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Influence of '*Candidatus* Phytoplasma prunorum' on primary and secondary metabolites of apricots

*Gabriela Trempetić[1], Eliška Zezulová[1], Tomáš Nečas[1], Petr Šnurkovič[2], Tomáš Kiss[1]**

[1]*Department of Fruit Science, Faculty of Horticulture, Mendel University in Brno, Brno, Czech Republic*
[2]*Department of Post-Harvest Technology of Horticultural Products, Faculty of Horticulture, Mendel University in Brno, Brno, Czech Republic*

**Corresponding author: tomas.kiss@mendelu.cz*

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Abstract: '*Candidatus* Phytoplasma prunorum' ('*Ca*. P. prunorum') is a causative agent of European stone fruit yellows (ESFY), an economically important decline disease of some stone fruit species (*Prunus* spp.). The present research focused on the influence of '*Ca*. P. prunorum' on primary and secondary metabolites in four apricot genotypes consisting of older trees (genotypes Nora, LEM 159, group 1) and younger trees (genotypes H 74 and H 177, group 2). The content of sugars (glucose, sucrose, fructose and sorbitol), total phenolic content (TPC), total flavonoid content (TFC), antioxidant capacity (AC), total soluble proteins (TSPC), mineral ions: potassium (K^+), sodium (Na^+), magnesium (Mg^{2+}) and calcium (Ca^{2+}), pigments: chlorophyll *a*, *b* and carotenoids, and indolic compounds content (ICC) were analysed in two time periods during the year in the leaves of tested trees. The results revealed that the presence of phytoplasma/ESFY symptoms significantly decreased the content of pigments in both groups during the summer sampling period. ESFY caused a decrease of TPC, TFC and ICC in the H 177 genotype. The phytoplasma decreased the TSPC and K^+ content in older trees during both sampling periods. The only increase caused by phytoplasma infection was observed in glucose content, but only in the group of older plants. The results of this study support the idea that '*Ca*. P. prunorum' affects metabolites in plants' defence system and manipulates basic metabolic processes during successful infection.

Keywords: plant metabolism; plant-pathogen interactions; *Prunus*; European stone fruit yellows

Phytoplasmas are wall-less prokaryotic plant pathogens in the class Mollicutes associated with several economically important diseases (Bertaccini & Lee 2018). Knowledge about phytoplasmas is scarce since they cannot be cultured *in vitro*, but with the use of appropriate bioinformatic analysis, molecular biology approaches, as well as indirect approaches, insights into the life of phytoplasmas are being revealed (Dermastia 2019; Gallinger et al. 2021; Hemmati 2021).

Phytoplasmas are obligatory parasites which inhabit the plant phloem and are vectored by phloem-feeding insects belonging to several Hemiptera families. Since they live in host-created nutrient-

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rich environments, they lack many important metabolic genes essential for free-living cells (Namba 2019). To achieve favourable conditions for infection, phytoplasmas are thought to modulate host plant cell metabolism (Prezelj et al. 2016; Dermastia et al. 2023).

Phytoplasmas induce various symptoms, such as phloem necrosis and decline, stunting, yellowing, witches' broom, phyllody, floral virescence, abnormal proliferation, and purple top (Namba 2019). Phytoplasma disease symptoms typically reflect the altered nutritional status of plants (Rossi et al. 2010). The early leaf yellowing or chlorosis, one of the most common symptoms of phytoplasma infection, is caused by chlorophyll degradation (Mittelberger et al. 2017). Several studies reported changes in mineral elements, such as increased levels of sodium, magnesium and potassium and a decrease in calcium in phytoplasma-infected plants (Al-Ghaithi et al. 2016; Raesi & Golmohammadi 2020) as well as increased carbohydrate and starch accumulation in infected leaves (Bertamini et al. 2002b; Giorno et al. 2013). Phytoplasmas inhibit hormone pathways, which leads to the expression of symptoms such as proliferation and witches' broom (Pertot et al. 1998).

It has been discovered that phytoplasmas can modify biochemical responses involved in defence mechanisms. Significantly higher total phenolic and flavonoid content in infected compared to healthy plants has been reported in different phytoplasma-plant pathosystems (Zafari et al. 2012; Negro et al. 2020). The accumulation of pathogenesis-related proteins (PR-proteins) contributes to the increase of total proteins in phytoplasma-infected tissues (Agrios 2005), and resistant hybrids accumulate higher protein content while extremely susceptible ones show a decrease in total soluble protein content (Musetti et al. 2007).

European stone fruit yellows (ESFY) is the common name of the decline disease of stone fruit species (*Prunus* spp.) caused by a bacterial organism '*Candidatus* Phytoplasma prunorum' ('*Ca*. P. prunorum') formerly known as the ESFY phytoplasma (Marcone et al. 2010). ESFY is the limiting factor in the production in several major stone-fruit-growing areas of Europe, where it causes considerable economic losses due to the high mortality of infected trees (Steffek et al. 2012; Nečas et al. 2015).

To date, no study has focused on the effect of '*Ca*. P. prunorum' on primary and secondary metabolites of apricot trees. Therefore, in this work, a trial of '*Ca.* P. prunorum' infected and healthy trees of different apricot genotypes in insect proof conditions was set up. Two groups of plants were studied, one consisting of older plants with already established infections and the other of young plants with newly established infections. The effect of '*Ca*. P. prunorum' on the content of primary and secondary metabolites (sugars: glucose, sucrose, fructose and sorbitol, TPC, TFC and AC, TSPC, mineral ions: K^+, Na^+, Mg^{2+} and Ca^{2+}, pigments: chlorophyll *a*, chlorophyll *b* and carotenoids, and ICC) was analysed throughout the year.

MATERIAL AND METHODS

Site and plant material. All tests were performed at the premises of the Faculty of Horticulture in Lednice, Mendel University in Brno. The area is located in the Lednice in the South Moravian region (Czech Republic) at an elevation of 176 m a.s.l. in a location with an average annual temperature of 9.7 °C.

The first group of three- to four-year-old plants (older plants, group 1) consisted of two apricot genotypes (Nora, LEM 159) which were prepared by chip-budding (1 bud per rootstock) from the phytoplasma-positive mother plants on GF 305 rootstocks (Nora in August 2020, LEM 159 in August 2019) and grown under insect-proof netting in pots in a 1:1 mixture of topsoil and garden peat (Sobeslav, CZ). From LEM 159 5 plants and from Nora 7 plants were used in the study.

The second group of plants (younger plants, group 2) consisted of one-year-old plants of two apricot genotypes (H 74, H 177), which were prepared by chip-budding (1 bud per rootstock) from the phytoplasma-positive mother plants (H 74 and H 177) on myrobalan (*Prunus cerasifera* Ehrh.) rootstocks in a nursery in August 2022. In the spring of 2023, the budded rootstocks were planted in the pots with a 3:4 mixture of topsoil and substrate (TS 3, Klassmann, Germany) and placed under insect-proof netting. From H 74 10 plants, and from H 177 14 plants were used in the study

The group 1 plants were irrigated by drip irrigation, and sprinklers irrigated the group 2 plants. Both groups were fertilised with Yara Mila Complex (Yara, Norway) according to the manufacturer's instructions.

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To distinguish the variants within plant groups (phytoplasma positive/negative or ESFY symptomatic/asymptomatic), the '*Ca*. P. prunorum' presence was investigated in DNA extracted from tested plants by real-time PCR as described in Kiss et al. (2024) two times (March and October) in 2023 (Table 1), and the appearance of leafroll and chlorosis, typical symptoms of ESFY, were recorded in the beginning of October 2023 (Table 1).

Sampling. The plant samples, consisting of approximately nine fully developed leaves per plant, were collected twice (in June and at the beginning of September) during the 2023 vegetation season (Table 1). The leaves were collected from the whole length of the annual shoot to make a representative sample. The samples were frozen at –80 °C and lyophilised at 20–50 Pa for 24 h. They were stored at –80 °C to preserve dryness and further used to determine analysed metabolites described below.

The analysis of pigment content: chlorophyll *a*, chlorophyll *b* and carotenoids. Pigments were determined using the spectrophotometric method according to Lichtenthaler et al. (1987), where 0.1 g of plant sample was ground using mortar and pestle, then mixed with 90% methanol and filtered using filter paper immediately to prevent pigment degradation. Extracts were transferred to 25 mL volumetric flasks and filled with 90% methanol until the mark. The absorbance was measured immediately after the extraction at 665.2, 652.4 and 470 nm using a Specord 50 Plus spectrophotometer (Analytik Jena, Germany). Each biological replicate was measured three times and then averaged. The pigment content was calculated according to the formulas:

$$C_a\ (\mu g/mL) = 16.82 \times A665.2 - 9.28 \times A652.4$$

$$C_b\ (\mu g/mL) = 36.92 \times A652.4 - 16.54 \times A665.2$$

$$C_{(x+c)}\ (\mu g/mL) = (1\,000 \times A470 - 1.91 \times C_a - 95.15 \times C_b)/225$$

Where: C_a – the concentration of chlorophyll *a*; C_b – the concentration of chlorophyll *b*; $C_{(x+c)}$ – the concentration of carotenoids; the results were expressed as milligrams of each pigment per gram of dry sample (mg/g DW)

Determination of total phenolic content, total flavonoids content and antioxidant capacity. The analysis of total phenolic content (TPC), total flavonoids content (TFC) and antioxidant capacity (AC) from the methanolic extracts prepared from 0.2 g of sample in 50 mL of 75% methanol was performed according to the methods described in Mrázová et al. (2021) using a Specord 50 Plus spectrophotometer (Analytik Jena, Germany). Each biological replicate was measured three times and then averaged. The results of TPC, TFC and AC were expressed as milligrams of gallic acid equivalent (GAE) per gram of dry sample (mg GAE/g DW), milligrams of catechin equivalent (CE) per gram of dry sample (mg CE/g DW), and milligrams of Trolox equivalent (TE) per gram of dry sample (mg TE/g DW), respectively.

Determination of sugar content. Sugar extracts were prepared using extraction method according to Zhu et al. (2018) with modifications. Plant samples weighing 0.2 g were ground using mortar and pestle and transferred into plastic vessels. To each sample, 8 mL of distilled water was added immediately, and the vessels were sonicated for 15 min using a TH-30 A sonicator (VEVOR, China) and centrifuged at 4 500 × g for 3 min in a Micro Star 17 centrifuge (VWR, USA). The supernatant was poured into a 25 mL volumetric flask, and this process was repeated three times with the same sample. The supernatant was adjusted with distilled water until the mark. The extract was filtered using 0.22 µm pore filters (Chromservis, Czech Republic) and stored at –20 °C in a freezer until the analysis.

Samples were subjected to high-performance liquid chromatography analysis (HPLC) using ECP 2000 (Ecom, Czech Republic) with an RI detector. Samples were run in a column (Polymer IEX Ca_SN8422, 250 × 8 mm, Watrex, Czech Republic) using an H_2O mobile phase with a flow rate of 0.5 mL/min and a column temperature of 80 °C

Table 1. Procedures performed on all plants during the test year 2023

Plant group	Apricot genotype	Schedule (month/year)			
		03/2023	06/2023	09/2023	10/2023
GROUP 1	Nora	Phytoplasma detection (real-time PCR)[a]	Sample collection for analysis of metabolites	Sample collection for analysis of metabolites	Phytoplasma detection (real-time PCR) and symptomatology[b]
	LEM 159				
GROUP 2	H 74				
	H 177				

[a]real-time PCR as described in Kiss et al. (2024); [b]presence of the typical ESFY symptoms (leafroll and chlorosis)

https://doi.org/10.17221/128/2024-PPS

and a pressure of 1.9 MPa. Each biological replicate was measured once, and the results were expressed as milligrams per gram of a dry sample (mg/g DW). Individual sugar results were quantified using external standards for sorbitol, fructose, glucose and sucrose (Sigma-Aldrich, USA).

Determination of cation content. Extracts for the cation analysis were obtained following the protocol by Blatny et al. (1997) with modifications. Extracts were prepared from 0.1 g of ground sample mixed with 10 mL of 0.1 M HCl in a plastic vessel. After sonication for 30 min, the samples were centrifuged at 4 500 × g at room temperature for 3 min. The supernatant was poured into a 25 mL volumetric flask and filled with 0.1 M HCl until the mark. The samples were filtered with a 0.45 μm pore-sized filter and were adjusted to a pH higher than 5 using 1 M Tris.

Potassium cation (K^+), sodium cation (Na^+) and calcium cation (Ca^{2+}) content were analysed by capillary isotachophoresis using IONOSEP 2003 (RECMAN, Czech Republic). As the leading electrolyte, the solution of 5 mM H_2SO_4 and 0.05% hydroxyethyl cellulose (HEC) was used, and as the terminating electrolyte, the solution of 5 mM CH_3COOH and 5 mM Tris. The concentrations were analysed according to the standard solution ($KCl+NaCl+CaCl_2+MgSO_4$). Each biological replicate was measured three times and then averaged. The final concentrations were recalculated into mg per 100 g dry sample (mg/100 g DW).

Determination of total soluble protein content. The sample preparation and analysis of total soluble protein content (TSPC) was performed according to a method by Deans et al. (2018), using the Pierce Bradford Protein Assay Kit (Thermo Fisher Scientific, USA). Protein assay reactions were prepared in triplicates in 96 well plates. The standards were prepared from six different concentrations of bovine serum albumin and one blank with 0.1 M NaOH. Each reaction consisted of 5 μL of sample or standard and 250 μL of Coomassie reagent. Reactions were left to equilibrate to room temperature for 10 min, then shaken for 30 sec, and the absorbance was measured at 595 nm using a PowerWave XS microplate reader (BioTek, USA). Each biological replicate was measured three times and then averaged. The results were expressed as grams of soluble protein per 100 g dry sample (g/100 g DW).

Determination of indolic compound content. Indolic compound content analysis was performed according to the methods of Gang et al. (2019) using the Salkowski reagent. The indolic compound content (ICC) was determined from 75% methanolic extract obtained from maceration of 0.1 g of plant sample overnight. The extracts were filtered using filter paper into a 25 mL volumetric flask and filled with methanol until the mark. After the 30 min reaction with the Salkowski reagent in the dark, the absorbance was measured at 530 nm. Each biological replicate was measured three times and then averaged. The concentrations were determined according to the standard curve with known concentrations of an auxin, the inole-3-acetic acid (IAA, Duchefa Biochemie, Netherlands). The results were expressed as mg of IAA per g of dried sample (mg IAA/g DW).

Statistical analysis. The average values were calculated using Microsoft Excel software, and the data were statistically analysed using Microsoft Excel and Statistica software ver. 14 (TIBCO Software Inc., USA). Only variants with at least two data points were used for statistical evaluation.

In the case of Ca^{2+}, the data were not normally distributed; therefore, a nonparametric Kruskal-Wallis test with subsequent multiple comparisons of mean ranks was used to analyse differences ($P < 0.05$) between the variants, separately for the group 1 and group 2 plants. In a specific sampling period, the analysis was focused only on the effect of the '*Ca*. P. prunorum' or ESFY on each genotype separately.

For the rest of the analysed parameters, the analysis of variance (ANOVA) in the general linear model (GLM) was used to study the effects ($P < 0.05$) of independent variables or their interaction. Due to focus on '*Ca*. P. prunorum' effect on analysed parameters in group 1 plants and ESFY effect in group 2 plants, only these two effects were used as independent variables and for interaction analysis, the combinations of '*Ca*. P. prunorum' or ESFY with sampling period and genotype were used. When a significant effect was observed in ANOVA, the differences between the variants were further analysed by post-hoc analysis with Tukey's test ($P < 0.05$).

RESULTS

Phytoplasma detection and ESFY symptom evaluation. In group 1 (older plants), both testing periods confirmed '*Ca*. P. prunorum' in 3 plants at each genotype (Nora and LEM 159) (Table 2). These positive plants also showed typical ESFY

https://doi.org/10.17221/128/2024-PPS

Table 2. Plant material of group 1 and 2 used in the study

Plant group	Apricot genotype	Rootstock	Year of chip-budding	Number of phytoplasma positive[a]/ ESFY symptomatic plants[b]	Number of phytoplasma negative[a]/ ESFY asymptomatic plants[b]
GROUP 1	Nora	GF 305	2020	3	4
	LEM 159	GF 305	2019	3	2
Total number of plants per group 1				6	6
GROUP 2	H 74	myrobalan seedling	2022	7	3
	H 177	myrobalan seedling	2022	10	4
Total number of plants per group 2				17	7

[a]applies for group 1 plants; [b]applies for group 2 plants

symptoms (Figure 1) and were considered phytoplasma-positive variants. The rest of the group 1 plants always tested phytoplasma-negative and did not show ESFY symptoms, thus being considered phytoplasma-negative variants.

In group 2 (younger plants), the real-time PCR tests in both testing periods resulted in negative reactions in all tested plants. However, as the ESFY symptom was manifested in some of the plants (H 74 7 plants and H 177 10 plants) (Figure 1, Table 2), the ESFY symptom was used for differentiation between variants (ESFY symptomatic/ESFY asymptomatic) in this group.

Figure 1. Symptomatic apricot plant (genotype H 177, group 2) with typical symptoms of ESFY showing leaf chlorosis and leafroll on the left and an asymptomatic plant of the same genotype on the right; picture taken in September 2023, 13 months after chip-budding on the rootstock

Photosynthetic pigments. Phytoplasma infection significantly decreased the content of all analysed pigments (chlorophyll *a*, *b* and carotenoids) in the leaves of group 1 plants (Table 3). However, subsequent analyses showed that the pigments were affected only during the summer (Figure 2). A decrease in all pigments during the summer was also observed in young group 2 plants, which showed ESFY symptoms (Figure 3). The effect of phytoplasma or ESFY presence on the content of the pigment was expected since leaf chlorosis is one of the main symptoms of ESFY disease and is more pronounced in the second half of the vegetation period. Apricot genotype did not contribute to phytoplasma or ESFY effect on pigment contents ($P > 0.05$).

Despite the normal distribution of chlorophyll *b* values in the group 1 plants (Kolmogorov-Smirnov test $P > 0.05$, Lilliefors test $P > 0.05$), the standard deviation (SD) value in the phytoplasma positive plants was higher than the mean value (Table 3). The observed variation was, however, caused by other effects (sampling season or genotype) than the '*Ca.* P. prunorum'.

Total phenolic content, total flavonoid content and antioxidant capacity. ESFY presence negatively affected TPC and TFC in young group 2 plants (Table 3). However, the effect of ESFY on these parameters depended on the apricot genotype, where only the H 177 genotype showed significantly lower TPC and TFC in symptomatic plants (Figure 4). TPC and TFC were not affected

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Table 3. Overall mean contents of primary and secondary metabolites in older, group 1 plants and younger, group 2 plants

Analysed parameter	Unit	Group 1				Group 2			
		Phytoplasma positive[1]		Phytoplasma negative[2]		ESFY symptomatic[3]		ESFY asymptomatic[4]	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Chlorophyll *a*	mg/g DW	1.19[a]	0.74	2.31[b]	0.69	2.03	0.98	2.19	0.71
Chlorophyll *b*	mg/g DW	0.31[a]	0.34	0.62[b]	0.24	0.42	0.28	0.49	0.22
Carotenoids	mg/g DW	0.37[a]	0.17	0.65[b]	0.18	0.60	0.24	0.62	0.18
TPC	mg GAE/g DW	42.74	10.40	42.94	12.54	53.20[a]	10.89	60.82[b]	10.81
TFC	mg CE/g DW	25.07	6.75	26.19	7.66	31.54[a]	6.12	37.07[b]	5.49
AC	mg TE/g DW	82.07	46.14	79.93	46.25	115.16	55.32	129.36	63.32
Sucrose	mg/g DW	17.93	10.91	19.55	10.16	18.53	9.64	18.20	8.37
Glucose	mg/g DW	17.52[b]	7.47	12.35[a]	5.57	12.61	6.46	10.31	7.28
Sorbitol	mg/g DW	73.41	9.40	70.29	15.78	67.98	21.01	61.49	15.01
Fructose	mg/g DW	12.63	4.45	12.28	4.33	12.41	7.78	10.76	7.08
K^+	mg/100 g DW	73.07[a]	61.98	91.78[b]	63.01	116.30	60.25	121.58	78.66
Na^+	mg/100 g DW	25.95	13.69	26.87	14.67	34.52	13.64	34.41	17.21
Ca^{2+}	mg/100 g DW	16.34	22.84	29.38	38.90	33.55	33.62	39.50	38.84
Mg^{2+}	mg/100 g DW	4.62	1.34	3.83	1.72	3.30	1.36	3.15	1.33
TSPC	g/100 g DW	1.64[a]	0.58	1.99[b]	0.61	2.67	0.92	2.56	0.48
Auxins	mg IAA/g DW	1.04	0.14	0.98	0.11	1.22	0.19	1.38	0.45

SD – standard deviation; TPC – total phenolic content; TFC – total flavonoids content; AC – antioxidant capacity; TSPC – total soluble protein content; ICC – indolic compounds content

[a,b]The letters indicate significant differences in analysed metabolites between phytoplasma positive and negative (group 1) or ESFY symptomatic and asymptomatic (group 2) plants according to ANOVA and subsequent grouping by Tukey's test ($P \leq 0.05$) where phytoplasma (group 1) or ESFY (group 2) were used as the main effects

[1]No. of plants (N) = 12, except for Mg^{2+} (N = 7)

[2]N = 12, except for Sucrose (N = 9) and Mg^{2+} (N = 5)

[3]N = 34, except fpr sucrose (N = 32), glucose (N = 29), Ca^{2+} (N = 33) and Mg^{2+} (N = 22)

[4]N = 14, except for glucose (N = 10), Ca^{2+} (N = 13) and Mg^{2+} (N = 10)

by phytoplasma presence in group 1 plants, and AC was not affected by phytoplasma or ESFY in both plant groups (Table 3).

Sugars. Phytoplasma presence significantly increased the glucose content in the leaves of older group 1 plants, while in symptomatic leaves of younger group 2 plants the glucose content was unaffected (Table 3). The other sugars (fructose, sorbitol and sucrose) were not affected by phytoplasma or ESFY presence (Table 3), and there was no influence of genotype or

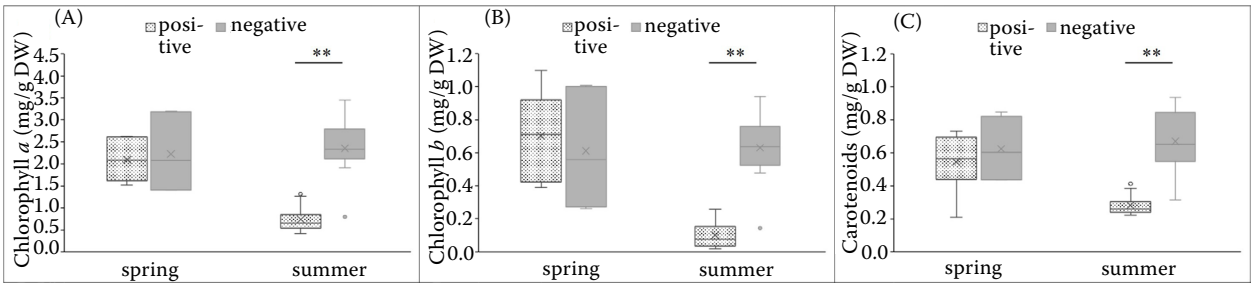

Figure 2. (A) Chlorophyll *a*, (B) chlorophyll *b*, (C) carotenoids content in spring and summer samples of phytoplasma positive and negative plants in group 1

The asterisk indicates a significant effect (*for $P < 0.05$, **for $P < 0.01$) based on post-hoc Tukey's test

https://doi.org/10.17221/128/2024-PPS

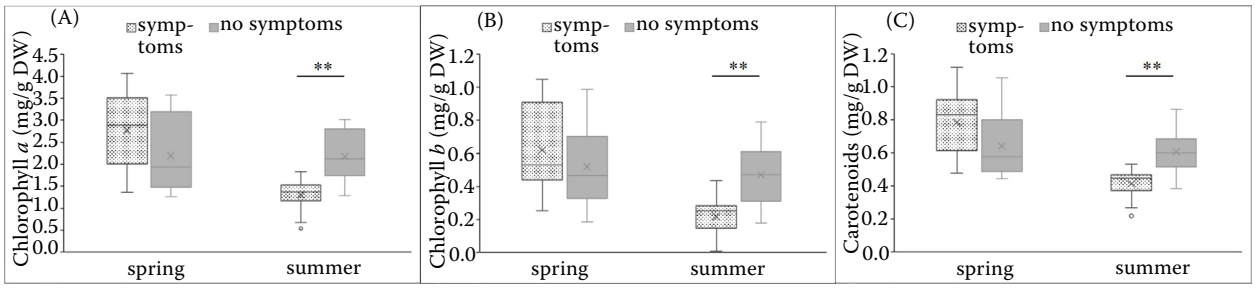

Figure 3. (A) Chlorophyll *a*, (B) chlorophyll *b*, (C) carotenoids content in spring and summer samples of ESFY symptomatic and asymptomatic plants in group 2

The asterisk indicates a significant effect (*for $P < 0.05$, **for $P < 0.01$) based on post-hoc Tukey's test

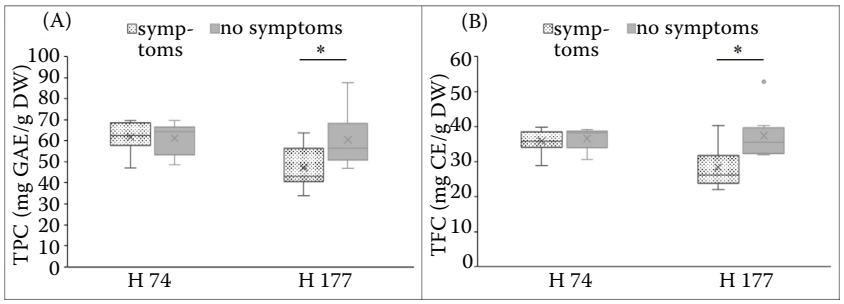

Figure 4. (A) Total phenolic content (TPC) and (B) total flavonoid content (TFC) in H 74 and H 177 genotypes of ESFY symptomatic and asymptomatic plants in group 2

The asterisk indicates a significant effect (*for $P < 0.05$, **for $P < 0.01$) based on post-hoc Tukey's test

sampling period on the effect phytoplasma or ESFY on the content of analysed sugars.

Ions content. Phytoplasma presence significantly affected the content of ions only in older group 1 plants (Table 3). In particular, the phytoplasma decreased the K^+ regardless of the sampling period (Table 3). The other ions (Ca^{2+}, Na^+, and Mg^{2+}) were not affected by the presence of phytoplasma or ESFY (Table 3), and the plant genotype or sampling period did not contribute to the effect of phytoplasma or ESFY on these ions.

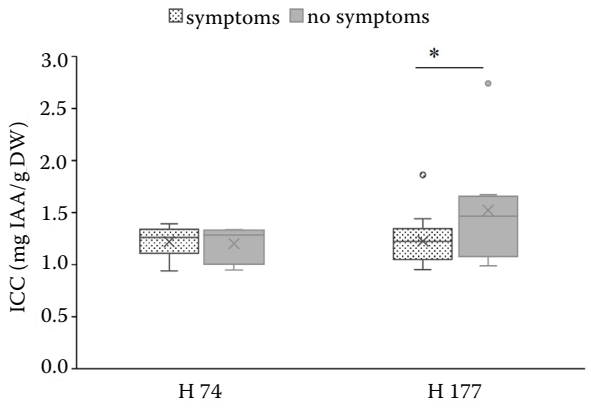

Figure 5. Indolic compound content (ICC) in H 74 and H 177 genotypes of ESFY symptomatic and asymptomatic plants in group 2

The asterisk indicates a significant effect (*for $P < 0.05$, **for $P < 0.01$) based on post-hoc Tukey's test

Total soluble protein content and indolic compound content. Phytoplasma presence decreased the total soluble protein content (TSPC) in older group 1 plants, while no effect of ESFY presence was observed in young group 2 plants (Table 3). The effect of phytoplasma or ESFY presence on TSPC was also not affected by genotype or sampling period.

Indolic compound content (ICC) was affected by ESFY presence only in young, group 2 plants (Table 3); however, the effect was dependent on the genotype, where a significant decrease was observed only in the H 177 genotype (Figure 5). Phytoplasma did not affect ICC in older group 1 plants (Table 3).

DISCUSSION

All plants used in this study were prepared by chip-budding of buds collected from phytoplasma-infected mother trees. Phytoplasma transmission rate by grafting is not 100% (Riedle-Bauer et al. 2012), so phytoplasma-negative and positive plants were expected. This was confirmed in the presented study. However, in group 2 (younger plants), despite visible ESFY symptoms in some plants, all plants resulted negative in phytoplasma detection. As the plants were tested 7 and 14 months (before and after the sampling period for metabolite analysis) after chip-budding, the negative result was probably

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caused by insufficient time between the chip-budding and the accumulation of a detectable amount of phytoplasmas. Other works also recorded this observation (Pastore et al. 2010; Riedle-Bauer et al. 2012). Due to the eradication of tested plants at the end of 2023 (16 months after chip-budding), no further phytoplasma detection was performed in group 2, and phytoplasma presence could not be confirmed in the following months. Thus, further development of phytoplasma presence in these trees could not be observed. It should also be remembered that, despite using positive and negative controls, the reliability of PCR testing and ESFY symptom manifestation does not always have to be 100%.

Leaf yellowing or chlorosis, caused by chlorophyll degradation, is the first visible symptom during the development of ESFY disease. Chlorophyll breakdown during natural senescence and phytoplasma infection proceeds via a common pathway in some members of the *Rosacea* family; however, the degradation of chlorophyll has a much earlier onset in phytoplasma-infected leaves (Mittelberger et al. 2017). In this study, both plant groups demonstrated decreased pigment content during summer, which was presented by visible chlorosis. The decreased total chlorophyll content is probably the result of enhanced chlorophyllase activity in infected leaves, but it has also been discussed that leaf chlorosis is a consequence of phytoplasma-induced sugar metabolism disorders (Wei et al. 2022). The effect of phytoplasma infection on photosynthetic pigments may depend on the susceptibility of the cultivar (Liu et al. 2016); however, this was not observed in this study, as no effect of genotype contribution was observed. Similar results have been reported in apple (*Malus pumila* Mill.) infected with '*Candidatus* Phytoplasma mali' (Bertamini et al. 2003), lime (*Citrus aurantifolia* Swingle*)* infected with '*Ca.* Phytoplasma aurantifolia' (Zafari et al. 2012) and sweet cherry (*Prunus avium* L.) affected by elm yellows disease (Tan et al. 2015).

Phenolics are well-known antifungal, antibacterial and antiviral compounds that accumulate in plants after infection and have been the subject of many studies on plant-defence mechanisms to pathogens (Gogoi et al. 2001; Mitrovic et al. 2021; Ayvaci et al. 2022; Davosir et al. 2023). Phenolic compounds exhibit antimicrobial and antioxidant properties, which have a role in evading bacterial pathogens (Cho & Lee 2015; Wang et al. 2018). They also protect tissues from the toxic effect of reactive oxygen species and are crucial in plant-microbe interaction and communications (Kumar et al. 2020). The dual function of phenolic compounds as attractants for symbiotic microbes and repellents for pests and pathogens depends on the surrounding environment (Pratyusha 2022). Phenolics and flavonoids in plant-pathogen interactions have been described as stress-induced compounds that act as a quencher of the host plant's defence reactions during pathogen attack (Naoumkina et al. 2010; Rusjan et al. 2014). Moreover, they enhance host cells' mechanical strength by synthesising lignin and suberin (Singh et al. 2014). Furthermore, multiple works also showed that the phenolic content and antioxidant capacity in plants of the genus *Prunus* can vary depending on specific cultivar/rootstock combinations (Usenik & Štampar 2000; Errea et al. 2000 Usenik et al. 2006). The effect of phytoplasma on different fruit species has been studied when mostly higher polyphenol contents were discovered in infected tissues than in healthy ones, for example, in apples, plums or lime (Musetti et al. 2000; Raiesi & Golmohamadi 2020). Musetti et al. (2000) analysed TPC in plum leptonecrosis (PLN) infected plum plants, where a greater amount of TPC was detected in young tissues, while no differences were detected in mature material. It has been discussed that the downregulation of the biosynthesis of phenolics and flavonoids could be a phytoplasma strategy to limit the production of protective compounds when the phytoplasma titer is still low (Davosir et al. 2023). This is in line with the results of the presented study, where the older (group 1) phytoplasma-positive plants already had a detectable amount of phytoplasma. Their TPC and TFC were not different from the phytoplasma-negative plants, while in the young (group 2) ESFY symptomatic plants, the TPC and TFC were decreased when compared to asymptomatic plants.

Phytoplasmas survival and multiplication depend on the organic matter present in the phloem tissue of the plant host. That is why the strongest impact is observed on carbohydrates' synthesis, distribution and utilisation. Carbohydrate metabolism and transport seem to be impacted by the inhibition of photosynthetic processes caused by phytoplasma infection (Musetti & Pagliari 2019). Symptomatic leaves fail to serve as source organs due to reduced photosynthetic function. Instead, starch accumulation in infected leaves demonstrates their transformation into strong sink organs (Tan et al.

https://doi.org/10.17221/128/2024-PPS

2015). Induced sugar mobilisation in the infected leaves results from increased activity of sucrose synthases and vacuolar invertases (Pagliarani et al. 2020). In this study, phytoplasma presence in the older plant group (group 1) significantly increased the glucose content in analysed tissues. Glucose content was not affected by the ESFY presence in the group of younger plants (group 2). The literature supports both findings. Increased content of glucose was reported in leaves of tomato (*Solanum lycopersicum* L.) plants affected by potato purple-top disease (Wei et al. 2022), jujube [*Ziziphus jujuba* (L). Mill] affected by jujube witches' broom (Xue et al. 2020) and grapevine (*Vitis vinifera* L.) leaves infected by '*Ca.* Phytoplasma solani' (Prezelj et al. 2016). No effect on the glucose content was reported in field-grown sweet cherries affected by Elm Yellows (Tan et al. 2015).

It can be assumed that the phytoplasma disease symptoms reflect the plant's altered nutritional status (Rossi et al. 2010). Potassium is essential for basic physiological functions such as the formation of sugars and the synthesis of proteins. Moreover, it is the most abundant cellular cation, essential for cellular growth, stomatal function, cytosolic pH homeostasis, and phloem solute transport (Very & Sentenac 2003). Potassium is also one of the crucial elements in increasing resistance to pathogen infections (Huber et al. 2012). In this study, K^+ content was significantly decreased only in the phytoplasma-positive plants from the group of older plants (group 1). Similar results have been reported in tomato plants infected by '*Ca.* P. solani' (Buoso et al. 2022). In lime infected by '*Ca.* P. aurantifolia' the potassium content was significantly increased (Al-Ghaithi et al. 2016; Raiesi & Golmahammadi 2020), while there was no effect of phytoplasma on the content of potassium in the '*Ca.* Phytoplasma asteris' infected cucumber (*Cucumis sativus* L.) (Wang et al. 2022). Phytoplasma infection causes the increase of Ca^{2+} levels in the sieve tubes, leading to sieve plate occlusion (Musetti & Favali, 2003; Musetti et al. 2013; van Bel & Musetti 2019). It has been discussed that the increase in the content of Ca^{2+} in the stem of phytoplasma symptomatic plants is due to the transportation role of the stem. At the same time, the leaves are responsible for the energy capture where the Ca^{2+} content can be decreased (Wang et al. 2022). Several reports proved that phytoplasmas decrease the content of Ca^{2+} in leaves, i.e. in lime infected by '*Ca.* P. aurantifolia' (Al-Ghaithi et al. 2016; Raiesi & Golmohamadi 2020) or tomato infected with '*Ca.* P. solani' (Buoso et al. 2022). In the presented study, no significant differences were observed despite slightly decreased Ca^{2+} content in the leaves in phytoplasma-positive plants in group 1 and ESFY symptomatic plants in group 2.

High protein content in pathogen-infected plants is associated with the activation of the host defence mechanism and the pathogen attachment mechanism (Agrios 2005). Effector proteins secreted by the pathogen are assumingly mediating the changes in host plant metabolism (Gross et al. 2022). Host plants under pathogen attacks produce pathogenesis-related proteins (PR-proteins), contributing to the infected tissue's total protein content. Resistant hybrids accumulate higher protein content than susceptible ones, while a decrease in the total soluble proteins could be caused by extreme susceptibility (Bertamini et al. 2002b). It was proposed that sugars amplify plant immunity responses since their accumulation activates PR-proteins and their genes (Rojas et al. 2014; Trouvelot et al. 2014; Albrecht et al. 2016). The decrease in total protein content in phytoplasma-infected leaves could result from the decreased synthesis of ribulose-1,5-biphosphate carboxylase, which is the major soluble protein of the leaf (Bertamini et al. 2002a). In this study, only the older plants (group 1) positive for phytoplasma infection demonstrated a significant decrease in TSPC. Similar results have been reported in field-grown grapevine infected by '*Ca.* P. solani' (Bertamini et al. 2002c) and Flavescence dorée (Musetti et al. 2007), field-grown apple infected by '*Ca.* P. mali' (Bertamini et al. 2003), tomatoes affected by '*Ca.* P. solani' (Favali et al. 2001), and lime infected by '*Ca.* P. aurantifolia' (Zafari et al. 2012).

An imbalance of host plant growth regulators could explain many symptoms associated with phytoplasma infection. Indole-3-acetic acid (IAA) is an indolic compound and the most abundant auxin in plants. It stimulates growth and is an important part of plant defence signalling pathways. Elevated IAA levels or auxin signalling promotes disease development in some plant-pathogen interactions (Kunkel & Harper 2018; Dermastia 2019). This study analysed the ICC instead of the specific IAA levels since the Salkowski reagent does not react with the IAA alone (Glickmann & Desaux 1995). ICC was decreased only in one genotype of apricots in the group of younger plants (group 2) dur-

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ing the summer sampling period, while no effect was observed in other groups. Due to the low sensitivity of the Salkowski reagent at higher concentrations, it might be that the detection of ICC is overestimated (Guardado-Fierros et al. 2024). However, the same pattern as in the presented study was reported in lime infected by '*Ca.* P. aurantifolie' (Zafari et al. 2012), which was analysed using the same Salkowski test and in the study on jujube infected with jujube witches' broom phytoplasma (Ye et al. 2017) obtained using HPLC-MS/MS.

CONCLUSION

According to the obtained results, not all of the analysed primary and secondary metabolites in the leaves of apricots were affected by '*Ca*. P. prunorum' or ESFY presence. The presence of phytoplasma or ESFY significantly decreased the content of the pigment in both groups of apricot trees only in the second sampling period, which is an expected result since leaf chlorosis is one of the main ESFY symptoms and its occurrence is mainly pronounced towards the end of the vegetation period. However, despite the decreased pigment content, the plants managed to maintain their photosynthetic activity and showed a significant increase in sugar content. Protective compounds like TPC, TFC, and the ICC were negatively affected by ESFY presence only in young plants and only in one genotype, showing that these compounds could be affected only in some apricot genotypes. It has also been demonstrated that phytoplasmas affect plants` nutritional status since the content of K^+ was significantly decreased in infected leaves. The results of this study support the idea that '*Ca*. P. prunorum' affects metabolites in plants' defence system and manipulates basic metabolic processes during successful infection. Further research and more advanced methods for analysis of plant primary and secondary metabolites are required to study the effect of '*Ca*. P. prunorum' in apricots.

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