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Degree of deterioration of the chemical composition of apricot fruits caused by Sharka disease

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Abstract

The objective of this study was to investigate the influence of Sharka disease on fruit weight, chemical composition and antioxidant activity of apricot fruit cv. 'Precoce de Thyrinte'. During two consecutive years, the presence of PPV-M strain was determined on leaves and fruits. This strain decreased the content of soluble solids content (SSC), invert (IS), reducing (RS), sucrose (SU) and total sugars (TS), pigments (chlorophylls and total carotenoids), total phenolics (TPC), total flavonoids (TFC), ripening (RI) and sweetness indexes (SI) in infected trees. The effect of PPV-M strain on pH juice and total antioxidant capacity (TAC) was not significant but increased ash content and both malic and citric acids. In general, a significantly greater deterioration in fruit quality was observed in the rainy 2023 year. However, the significant effect interaction viral status × year indicated the complex nature of the deterioration in fruit quality depending on PPV-M infection and weather conditions during fruit development and its maturity.

Keywords: Antioxidant activity; fruit weight; PPV-M strain, *Prunus armeniaca* L., primary and secondary metabolites

Zusammenfassung

Ausmaß der Verschlechterung der chemischen Zusammensetzung von Marillen durch Scharka-Erkrankung. Ziel dieser Studie war es, den Einfluss der Scharka-Krankheit auf das Fruchtgewicht, die chemische Zusammensetzung und die antioxidative Aktivität der Marillensorte *Precoce de Thyrinte* zu untersuchen. Über zwei aufeinanderfolgende Jahre hinweg wurde das Vorkommen des PPV-M-Stammes (Plum pox virus, M-Stamm) an Blättern und Früchten nachgewiesen. Dieser Virusstamm verringerte den Gehalt an löslichen Feststoffen (SSC), Invertzucker (IS), reduzierenden Zuckern (RS), Saccharose (SU) und Gesamtzuckern (TS) sowie an Pigmenten (Chlorophyllen und Gesamtcarotinoiden), Gesamtphenolen (TPC), Gesamtflavonoiden (TFC), Reifungsindex (RI) und Süßeindex (SI) bei infizierten Bäumen. Der Einfluss des PPV-M-Stammes auf den pH-Wert des Fruchtsaftes und die Gesamtantioxidative Kapazität (TAC) war nicht signifikant, jedoch stieg der Aschegehalt sowie der Gehalt an Äpfel- und Zitronensäure an. Insgesamt wurde im niederschlagsreichen Jahr 2023 eine signifikant stärkere Qualitätsminderung der Früchte beobachtet. Die signifikanten Effekte der Interaktion zwischen Virusstatus und Jahr verdeutlicht die Komplexität der Qualitätsveränderung, die sowohl von der PPV-M-Infektion als auch von den Witterungsbedingungen während der Fruchtentwicklung und -reife abhängig ist.

Schlagwörter: antioxidative Aktivität, Fruchtgewicht, PPV-M-Stamm, *Prunus armeniaca* L., primäre und sekundäre Metaboliten

Introduction

Plum pox virus, PPV (genus *Potyvirus*, family *Potyviridae*) is considered a most detrimental virus of *Prunus* species causing Sharka disease. As known, the first symptoms of “Sharka” were observed in the south-west region of Bulgaria (Atanasov, 1932) by some plum growers from Zemen district between 1915 and 1918, at the close of World War I, although some reports indicate symptoms were seen in North Macedonia as early as 1910 (Fuchs et al., 2008). D. Atanasov has seen the diseased tree around 1926 in some villages close to the mentioned Zemen village (Kamenova and Milusheva, 2005). In the mentioned district, Sharka caused enormous damage on plum, primarily on ‘Kyustendilska Sinya Sliva’ cultivar since it was the most widespread in Bulgaria. In Serbia, the presence of Sharka was reported in the mid-1930s by Josifović (1937). On apricot, Sharka was first discovered also in Bulgaria in 1933 (Christoff, 1938), in Serbia in 1936, probably alongside with detection on the plum (Josifović, 1937). Based on biological, serological, molecular and epidemiological properties, today ten PPV strains were described [PPV-D (Dideron), PPV-M (Marcus), PPV-EA (El Amar), PPV-C (Cherry), PPV-Rec (Recombinant), PPV-W (Winona), PPV-T (Turkey), PPV-CR (Cherry Russian), PPV-AM (Ancestor Marcus), and PPV-CV (Cherry Volga)] (Rodamilans et al., 2020). Out of all known, three strains (PPV-D, PPV-M, and PPV-Rec) are considered major strains that are widely distributed in numerous countries (Sochor et al., 2012), including Serbia (Jevremović, 2013).

PPV causes enormous damage in susceptible plum, apricot, peach and nectarine cultivars. It also negatively affects sour (Kalashyan et al., 1994; Sheveleva et al., 2021) and sweet cherries (Crescenzi et al., 1997; Myrta and Savino, 2008) and has been experimentally transmitted to almond (Dallot et al., 1996). The PPV also can infect wild and ornamental *Prunus* species (Sebestyen et al., 2008).

Apricots are susceptible to all three major strains (Garcia et al., 2014). In sensitive apricot cultivars such as for example ‘Goldrich’, ‘San Castrese’, ‘Chuang Zhi Hong’ (Polák et al., 1997) or ‘Hungarian Best’ (Milošević et al., 2019), PPV induces chlorotic spots and rings on fruits that darken with time, the tissue beneath them necroses and the fruits become deformed and fall off prematurely. Such fruits are smaller, lighter and tasteless. PPV symptoms on leaves of most apricot cultivars are in most cases faint, with no visible symptoms on fruit skin, whereas all cultivars show clear PPV symptoms on stone (Németh, 1986). Hence, Sharka disease is a serious limiting factor for stone fruit production areas that are affected (Kölber, 2001). For these reasons, the need to cultivate PPV-resistant apricot cultivars is imperative; however, a big problem is their limited ability to adapt to local environmental conditions, especially in the temperate-continental climate zone (Milošević et al., 2010).

Apricot is a very important fruit type for the Serbian agricultural sector and has a long tradition. However, apart from the known limiting factors of its cultivation in this country (Milošević et al., 2010), in recent few decades more plantations have been infected with PPV (Milošević and Milošević, 2019; Milošević et al., 2019). Although Sharka disease is spread in some Serbian apricot plantations, there are no data about number of trees infected by PPV, financial damages for any period and level of fruit quality deterioration. Generally, the PPV symptomatic apricot fruits are rejected by consumers at markets and bazaars as the infected trees produce blemished and unmarketable fruits (Iwanami and Tomimura, 2017).

After World War II, over a thousand apricot cultivars from Europe and North America, some from Asia, were introduced to Serbia. However, as a rule, they failed to adapt to the ecological conditions that are largely unfavorable for its survival and eventual cultivation. Today, the predominant cultivar in Serbia is 'Hungarian Best', which is very sensitive to PPV (Milošević et al., 2019). On a smaller scale, new Serbian cultivars and selections are grown, and sporadically also some foreign ones, including the old Greek cultivar 'Precoce de Thyrinte', chosen due to its early ripening and good adaptation capability to Serbian environmental conditions.

In the last 100 years, a lot of research has been conducted on Sharka disease and thousands of articles have been written with different approaches such as detection techniques, genome characterization and organization, gene expression, transmission, and the description of candidate genes involved in PPV resistance (Espinoza et al., 2021; García et al., 2024). However, in the available literature there are very few articles related to the degree of deterioration of fruit size, chemical composition and antioxidant capacity. For those reasons, the main goal of the present study was to assess the fruit quality characterization of 'Precoce de Thyrinte' picked from PPV infected trees and their comparison with fruit quality from uninfected trees during two consecutive years. We believe that the results will contribute to the knowledge about degree of the deterioration (derogation) of fruit size and chemical composition caused by Sharka disease.

Materials and methods

Experimental layout and plant material

This trial was carried out in private commercial plantation located in Prislonica vilage near Čačak city, western Serbia in 2023 and 2024. Main weather conditions during investigation and long-term average values (monthly air temperature in °C and sum of precipitation in mm) are presented in Tab. 1. Study area is located at 340 m above sea level, 43°53' N latitude, 20°21' E longitude. Orchard was established in 2015 with 19 apricot cultivars originating from different world regions, including 'Precoce de Thyrinte' as plant material. Before grafting, scion shoots of all cultivars were tested on all known PPV strains using IC-RT-PCR procedure and declared as virus-free. After this procedure, scion genotype was grafted onto Myrobalan seedlings in summer 2014 and planted in autumn 2015 with spacing of 5.5 m × 3.0 m. Standard cultural practices were used, except irrigation.

The trial was set up in a randomized block design with four replications, each containing five trees of the tested 'Precoce de Thyrinte' cultivar (n = 20).

As a result of natural infection, the first visible symptoms of PPV presence on this cultivar were observed on fruits and stones in 2020 and 2021 on six trees in total. The infected trees were marked and subjected to observation and analysis and compared with 6 PPV uninfected trees in 2023 and 2024. Leaf samples (average 20 per tree) for presence of PPV were taken randomly in mid-May from all parts of the canopy from 6 trees with visible PPV symptoms. By random selection, 6 trees with no visible PPV symptoms were marked and 20 leaves per tree were collected at the same time.

Tab. 1: Weather conditions of the Čačak region (Western Serbia) for 2023 and 2024*

Months	Average monthly air temperature (°C)			Total precipitation (mm)		
	2023	2024	LTA	2023	2024	LTA
Jan	3.4	2.0	0.03	90.0	56.0	44.1
Feb	3.3	9.0	2.3	37.2	7.8	38.9
Mar	7.5	10.0	6.8	65.0	49.6	46.2
Apr	11.7	13.9	11.5	6.6	48.2	51.6
May	16.2	15.9	16.8	20.0	92.4	72.7
June	19.4	22.1	20.0	140.8	61.4	87.3
July	23.1	24.8	21.5	88.0	58.8	79.1
Aug	21.9	25.3	21.2	49.6	18.4	58.0
Sep	19.5	18.0	16.7	75.2	86.0	56.2
Oct	15.1	12.9	11.4	11.8	37.0	51.1
Nov	7.1	4.7	6.0	134.6	66.8	55.9
Dec	5.5	2.5	1.4	30.2	66.8	50.4
Mean or total	12.8	13.4	11.3	749.0	649.2	690.2

*Source: Republic Hydro-meteorological Bureau in Belgrade, Serbia (http://www.hidmet.gov.rs)
LTA: normal refers to the long-term average (45-year average, i.e. 1965–2010 period)

Detection of presence/absence of PPV

PPV detection and characterization was carried out with Immunocapture Reverse Transcription Polymerase Chain Reaction (IC-RT-PCR) in May 15, 2020 on apricot leaves. Polyclonal antibodies (0.5 mg mL^{-1}) produced at the Fruit Research Institute, Čačak were used for PCR plate coating in immunocapture step. Reverse transcription was performed with random hexamer primers using Maxima Reverse Transcriptase (Thermo Fisher Scientific, USA). The obtained cDNA was used for further PCR analyses. Each sample was tested in three separate PCR reactions using primers designed by Šubr et al. (2004). Primer sets mD5/mD3, mM5/mM3, and mD5/mM3, that amplify the 3’NIb-5’CP region, were used for specific detection of PPV-D, PPV-M and PPV-Rec isolates, respectively. PCR mixture of 20 µl consisted of 2µl10 × DreamTaq Green Buffer, 2 µl 2.5 mM dNTPs, 1.2 µl 25 mM $MgCl_2$, 0.2 µl of each 10 mM primer, and 0.2 µl 5U of Green Taq DNA polymerase (Thermo Fisher Scientific, USA). The thermal cycling conditions were as follows: 3 min at 94°C; 30 cycles each of 45 s at 94°C, 30 s at 60°C, and 60 s at 72°C; and a final step of 7 min at 72°C. All PCRs were carried out in TPersonal thermal cycler (Biometra, Germany). Obtained PCR products were analyzed in 1.5% agarose gel, stained with ethidium-bromide, and visualized in a Gel Doc EZ System (Biorad, USA). The presence of the PCR fragment of the expected size was considered a positive reaction.

Determination of fruit weight, chemical composition and antioxidant activity

For each sampling, 10 PPV-uninfected fruits were randomly hand-picked from trees showing no visible PPV symptoms (*n* = 40), and 10 symptomatic fruits (Fig. 1) were collected from PPV-infected trees. Fruits were sampled from all parts of the canopy at full ripening stage (approximately mid-June in both years), with four replications per group (*n* = 40).

Fruit weight (FW, g) was measured using digital balance VK-TECH (Daejeon, South Korea). The juice for chemical analyses was extracted using kitchen extractor from 10 randomly selected whole fruits (skin + pulp) from PPV uninfected and PPV infected trees. The SSC (°Brix) was assessed with a hand refractometer CZ 32-G (Carl Zeiss, Jena, Germany) at 20 °C. For both malic (%) and citric acid (%), prepared juice was titrated with 0.1 mol L^{-1} NaOH, up to pH 8.1 using 1-2 drops of phenolphthalein as an indicator to pink. Once the SSC and malic acid content were assessed, the ripening index (RI) or SSC/malic acid ratio was calculated. Juice pH was measured with a pH meter MA 5740 (Iskra, Kranj, Slovenia). For ash content (%) determination, fruits were incinerated at 550°C in oven during 8-10 h. Sugar contents (TS, RS, IS and SU (all in % of fresh weight, fw)] were determined according to the Luff-Schoorl method (AOAC, 1995). The SI was expressed as TS/malic acid ratio.

Fig. 1: Typical symptomatic fruits on a PPV-M-infected apricot cultivar 'Precoce de Thyrinte' tree in Prislonica village near Čačak city, Serbia (photo taken in mid-June 2024 by T. Milošević).

Spectrophotometric measurements for chlorophyll (*a* and *b*), total carotenoids, TPC, TFC and TAC were performed by Cary 300 UV/Vis spectrophotometer (Agilent Technologies, Santa Clara, CA) on fw basis. Contents of chlorophyll (*a* and *b*) and total carotenoids were determined by methods proposed by Lichtenthaler and Wellburn (1983), and the results were expressed as μg 100 g^{-1}. The TPC (mg 100 g^{-1}) was determined using the Folin-Ciocalteu method (Singleton et al., 1999) whereas TFC (mg 100 g^{-1}) was estimated by the $AlCl_3$ method (Quettier et al., 2000). The TAC (mg 100 g^{-1}) was evaluated by the phosphor-molybdenum method (Prieto et al., 1999).

The values presented for each measurement are the means of triplicate measures on equidistant points of each fruit and expressed as the mean ± standard error (SE).

Statistical analysis

The results were processed statistically by two-way analysis of variance (ANOVA), model 2 × 2, using MS-Excel software (Microsoft Corporation, Redmond, WA, USA). Source of variations was fruit viral status (A) and year (B). When the *F* test was significant, means were compared with the LSD test at $P \leq 0.05$.

Results and discussion

Presence/absence of PPV

Results of the IC-RT-PCR analysis revealed that only PPV-M strain was detected in analyzed samples. PPV was confirmed in all samples showing Sharka-like symptoms on fruits and leaves. In asymptomatic samples, the PPV presence was not confirmed. The PPV symptoms on apricot fruits in our study are given in Fig. 1. PPV-M strain is present in many European countries. It is considered an epidemic strain that is efficiently transmitted by a number of aphid species (Dallot et al., 2003; Capote et al., 2010). Otherwise, susceptible apricot cultivars were characterised by high relative PPV concentration (9.8×10^{-5}) whereas cultivars resistant to PPV were characterised by very low relative PPV concentration (5×10^{-2}) (Polák, 1998).

Fruit quality performances and antioxidant activity

Data in Tab. 2 showed that FW from PPV symptomatic trees was lower by 22.64% on average compared to PPV asymptomatic trees. Several authors reported that diseased apricot fruits are smaller and deformed (Llacer and Cambra, 1986; Poggi Pollini et al., 2008) with deformations accompanied by typical rings and mottling. On the other hand, FW was higher in first season of trial than in second. Interaction viral status × year was not significant. Other authors emphasize the significant influence of climatic factors (air temperature, amount of precipitation) on the spread of PPV and intensity of infection (Špánik et al., 1998; Glasa et al., 2003). In our earlier study, we found that there were no significant differences in FW of 'Hungarian Best' (clone 'T-14') harvested from PPV infected and healthy trees, although a mixed infection with PPV-D and PPV-Rec strains was detected in this apricot (Milošević et al., 2019).

Tab. 2: Impact of Sharka disease on fruit weight of apricot fruits from PPV uninfected and PPV infected trees

Source of variation	Fruit weight (g)
Viral status (A)	
PPV unifected	43.20 ± 1.89 a
PPV infected	33.42 ± 1.47 b
Year (B)	
2023	39.44 ± 1.97 a
2024	37.18 ± 1.39 b
ANOVA (*F* test)	
A	*
B	*
A×B	ns

No statistically significant differences between averages by LSD test at $P \leq 0.05$ are indicated by the same letter in columns. Asterisks in columns indicate significant differences at $P \leq 0.05$ by *F* test. ns: not significant.

Healthy fruits had higher SSC and better RI value than diseased ones, whereas diseased fruits had higher values of both malic and citric acids and ash content (Tab. 3). There were no significant differences between PPV uninfected and PPV infected fruits in pH juice. All sugar contents and SI were higher in fruits picked from uninfected trees (Tab. 4). For example, healthy fruits contained 13.67% more TS than PPV infected ones. These data are in agreement with results from Usenik et al. (2015) and Espinoza et al. (2021) who reported that PPV infection can modify the ripening process, induced by an alteration of the primary metabolism, including organic acids, soluble solids and sugars. Similarly, previous studies recorded that PPV infected fruits were lower in sugars (Šutić et al., 1999; Usenik and Virscek Marn, 2017), especially in sucrose

(Kamenova and Milusheva, 2005) and higher in acidity, resulting in inferior taste of diseased fruits (Milošević et al., 2019). On the other hand, Poggi Pollini et al. (2008) reported that fruit quality in general was only negligibly influenced by the infection but only SSC appeared to be slightly higher in fruits from PPV unifected trees compared to the symptomatic fruits of the same genotype. Probably, intensity and duration of infection, fruit type, genotype, crop load, nutrient status of the plant and tree age may be the reason for the discrepancies between the results of the mentioned authors as previously reported by Vanek et al. (1992).

Tab. 3: Soluble solids content, titratable acidity, pH juice, ripening index and ash content in fruits from PPV uninfected and PPV infected apricot trees

Source of variation	**Soluble solids content (°Brix)**	**Titratable acidity (%)**		**pH juice**	**Ripening index**	**Ash (%)**
		Malic acid	**Citric acid**			
Viral status (A)						
PPV unifected	12.86 ± 0.06 a	1.80 ± 0.01 b	1.72 ± 0.01 b	3.46 ± 0.01 a	7.40 ± 0.04 a	0.63 ± 0.01 b
PPV infected	11.45 ± 0.03 b	1.89 ± 0.01 a	1.80 ± 0.01 a	3.47 ± 0.01 a	6.06 ± 0.04 b	0.70 ± 0.00 a
Year (B)						
2023	12.66 ± 0.03 a	1.68 ± 0.00 b	1.61 ± 0.00 b	3.50 ± 0.01 a	7.67 ± 0.02 a	0.72 ± 0.01 a
2024	11.64 ± 0.06 b	2.01 ± 0.01 a	1.92 ± 0.01 a	3.42 ± 0.01 b	5.78 ± 0.06 b	0.62 ± 0.00 b
ANOVA (*F* test)						
A	*	*	*	ns	*	*
B	*	*	*	*	*	*
A×B	*	*	*	*	*	*

No statistically significant differences between averages by LSD test at $P \leq 0.05$ are indicated by the same letter in columns. Asterisks in columns indicate significant differences at $P \leq 0.05$ by *F* test. ns: not significant.

Tab. 4: Impact of Sharka disease on sugar contents and sweetness index in apricot fruits

Source of variation	**Invert sugars (%)**	**Reducing sugars (%)**	**Sucrose (%)**	**Total sugars (%)**	**Sweetness index**
Viral status (A)					
PPV unifected	7.99 ± 0.09 a	1.81 ± 0.02 a	7.59 ± 0.01 a	9.80 ± 0.01 a	5.60 ± 0.01 a
PPV infected	7.62 ± 0.01 b	0.81 ± 0.01 b	7.24 ± 0.01 b	8.46 ± 0.01 b	4.50 ± 0.05 b
Year (B)					
2023	7.92 ± 0.01 a	1.10 ± 0.01 b	7.53 ± 0.01 a	9.02 ± 0.01 b	5.49 ± 0.01 a
2024	7.68 ± 0.02 b	1.52 ± 0.01 a	7.30 ± 0.01 b	9.23 ± 0.02 a	4.62 ± 0.05 b
ANOVA (*F* test)					
A	*	*	*	*	*
B	*	*	*	*	*
A×B	*	*	*	*	*

No statistically significant differences between averages by LSD test at $P \leq 0.05$ are indicated by the same letter in columns. Asterisks in columns indicate significant differences at $P \leq 0.05$ by *F* test.

Comparing the results by years of research, statistically higher SSC, pH juice, ash content, IS, SU and both ripening and sweetness indexes were observed in 2023, whereas amounts of both malic and citric acids, RS and TS were higher in 2024. These results can be connected to the influence of weather conditions (Vanek et al., 1992), especially temperature on pathogenicity (aggressivity) of PPV (Glasa et al., 2003).

Data in Tab. 5 and 6 showed that content of pigments and secondary metabolites evaluated were higher in fruits picked from uninfected trees than in infected fruits. For example, there were 1.56- and 1.21-fold differences between higher and lower values of total chlorophyll and total carotenoid contents, respectively i.e. between healthy and diseased fruits. According to Hernández et al. (2006), PPV infections produce alterations in the chlorophyll fluorescence parameters in susceptible apricot cultivars such as ‘Real Fino’ which was confirmed by our results. In addition, the alteration of the photosynthesis provoked by a PPV infection could affect the primary metabolism in the plant (Clemente-Moreno et al., 2014).

Tab. 5: Content of chlorophyll and total carotenoids in apricot fruit

Source of variation	**Chlorophyll a (μg 100 g^{-1})**	**Chlorophyll b (μg 100 g^{-1})**	**Total chlorophyll (a+b) (μg 100 g^{-1})**	**Total carotenoids (μg 100 g^{-1})**
Viral status (A)				
PPV unifected	172.88 ± 0.72 a	78.04 ± 2.49 a	250.92 ± 2.92 a	939.49 ± 4.36 a
PPV infected	92.53 ± 0.65 b	68.11 ± 2.55 b	160.64 ± 2.13 b	775.03 ± 2.67 b
Year (B)				
2023	166.97 ± 0.85 a	35.00 ± 1.77 b	201.97 ± 1.90 a	846.17 ± 3.69 b
2024	98.44 ± 0.52 b	111.16 ± 3.26 a	209.59 ± 3.15 a	868.35 ± 3.34 a
ANOVA (*F* test)				
A	*	*	*	*
B	*	*	*	*
A×B	*	*	*	*

No statistically significant differences between averages by LSD test at $P \leq 0.05$ are indicated by the same letter in columns. Asterisks in columns indicate significant differences at $P \leq 0.05$ by *F* test.

Tab. 6: Content of total phenolics and total flavonoids in apricot fruit and their antioxidant capacity

Source of variation	**Total phenolic content (mg 100 g^{-1})**	**Total flavonoid content (mg 100 g^{-1})**	**Total antioxidant capacity (mmol 100 g^{-1})**
Viral status (A)			
PPV unifected	26.19 ± 0.34 a	7.24 ± 0.12 a	0.35 ± 0.01 a
PPV infected	22.13 ± 0.18 b	4.93 ± 0.11 b	0.35 ± 0.00 a
Year (B)			
2023	25.87 ± 0.25 a	7.04 ± 0.11 a	0.36 ± 0.01 a
2024	22.46 ± 0.27 b	5.12 ± 0.12 b	0.34 ± 0.00 b
ANOVA (*F* test)			
A	*	*	ns
B	*	*	*
A×B	*	*	*

No statistically significant differences between averages by LSD test at $P \leq 0.05$ are indicated by the same letter in columns. Asterisks in columns indicate significant differences at $P \leq 0.05$ by *F* test.

As known, TPC and TFC are important as they provide information on the overall quality of apricot fruits, especially as human health-promoting compounds. Previous research has shown that Sharka disease significantly modifies the above mentioned phytochemicals in sensitive stone fruits, especially plum (Usenik et al., 2015). This may be associated with the enhanced synthesis and accumulation of these compounds, especially flavonoids, in order to defend plants against stresses of biotic and abiotic origin. In light of this, Horsáková et al. (2013) reported that increased content of antioxidant activity in peach fruits of PPV infected trees is probably caused by the function of protective systems which regulate the production of reactive oxygen species and thus protect cells from oxidative damage. However, the results in our work showed the opposite trend, i.e. healthy fruits were richer in TPC and TFC than diseased ones. In our previous study on 'Hungarian Best' apricot (Milošević et al., 2019), we also determined significantly higher TPC and TFC content in healthy fruits compared to PPV infected ones. Interestingly, total antioxidant activity in our study was equal in both uninfected and infected fruits (Tab. 6). It seems that fruit type, cultivar and PPV strain play important roles in the degree of the plant's reaction to its presence.

Significant differences were observed in the effects of the year on pigments and phenolic compounds content and antioxidant activity (Tab. 5 and 6). The chlorophyll a (Chl a), TPC, TFC and TAC were higher in 2023 than in 2024, whereas chlorophyll b (Chl b) and total carotenoids were higher in 2024. Total chlorophyll content was similar in both years of investigation. Year-to-year variations in pigments and secondary metabolites content in PPV infected fruits such as peach and/or plums have been well documented in previous studies (Samara et al., 2017; Miletic et al., 2022). In addition, Miletic et al. (2022) reported that plum fruit infected with PPV was richer in some phytochemicals under heavy rainfall during summer months. In the present study, a very high amount of precipitation during May and June i.e. in the last stage of fruit development and ripening was registered (Tab. 1) and such weather conditions could have affected the content of these compounds.

However, the significant interaction viral status × year demonstrates the complex nature of the trends in the sources of variation for all primary and secondary metabolites (Tab. 5 and 6). Namely, the tendency that viral status was a key category in determining the content of primary and secondary metabolites over the years is not consistent. Contrary to our results, Miletic et al. (2022) reported that interactions viral status × year were not significant for TPC and TFC in plum cv. 'Čačanska Lepotica'. These discrepancies may be related to the fruit type, cultivar, PPV strain and its virulence capacity, duration of infection, tree age, weather conditions and orchard management as previously reported (Vanek et al., 1992; Glasa et al., 2003; Usenik et al., 2015; Milošević et al., 2019). For example, mineral nutrition can reduce or enhance the intensity of PPV symptoms (Nemeth, 1986).

Conclusion

Results of the IC-RT-PCR analysis in our study revealed that only PPV-M strain was detected in analyzed samples. This strain was confirmed in all fruit and leaf samples and showing Sharka-like symptoms in naturally infected trees of 'Precoce de Thyrinte' apricot. The PPV infected trees produced fruits with deteriorated appearance, reduced fruit weight and also significantly altered phytochemical composition in comparison to uninfected trees. In addition to viral status, year and interaction viral status × year also had a significant impact on most of the examined traits. Taking into account all these experimental results, it is evident that 'Precoce de Thyrinte' can be considered as an apricot cultivar that is highly sensitive to PPV-M strain. Virus free planting material, control of aphids as PPV vectors, and destruction of virus host plants are imperative for the successful cultivation of susceptible apricot cultivars.

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