did not identify a significant difference between the infestation rates of the two species (Fisher's exact test, $p=0.06$).

Molecular identification of *Cacopsylla* psyllids

The species identification of psyllids morphologically classified as *C. pruni* and *C. crataegi*, which were infected with 'Ca. Phytoplasma prunorum', was confirmed by molecular analysis. Based on biotype analysis, only the 'B' biotype was present among the collected *C. pruni* individuals.

Infection of *Cacopsylla* host plants with 'Candidatus Phytoplasma prunorum'

Among the surveyed host plants, 28.6% of rootstock suckers of apricot trees, 26.7% of blackthorns, and 27.3% of hawthorn were infected with the 'Ca. Phytoplasma prunorum' pathogen.

Identification of *Cytospora* isolates and plant protection against *C. sorbicola*

Identification of *Cytospora* isolates

Based on the ITS2 region, the species of *Cytospora* isolates obtained from apricot trees could not be accurately determined. However, analysis of the β -tubulin gene sequences revealed that among the isolates, 4 were identified as *C. cincta*, 14 as *C. leucostoma*, and 9 as *C. sorbicola*.

Effect of fungicidal substance agents against *Cytospora sorbicola* under *in vitro* conditions

At authorised application dose levels of the fungicides tested, there was no mycelial growth of isolates, except for the active substance mancozeb. However, at half and one-tenth of the application dose level, several isolates started to exhibit growth, except for captan (**Table 3**).

Table 3. The number of *Cytospora sorbicola* isolates that grew with the active substances used in the experiment

| Active agents of fungicide | Number of <i>Cytospora sorbicola</i> with mycelial growth | | |
|-----------------------------|---|--|---|
| | Authorised application dose | Authorised application dose diluted half | Authorised application dose diluted tenfold |
| captan | 0 | 0 | 0 |
| tribasic copper sulfate-„1” | 0 | 1 | 4 |
| tribasic copper sulfate-„2” | 0 | 0 | 2 |
| copper + mancozeb | 0 | 1 | 4 |
| copper hydroxide | 0 | 1 | 4 |
| mancozeb | 1 | 1 | 1 |

Space competition and parasitization ability of *Trichoderma asperellum* 'T34' against *Cytospora sorbicola* under *in vitro* conditions

The inhibitory effect of *T. asperellum* 'T34' on the growth of *C. sorbicola* was between 20% and 44.9% in the case of simultaneous inoculation and between 0.9% and 24.8% in the delayed method. In both inoculation methods,

Trichoderma inhibited sporulation of the pathogen. Furthermore, the hyperparasite completely covered the colonies of the pathogen and then sporulated on them. Microscopic examination of the contact zone between the mycoparasite and the pathogen colonies revealed that *T. asperellum* 'T34' was capable of parasitizing the hyphae of *C. sorbicola*: the hyphae of *T. asperellum* coiling around and penetrated the fungal filaments of the pathogen.

Evaluation of apricot cultivars and rootstock variants regarding susceptibility to apoplexy

On the trees we surveyed, we often observed '*Ca. Phytoplasma prunorum*', *Cytospora* spp., and *P. syringae* pathogens.

Assessment of apricot cultivar susceptibility

Érd orchard

From the three cultivars tested, the 'Zebra' showed the lowest disease frequency (Marascuilo test, $p < 0.05$), as well as the lowest symptom severity index and tree mortality (Marascuilo test, $p < 0.05$).

Sóskút orchard

Comparison of disease incidence among the apricot cultivars on 'Myrobalan', significantly lower disease frequency was identified in the 'Bergeron' cultivar compared to 'Gönci magyar kajszi', 'Magyarkajszi C.235', and 'Tomcot' cultivars. However, no differences were identified between 'Bergeron' and 'Mandulakajszi' (Marascuilo test, $p < 0.05$). The 'Mandulakajszi' cultivar exhibited the lowest symptom severity index and tree mortality (Marascuilo test, $p < 0.05$).

No significant difference was identified in disease frequency among the apricot cultivars on plum inter-stem on 'Myrabolan' rootstock (Marascuilo test, $p < 0.05$). The 'Mandulakajszi' cultivar had the lowest symptom severity index. The 'Bergeron' and 'Mandulakajszi' had significantly lower tree mortality compared to the 'Gönci magyar kajszi' cultivar (Marascuilo test, $p < 0.05$).

Between the apricot cultivars on wild apricot rootstock, the 'Bergeron' cultivar had the lowest disease frequency, while the 'Mandulakajszi' cultivar had the lowest tree mortality (Marascuilo test, $p < 0.05$). The 'Bergeron' and

'Mandulakajsi' cultivars showed similar symptom severity indexes, which were significantly lower than that of the 'Gönci magyar kajszi' cultivar.

Evaluation of rootstock variants' susceptibility regardless of apricot cultivars

The comparison among 'Myrobalan', plum inter-stem on 'Myrobalan', and wild apricot rootstocks did not show significant differences in disease frequencies (Marascuilo test, $p < 0.05$). The highest symptom severity index was identified in the wild apricot, which was 5% higher than that of the two 'Myrobalan' rootstock variants. The plum inter-stem on 'Myrobalan' rootstock had significantly lower tree mortality than the other two rootstock variants (Marascuilo test, $p < 0.05$).

Evaluation of rootstock variants' susceptibility for each apricot cultivar

For the 'Myrobalan' and wild apricot 'Bergeron' trees, disease frequency (Marascuilo test, $p < 0.05$) and symptom severity index were lower than those for the plum inter-stem on 'Myrobalan' rootstock. However, the latter rootstock type had the lowest tree mortality (Marascuilo test, $p < 0.05$).

The disease frequency of the 'Gönci magyar kajszi' on two 'Myrobalan' rootstock variants was significantly lower than that on wild apricot rootstock (Marascuilo test, $p < 0.05$). The plum inter-stem on 'Myrobalan' rootstock type had the lowest symptom severity index and tree mortality (Marascuilo test, $p < 0.05$).

When combined with three rootstock variants, the disease frequency of the 'Mandulakajsi' cultivar did not differ, and the symptom severity index of the two 'Myrobalan' rootstock types was nearly identical, both of which were slightly lower than that of the wild apricot rootstock. The plum inter-stem on 'Myrobalan' rootstock had the lowest tree mortality rate.

4. CONCLUSIONS AND PROPOSALS

The significance and impact of apoplexy pathogens on the appearance of symptoms and the disease in apricot trees

The frequency of the detected pathogens '*Ca. Phytoplasma prunorum*', *Cytospora* spp., and *P. syringae* corresponded to several previous studies (Scortichini, 2006; Pokharel and Larsen, 2009a,b; Ami *et al.*, 2016; Yildiz *et al.*, 2016; Ivić *et al.*, 2017). The number of infected trees significantly increased (from 9-16% to 52.5%) when we applied a combined evaluation instead of independent monitoring of the three examined pathogens. Of the infected trees, 28.6% contained more than one pathogen. These results highlight the need to consider apricot apoplexy in a complex manner, taking into account multiple pathogens. Although our findings suggest that the presence of pathogens may significantly vary in apricot trees.

The quantity of identified pathogens varied from orchard to orchard in some cases, leading us to conclude that the presence and spread of pathogens may also be influenced by the environmental and cultivation conditions of the orchards. This conclusion is supported by observations of varying pathogen presence and symptom incidence rates in other studies (Pokharel, 2013; Nečas *et al.*, 2015; Ami *et al.*, 2016; Ivić *et al.*, 2017; Riedle-Bauer *et al.*, 2019; Doolotkeldieva and Bobusheva, 2020).

The investigation into the relationship between symptom appearance and infection revealed that in most cases, infection can be recognized based on symptoms alone. However, determining the responsible pathogen(s) based on visual inspection is not always feasible because certain apoplexy symptoms can be characterized by the presence of multiple pathogens (Klement, 1977a; Morvan, 1977; Rozsnyay, 1977; Biggs and Grove, 2005; Kennelly *et al.*, 2007; Lamichhane *et al.*, 2014; Žežlina *et al.*, 2016). Interestingly, certain individual and mixed (*P. syringae*; *P. syringae* + *Cytospora* sp.) infections result in less pronounced symptoms in trees. Therefore, supplementing symptom observations with sensitive molecular pathogen detection methods is advisable, as it enables a more accurate diagnosis of apoplexy pathogens.

Identification of collected individuals of *Cacopsylla pruni* and *C. crataegi*

Based on morphological examination, the majority of collected psyllids belonged to the species *Cacopsylla pruni* and *C. crataegi*. Biotype of *C. pruni* individuals confirmed the results of morphological identification, as the PCR-based method applied was specific to *C. pruni* (Peccoud *et al.*, 2013). Among the examined individuals, only biotype ‘B’ was identified, similar to previous studies by Viczián *et al.* (2017) and Lepres *et al.* (2018). According to the analysis, *C. pruni* individuals can be classified into cluster “2”. In the examination of *C. crataegi* individuals carrying the pathogen, digestion with the *AluI* enzyme resulted different fragment lengths from the expected. The analysis of the sequences confirmed that there is one more restriction site in the examined region compared to previous studies (Oettl and Schlink, 2015). The phylogenetic analysis of *C. crataegi* separated our samples and the Italian samples into distinct clades, but the two groups shared a common ancestor. Thus, it is conceivable that within *C. crataegi*, like *C. pruni*, there may be several variants, possibly biotypes.

Distribution and infection of collected *Cacopsylla pruni* and *C. crataegi* individuals and infection of their host plants

Examining the vector role of *C. pruni*, we found that 6.6% of individuals were infected by the ‘*Ca. Phytoplasma prunorum*’ pathogen, which was close to the proportions obtained in several previous studies (0.8–4.8%) (Jarausch *et al.*, 2008; Etropolska *et al.*, 2015; Warabieda *et al.*, 2018; Jarausch *et al.*, 2019; Marie-Jeanne *et al.*, 2020). This suggests, *C. pruni* played a significant role in the spread of phytoplasma in our study sites. We found that pathogen transmission was independent of sex, similar to previous results by Ermacora *et al.* (2011) and Peccoud *et al.* (2013).

C. pruni was collected in large numbers from apricot, similar to studies of Jarausch *et al.* (2008) and Lepres *et al.* (2018). In contrast, other studies collected few or no individuals from apricot (Jarausch *et al.*, 2001; Labonne and Lichou, 2004; Viczián *et al.*, 2017). Based on these contradictions, it would be worthwhile to identify the role of apricot in the life cycle of plum psyllids.

C. crataegi was also collected in large numbers from apricot, similar to the results of Dér (2005). An important question is whether apricot is host or food plant for hawthorn psyllids?

The 2.6% of the collected *C. crataegi* individuals carried the 'Ca. Phytoplasma prunorum' pathogen independent of sex. No previous data on phytoplasma infection of this species were found in the national or international literature. We would like to emphasize that no significant differences were identified between the 'Ca. Phytoplasma prunorum' infection rates of *C. crataegi* and *C. pruni*. Based on these results, we consider it important to perform a 'Ca. Phytoplasma prunorum' pathogen transmission experiment with *C. crataegi*.

It is also interesting that approximately one-third of the *C. monogyna* shrubs included in the collections and testing showed phytoplasma infection confirmed by molecular testing. On the basis of the literature reviewed, it seems that the monocotyledonous hawthorn serves as a new host plant and reservoir for the 'Ca. Phytoplasma prunorum' pathogen. Furthermore, *C. crataegi* individuals infected by 'Ca. Phytoplasma prunorum' have been collected from hawthorns. It is possible that we have discovered a new mode of persistence and spread of the pathogen. In order to prove this, the previously mentioned phytoplasma transmission study with *C. crataegi* species has to be performed.

Importance of *Cytospora* species in apoplexy

We found that *Cytospora* species continue to play an important role in apricot apoplexy, consistent with earlier observations by Klement (1977b) and Rozsnyay (1977). Among the *Cytospora* species isolated, *C. cincta* (15%), *C. leucostoma* (52%), and *C. sorbicola* (33%) were identified. The presence of *C. sorbicola* in Hungary was detected for the first time in our study. Based on artificial inoculation experiments, *C. sorbicola* was found to be pathogenic and virulent to apricot. The pathogen induces significant necrosis in the bark tissue, phloem, and xylem. These results suggest that, *C. sorbicola* is a primary pathogen of apricot.

In vitro* efficacy of fungicides against the pathogen *Cytospora sorbicola

In our *in vitro* experiment, authorised application doses of captan, copper hydroxide, copper + mancozeb, and tribasic copper sulphate were capable of completely inhibiting the growth of the mycelium. Therefore, it is worth including these active ingredients in *in vivo* experiments, except for those containing mancozeb, as their application permits have since been withdrawn. Captan was also able to inhibit the growth of pathogen isolates at reduced doses, suggesting that it may be worthwhile to conduct *in vivo* studies with reduced-dose treatments

using this active ingredient for the development of environmentally friendly technologies. At all doses of mancozeb, one isolate showed growth. Collina *et al.* (2006) also found mancozeb to be ineffective against *C. vitis* under *in vitro* conditions. However, the use of mixed active ingredient formulations (copper + mancozeb) has proven to be fungicidal, highlighting the significant role of combined active ingredient formulations and fungicide rotation in plant protection treatments.

During the application of reduced active ingredient doses (except for captan), several isolates have been shown to develop. Thus, when spraying with these products, particular attention should be paid to their application at the correct dose.

Space competition and parasitization ability of *Trichoderma asperellum* ‘T34’ against *Cytospora sorbicola* under *in vitro* conditions

The results indicate that *Trichoderma asperellum* ‘T34’ has significant space-competitive capability and parasitic ability potential against *Cytospora sorbicola* under *in vitro* conditions. The hyperparasite limited the growth and sporulation of the pathogen cultures in both simultaneous and delayed inoculations, moreover covered them and sporulated intensively on them. Furthermore, we observed that the hyphae of *T. asperellum* coiling around and penetrate the hyphae of the pathogen. Based on promising laboratory results, the mycoparasite can be included in *in vivo* experiments. These experiments, based on the results obtained from simultaneous and delayed inoculations of the mycoparasite, could be preventive and curative in nature (e.g., protection of pruning wounds, treatment of cancerous wounds).

Evaluation of susceptibility to apoplexy of apricot cultivars and rootstock varieties

Our studies suggest that apricot cultivars have different susceptibilities to apoplexy, which is consistent with the results of previous studies on variety susceptibility conducted with ‘*Ca. Phytoplasma prunorum*’, *P. syringae*, and *Cytospora* spp. pathogens (Audergon *et al.*, 1991; Brun *et al.*, 2011; Gormez *et al.*, 2013; Yilmaz and Erincik, 2017; Nečas *et al.*, 2018; Moale and Septar, 2019). In our study, the ‘Zebra’ showed outstanding resistance to symptom appearance and tree mortality, furthermore the ‘Mandulakajsz’ grafted onto ‘Myrobalan’,

plum inter-stem on 'Myrobalan' and wild apricot rootstocks had significant tolerance.

The rootstock variants did not show any difference in disease frequency. Furthermore, both 'Myrobalan' and plum inter-stem on 'Myrobalan' only slightly reduced the severity of symptoms compared to wild apricot. However, significant differences were identified when comparing rootstocks according to the scion cultivar: different rootstock variants reduced disease incidence and symptom severity depending on the apricot cultivar. From this, we concluded that the sensitivity of trees to apoplexy is determined by the interaction of the scion and rootstock. Thus, the selecting the appropriate rootstock-scion combination, can significantly reduce symptoms. Among the rootstocks, the plum inter-stem on 'Myrobalan' significantly reduced the rate of tree mortality, which was also observed in the studies conducted with both rootstock and cultivar. These results are consistent with previous studies that demonstrated the tolerance of plum rootstocks to '*Ca. Phytoplasma prunorum*', *Pseudomonas*, and *Cytospora* species (Nyujtó and Tomcsányi, 1959; Rozsnyay, 1963; Nyujtó and Surányi, 1981; Prunier *et al.*, 1999; Nagy and Lantos, 1998; Kison and Seemüller, 2001). Therefore, we consider it worthwhile to evaluate the effect of plum inter-stem on 'Myrobalan' rootstock on other scion cultivars as well.

NEW SCIENTIFIC FINDINGS

1. After the monitoring of the main pathogens causing apoplexy in apricot orchards around Budapest, we found that among their individual infections, '*Candidatus Phytoplasma prunorum*' was the most common, while among mixed occurrences, '*Ca. Phytoplasma prunorum*' and *Cytospora* sp. were predominant.
2. We first confirmed the presence of the '*Ca. Phytoplasma prunorum*' pathogen in *Cacopsylla crataegi* psyllid individuals and found that both males and females carry the pathogen.
3. We described *Crataegus monogyna* as a new host plant for the '*Ca. Phytoplasma prunorum*' pathogen.
4. We identified the *Cytospora sorbicola* pathogen for the first time in Hungary.
5. We found the captan, copper hydroxide, copper + mancozeb, and tribasic copper sulfate contact fungicides to be effective against *C. sorbicola* *in vitro*.
6. *Trichoderma asperellum* 'T34' strain effectively parasitizes the *C. sorbicola* pathogen under *in vitro* conditions.
7. The combination of apricot tree cultivar and rootstock type collectively influences susceptibility to apoplexy pathogens. We identified the 'Zebra'- 'Myrobalan', 'Mandulakajsi'- 'Myrobalan', 'Mandulakajsi' plum interstem on 'Myrobalan', and 'Mandulakajsi'-wild apricot cultivar-rootstock combinations as tolerant.

5. LITERATURE CITED

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Elektronikus hivatkozások

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6. PUBLICATIONS RELATED TO THE THESIS

1. Publication in impact factor journal

Koncz, L. S., Petrőczy, M., Péntes, B., Ladányi, M., Palkovics, L., Gyócsi, P., Nagy, G., Ágoston, J., Fail, J. (2023). Detection of '*Candidatus* Phytoplasma prunorum' in Apricot Trees and its Associated Psyllid Samples. *Agronomy*, 13(1), 199. IF érték: 3,7

2. Publications in peer-reviewed journals

Koncz. L. S. Kiss A., Ladányi, M., Petrőczy, M.; Palkovics, L., Nagy, G. (2021). Algartartalmú növénykondicionálók közvetett hatása a *Cytospora leucostoma* kórokozóra. *Növényvédelem*, 82(7), 287-296.

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3. Conference summaries

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Fórum, 2023. 10. 24-26. Debrecen.