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How does sharka affect the phenolics of plum fruit (*Prunus domestica* L.)?

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Abstract

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Plum pox virus (PPV), the causal agent of the sharka disease, is the most important viral disease in plums. To understand plant defense response against PPV-infection, changes in the composition of phenolics were studied in plum fruit (*Prunus domestica* L.). The phenolics were determined in visually undeformed and necrotic tissue during the last three ripening stages. The results indicated a significantly modified composition of anthocyanins, flavonols and hydroxycinnamic acids, in necrotic tissue the most. The phenolics differed significantly also between developmental stages and give insight into the phenolic profile of fallen unripe fruit. This study shows how PPV infection induces the biosynthesis of flavonoids in plum fruit.

Keywords: *Plum pox virus*; anthocyanins; flavonols, hydroxycinnamic acids; necrosis

The most important viral disease in plums, sharka, causes severe economic losses, serious fruit production problems and high costs associated with management of this disease – to the extent of more than 10,000 million euros worldwide over the last 30 years (Cambra et al. 2006). *Plum pox virus* (PPV), the causal agent of the sharka disease, is one of the most fully studied plant viruses; however, information concerning plant response to PPV infection is scarce (Clemente-Moreno et al. 2015). In susceptible plum cultivars, infection causes severe losses in yield quantity and quality (Kamenova, Milusheva 2006; Lácer, Cambra 2006). PPV usually affects both the leaves (leaf symptoms) and the fruit (fruit symptoms) of plants. Typical sharka symptoms on plum include chlorotic spots or rings, oak-leaf patterns and vein clearing on leaves, shallow rings and arabesque depressions on fruit, sometimes with brownish or reddish necrotic flesh (Sochor et al. 2012). Fruit from infected trees may drop prematurely (Clemente-Moreno et al. 2015).

Despite all the knowledge about PPV-based approaches, only a few studies have focused on the PPV-infected plant responses that are discussed in the review by Clemente-Moreno et al. (2015). These few works have shown that the most affected were the chloroplast and the photosynthetic machinery of the plant.

The main objective of this study was to evaluate changes in the composition of phenolics in ne-

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crotic and visually undeformed fruit tissue due to PPV-infection. The composition of phenolics at different phases of fruit ripening will give an insight into the phenolic profile of prematurely dropped plums, given that the greatest effect of PPV infection in sensitive plum cultivars is premature fruit drop (Milosevic et al. 2010).

MATERIAL AND METHODS

Plum fruit of the susceptible local cv. 'Domača češplja' (*Prunus domestica* L.) (Usenik et al. 2007) was collected from a plum orchard in Slovenia. Trees were selected according to the expression of sharka symptoms in the orchard. Infected trees showed severe symptoms on leaves and fruit as well as premature fruit dropping. There were no symptoms of PPV infection on the healthy trees. The fruit was picked on August 20 (*t*0), August 29 (*t*9), and September 11 (*t*22), 2013.

The presence/absence of PPV and other viruses was checked in buds collected from the canopy, using a double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) on March 12, 2014. Total RNA isolated from two PPV positive samples was used in reverse transcriptase polymerase chain reaction (RT-PCR) using PPV-3MODIF/P3M and PPV3MODIF/P3D primer pairs (Virscek Marn et al. 2012). The resulting amplicons were directly sequenced. Strain designations for all sequences were determined based on 99% nucleotide identity with sequences in the National Centre for Biotechnology Information (NCBI) database, using the Basic Local Alignment SearchTool (BLAST) .

For each sampling date, 30 fruit samples were selected randomly from infected and healthy trees. They were immediately transferred to the laboratory for further analysis. Each of the 30 fruit samples was randomly divided into five subsamples. The tissue for the determination of phenolic compounds was taken from four sides of each of the 6 fruit samples using a round stainless steel probe (diameter 6 mm) from the peel through the flesh. Fruit from infected trees were considered as symptomatic fruit. Two types of tissue were taken from symptomatic fruit: visually unaffected tissue (undeformed) (*und*) and tissue from the area of necrosis (necrotic tissue) (*nec*). The *und* and *nec* were taken from the same fruit. The extent of the tissue collected from each fruit in the case of *nec* was dependent on the area of necrosis in order to obtain the final weight of 3 g per subsample. Samples from healthy trees were considered as a healthy tissue (*h*) and used as control. Tissue of each subsample was immediately frozen and kept at –20°C until extraction.

Plum tissue was ground to a fine paste in a mortar chilled with liquid nitrogen. One g was extracted with 2.4 ml of methanol containing 3% (v/v) formic acid and 1% (w/v) 2.6-di-tert-butyl-4-methylphenol (BHT) in a cooled ultrasonic bath for 1 hour. After extraction, samples were centrifuged at 12,800 g for 5 min at 4°C and filtered through Chromafil A0-20/25 polyamide filters (Macherey-Nagel, Düren, Germany).

Determination of phenolics. The analysis was performed immediately after extraction on a Surveyor HPLC system with a photodiode array (PDA) detector (Thermo Scientific, San Jose, USA). The column was a Gemini C_{18} (150 × 4.6 mm; 3 µm; Phenomenex, Torrance, USA) protected with a Phenomenex security guard column and operated at 25°C. The elution solvents were aqueous 0.1% formic acid in double-distilled water (A) and 0.1% formic acid in acetonitrile (B). Samples were eluted using a linear gradient (Wang et al. 2002), with an injection amount of 20 µl and a flow rate of 0.6 ml/min.

Phenolic compounds were identified using a mass spectrometer (Thermo Scientific, LCQ Deca XP MAX) with an electrospray ionization (ESI) operating in positive (anthocyanins) and negative (all the other phenolics) ionization mode. Analyses were carried out using full -scan, data-dependent MS^n scanning from m/z 100 to 1,000. The capillary temperature was 250°C, the sheath gas and auxiliary gas were 60 and 15 units, respectively, and the source voltage was 3 kV for negative and 4 kV for positive ionization.

The identification of compounds was confirmed by comparing retention times, spectra characteristics, and by fragmentation. The concentrations of neochlorogenic acid (3-*O*-caffeoylquinic acid) (NA), chlorogenic acid (5-*O*-caffeoylquinic acid), rutin (quercetin-3-*O*-rutinoside), quercetin-3-*O*-galactoside, quercetin-3-*O*-glucoside, and peonidin-3-*O*-glucoside were calculated from peak areas of samples and corresponding standards. Quantification of compounds lacking standards was carried out using similar compounds. The concentrations of *p*-coumaroylquinic acid (4-*O*-*p*-coumaroylquin-

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ic acid) was calculated as equivalents of a *p*-coumaric acid, cryptochlorogenic acid (4-*O*-caffeoylquinic acid) as equivalents of a chlorogenic acid, kaempferol-3-*O*-rutinoside as equivalents of a kaempferol-3-*O*-glucoside, isorhamnetin-3-*O*-rutinoside as equivalents of a isorhamnetin-3-*O*-glucoside, cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside as equivalents of a cyanidin-3-*O*-galactoside, and peonidin-3-*O*-rutinoside as equivalents of a peonidin-3-*O*-glucoside. Hydroxycinnamic acids were detected and quantified at 280 nm, flavonol derivatives at 350 nm, and anthocyanins at 530 nm.

The average content of compounds was expressed in grams per kilogram of fresh weight (g/kg FW). For the general presentation, the sum of hydroxycinnamic acids (HCA), the sum of flavonols (FLAVO), the sum of anthocyanins (ANTH) and the sum of all phenolics were calculated.

Statistical analysis. The data were analysed using the R program (R Development Core Team, 2013). A two-way analysis of variance (ANOVA) was carried out to determine the influence of infection/tissue – tissue (*und*, *nec*, *h*) and time (harvesting date) on the phenolic profile. The influence of tissue type during the last stages of ripening on the composition of phenolics (relative values) in different types of tissue was examined on the basis of compositional data analysis, using isometric log-ratio transformation (*ilr*) based on sequential binary partition. Multivariate (MANOVA) and further univariate analysis of variance (ANOVA) were performed on *ilr* coordinates.

RESULTS AND DISCUSSION

The presence of PPV-Rec was confirmed in the trees showing sharka symptoms, but the symptomless trees proved to be PPV-free. All differences between infected and healthy plums were ascribed to PPV infection, given that other viruses that can affect stone fruit were not found.

Groups of phenolics

Three main groups of phenolics were detected in the edible part of plum fruit: anthocyanins, flavonols and, most commonly, hydroxycinnamic acids. All phenolics reported were present in all plum samples. The highest sum of phenolics was determined in *nec* on all sampling dates. HCA represented the predominant group of phenolics. It was followed by ANTH and FLAVO, but the content level of ANTH often exceeded the values of HCA of *nec* (Table 1, Fig. 1a).

The average content of HCA was significantly influenced by tissue type (Table 1). Symptomatic fruit had a significantly lower content level of HCA than that of *h*. This finding is contrary to previous reports about the accumulation of HCA as a plant response to pathogens (Mikulic Petkovsek et al. 2011) or to Ca^{2+} deficiency disorder (Zupan et al. 2013) in apple. During ripening, the relative content of HCA decreased as reported also Gordon et al. (2012). The interpretation could relate to the

Table 1. Average contents with standard errors (SE) (g/kg FW) of HCA, ANTH, FLAVO, and sum of phenolics for healthy (*h*), undeformed (*und*) and necrotic (*nec*) plum tissue during ripening time; $n = 5$

Time	Tissue	HCA		ANTH		FLAVO		Sum of phenolics
			SE		SE		SE	
*t*0	*h*	0.4^{aA}	0.08	0.04^{cC}	0.01	0.04^{cB}	0.007	0.5
	und	0.3^{bA}	0.03	0.12^{bC}	0.03	0.04^{bB}	0.009	0.5
	nec	0.3^{bA}	0.04	0.22^{aC}	0.06	0.06^{aB}	0.018	0.6
*t*9	*h*	0.4^{aA}	0.03	0.07^{cB}	0.02	0.04^{cA}	0.020	0.5
	und	0.2^{bA}	0.09	0.23^{bB}	0.05	0.09^{bA}	0.029	0.6
	nec	0.3^{bA}	0.04	0.49^{aB}	0.06	0.11^{aA}	0.017	0.9
*t*22	*h*	0.4^{aA}	0.04	0.14^{cA}	0.01	0.04^{cA}	0.013	0.6
	und	0.3^{bA}	0.05	0.34^{bA}	0.06	0.08^{bA}	0.016	0.7
	nec	0.3^{bA}	0.05	0.66^{aA}	0.11	0.10^{aA}	0.020	1.0

^{a,b}no statistically significant differences among averages marked with the same letter (Tukey's test); small letters indicate differences among tissues and capital letters among time points; time: *t*0 – August 20, *t*9 – August, *t*22 – September 11, 2013

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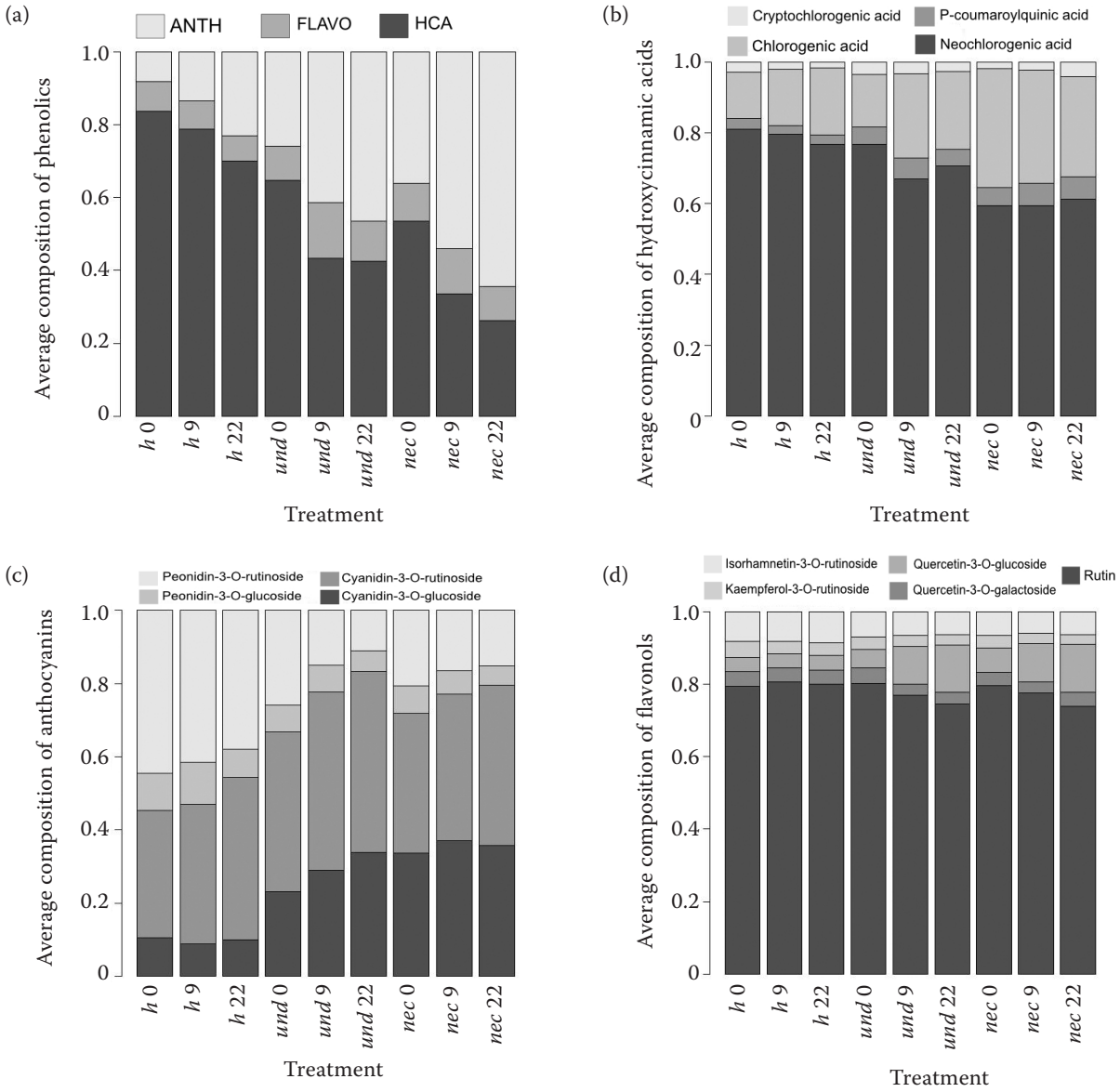

Fig. 1. The average composition of phenolics (a), hydroxycinnamic acids (b), anthocyanins (c), and flavonols (d) in plum fruit according to the infection/tissue and time

h – healthy tissue; *und* – visually unaffected tissue; *nec* – necrotic tissue

biosynthetic pathway of flavonoids, to which anthocyanins and flavonols belong. Flavonoid biosynthesis is initiated from of 4-coumaroyl-CoA (Falcone Ferreyra et al. 2012), which is the final product of the phenylpropanoid pathway. It is possible that stress-induced metabolic channeling determines increased synthesis of flavonoids, on account of reduced synthesis of hydroxycinnamic acids.

The infection caused a 4.6–6.8 times greater increase of ANTH and 1.6–2.6 times greater increase of FLAVO in *nec* when compared to *h*. PPV infection caused stress (Clemente-Moreno et al. 2015), which induced the biosynthesis of phenolics (Naczk and Shahidi 2004). Ripening significantly increased ANTH; the most in *nec*, and in *und* more than in *h*. The higher ANTH content in symptomatic fruit shows the stress-protective role of anthocyanins in the plant (Mikulic Petkovsek et al. 2011, 2013a,b). It is well known that a riper plum contains more anthocyanins (Diaz-Mula et al. 2008; Usenik et al. 2013). The same is true for FLAVO. The increased synthesis of phenolic compounds may be a result of higher enzymatic activity, a consequence of stimulated phenylalanine

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ammonialyase (PAL) enzyme activity under stress conditions (Slatnar et al. 2010).

The relative content of HCA during ripening decreased: 70–84 (*h*), 43–65 (*und*) and 26–54% (*nec*), and increased for ANTH: 8–23 (*h*), 26–46 (*und*), and 36–64% (*nec*) and FLAVO: 7.0–8.3 (*h*), 9.3–15.2 (*und*) and 9.5–12.5% (*nec*).

Our results show that the phenolic composition of *h* significantly differed from both *und* and *nec*. The average composition according to groups of phenolics is shown in Fig. 1a. The composition was analysed through two *ilr* coordinates representing the log-ratio between FLAVO and HCA (ilr_1 = $const_1$ln(FLAVO/HCA)) and the log-ratio between ANTH, HCA and FLAVO (ilr_2 = $const_2$ln(ANTH / g(HCA´FLAVO)). The coordinate ilr_1 was significantly influenced by the tissue type – the highest was that of *nec*, owing to the lower relative content of HCA. Ripening significantly increased the ilr_1 of symptomatic fruit, owing to a decrease in the relative content of HCA and an increase in FLAVO. This resulted in significantly higher ilr_1 in symptomatic fruit when compared to *h*. The interaction between tissue and time was statistically significant for ilr_2. The ilr_2 significantly increased during ripening, owing to the increase in ANTH and decrease in HCA, evidently the greatest in *nec* and the least in *h*.

Hydroxycinnamic acids

Four derivatives in this group were identified (Table 2): the predominant neochlorogenic acid (NA), chlorogenic acid (CA), *p*-coumaroylquinic acid (PCA), and cryptochlorogenic acid (CRY). The average content of NA and PCA was influenced only by tissue type. The *h* had a higher content level of NA than both *und* and *nec*. Contrary to our results, Mikulic Petkovsek et al. (2011) found that the *Xanthomonas arboricola pv. juglandis* (*Xaj*) infection in green walnut husk caused an increase in NA. The infection significantly increased the level of PCA, the most in *nec*. Thus, PCA can be proposed as a good indicator of stress, given that similar results were found in strawberry fruit infected with *Colletotrichum nymphaeae* (*Cn*) (Mikulic Petkovsek et al. 2013a), in green walnut husk infected with *Xaj* (Mikulic Petkovsek et al. 2011), and in apple pulp affected with bitter pit (Zupan et al. 2013). The content levels of CA and CRY depended on tissue type and ripening stage. Significantly more CA than in *h* was measured in *nec* on *t*0 and *t*9. There were no significant differences in CA between *h* and *und*. The CA in *nec* decreased significantly between *t*0 and *t*22.

The relative concentration ranges of individual hydroxycinnamic acids during ripening were these: 81–77 (*h*), 77–67 (*und*), and 59–61% (*nec*) for NA,

Table 2. Average contents with standard errors (SE) (g/kg FW) of individual hydroxycinnamic acids for healthy (*h*), undeformed (*und*) and necrotic (*nec*) plum tissue during the ripening time; $n = 5$

Time	Tissue	Neochlorogenic acid	SE	Chlorogenic acid	SE	*p*-coumaroylquinic acid	SE	Cryptochlorogenic acid	SE
*t*0	*h*	0.32^{aA}	0.06	0.05^{de}	0.01	0.01^{cA}	0.002	0.011^{a}	0.005
	und	0.22^{bA}	0.03	0.04^{e}	0.05	0.01^{bA}	0.001	0.009^{ab}	0.002
	nec	0.19^{bA}	0.02	0.11^{a}	0.02	0.02^{aA}	0.002	0.006^{b}	0.002
*t*9	*h*	0.33^{aA}	0.03	0.07^{cde}	0.01	0.01^{cA}	0.001	0.008^{ab}	0.000
	und	0.17^{bA}	0.07	0.06^{cde}	0.02	0.01^{bA}	0.003	0.008^{ab}	0.004
	nec	0.18^{bA}	0.03	0.10^{ab}	0.01	0.02^{aA}	0.002	0.007^{b}	0.001
*t*22	*h*	0.34^{aA}	0.03	0.08^{abc}	0.00	0.01^{cA}	0.000	0.006^{b}	0.002
	und	0.22^{bA}	0.05	0.07^{cde}	0.00	0.01^{bA}	0.000	0.008^{ab}	0.001
	nec	0.16^{bA}	0.03	0.08^{bcd}	0.02	0.02^{aA}	0.002	0.011^{a}	0.003

^{a,b}no statistically significant differences among averages marked with the same letter (Tukey's test); only small letters are used for indication of differences among treatments for the variables with statistically significant interaction between tissue and time. If the interaction was not statistically significant, small letters indicate differences among tissues and capital letters among time points. time: *t*0 – August 20, *t*9 – August, *t*22 – September 11, 2013

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13–19 (*h*), 15–24 (*und*), and 34–28 % (*nec*) for CA, 2.5–3.1 *(h*), 4.7–6.0 (*und*), and 5.1–6.3% (*nec*) for PCA, and 1.5–2.8 (*h*), 2.7–3.4 (*und*), and 1.8–4.1% (*nec*) for CRY (Fig. 1b).

The composition of hydroxycinnamic acids was analysed through three *ilr* coordinates, representing the log-ratio between PCA and NA: (ilr_1 = $const_1$ln(PCA/NA)), ilr_2 = $const_2$ln(CRY/CA) and ilr_3 = *const* ln(g(NA × PCA) / *g*(CA´CRY)). The ilr_1 coordinate was influenced only by tissue type – the highest was that for *nec* and the lowest that for *h*, owing to the higher relative content of NA. The interaction between tissue type and time was statistically significant for the ilr_2 coordinate: the highest was that for *h* and the lowest that for *nec* because of a higher relative content of CA in *nec*. The present results show a significant effect of tissue type and a negative time trend for the ilr_3 coordinate Symptomatic fruit had a lower relative content level of NA and a higher relative content level of CA compared to *h*.

Anthocyanins

In this group, cyanidin-3-*O*-glucoside (CG), cyanidin-3-*O*-rutinoside (CR), peonidin-3-*O*-glucoside (PG), and peonidin-3-*O*-rutinoside (PR) were identified (Table 3). Cyanidin-3-*O*-rutinoside has been ascertained as the main anthocyanin in the *Prunus domestica* plum, but the infection altered the most cyanidin-3-*O*-glucoside.

The relative concentration of anthocyanins in symptomatic fruit was evidently higher than that in *h*, with the highest levels in *nec*. The higher level of anthocyanins in *nec* is also consistent with the visual appearance; depressions on the fruit were darker than surrounding tissues. Fruit accumulate anthocyanins during maturation (Diaz-Mula et al. 2008; Usenik et al. 2009) and additionally under stress conditions (Valentinuzzi et al. 2015). Anthocyanins are involved also in internal regulation of plant cell physiology and signalling (Falcone Ferreyra et al. 2012).

On all sampling dates, a higher content of CR and PR was determined in *nec* when compared to *h*. The *und* shows similarities with *h*, except on *t*9 and *t*22 when a higher content of CR was measured in *und*. The content of both compounds increased with ripening only in *nec*. Tissue and time significantly influenced the content of CG and PG. Symptomatic fruit had significantly higher content levels of both anthocyanins, with *nec* higher than *und*. This can be attributed to the forced ripening of symptomatic fruit. Ripening increased the levels of CG and PG from *t*0 to *t*9.

The relative concentration ranges of individual anthocyanins increased with time: 35–44 (*h*), 44–49 (*und*), and 38–44% (*nec*) for CR, 9–11 (*h*), 23–34 (*und*), and 34–37% (*nec*) for CG, 38–44 (*h*), 11–26 (*und*), and 15–21% (*nec*) for PR, 8-12 (*h*), 5.6–7.4 (*und*), and 5.3–7.5% (*nec*) for PG (Fig. 1c). Anthocyanins are responsible for the colour changes associated with ripening (Usenik et al. 2009).

Table 3. Average contents with standard errors (SE) (g/kg FW) of individual anthocyanins for healthy (*h*), undeformed (*und*) and necrotic (*nec*) plum tissue during ripening time; *n* = 5

Time	Tissue	Cyanidin-3-rutinoside		Cyanidin-3-glucoside		Peonidin-3-rutinoside		Peonidin-3-glucoside	
			SE		SE		SE		SE
	h	0.01^{f}	0.01	0.004^{cC}	0.001	0.02^{e}	0.003	0.004^{cB}	0.001
*t*0	*und*	0.05^{def}	0.02	0.026^{bC}	0.006	0.03^{de}	0.006	0.009^{bB}	0.002
	nec	0.08^{de}	0.03	0.077^{aC}	0.031	0.04^{cd}	0.007	0.016^{aB}	0.004
	h	0.03^{ef}	0.00	0.006^{cB}	0.002	0.03^{de}	0.015	0.009^{cA}	0.004
*t*9	*und*	0.11^{cd}	0.03	0.065^{bB}	0.014	0.03^{d}	0.009	0.017^{bA}	0.003
	nec	0.20^{b}	0.05	0.181^{aB}	0.018	0.08^{b}	0.009	0.032^{aA}	0.009
	h	0.06^{def}	0.01	0.014^{cA}	0.004	0.05^{c}	0.005	0.012^{cA}	0.005
*t*22	*und*	0.17^{bc}	0.04	0.113^{bA}	0.021	0.04^{cd}	0.007	0.020^{bA}	0.006
	nec	0.29^{a}	0.05	0.240^{aA}	0.067	0.10^{a}	0.007	0.035^{aA}	0.005

for explanation see Table 2

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Three *ilr* coordinates were calculated: ilr_1 = $const_1$ln(CR/CG), ilr_2 = $const_2$ln(PR/PG) and ilr_3 = $const_3$ln(g(CG´CR)/g(PG × PR)). The interaction between tissue type and time was significant (P = 0.0727) for the ilr_1; the log-ratio of *und* during ripening decreased, owing to the relative increase in CG, but increased slightly in *h* and *nec*, owing to a relative increase in CR. The ratio between peonidins (ilr_2) was significantly affected by the interaction between tissue type and time. The highest was that for *h*, owing to the higher relative content of PR. The ratio shows similarities between *und* and *nec*. The ilr_3 coordinate was significantly influenced by tissue type. It was the highest for *nec* and the lowest for *h*, owing to the higher relative content of both peonidins. Ripening increased the ilr_3 log-ratio and resulted in a higher relative content level of cyanidins in *und* and *nec*. The ilr_3 coordinate shows evident differences between *h* and symptomatic fruit, where cyanidins predominate (83 in *und* and 79% in *nec* on *t*22) (Fig. 1c). The cyanidins of *h* represented 54% at the date of last sampling.

Flavonols

Here the prevalent rutin (RU), quercetin-3-*O*-galactoside (QGA), quercetin-3-*O*-glucoside (QGL), kaempferol-3-*O*-rutinoside (KR) and isorhamnetin-3-*O*-rutinoside (IR) were identified (Table 4). The greatest increase due to infection was recorded in quercetin-3-*O*-glucoside (2.7–7.3 times greater in *nec* and 1.4–6.0 times greater in *und*).

The results show significantly higher levels of QGL in symptomatic fruit on *t*9 and *t*22, which is further enhanced by maturation. The average content of all other identified flavonols was influenced by both tissue type and time. The symptomatic fruit had significantly more RU (1.1 to 1.9 times greater in *und* and 1.6–2.5 times greater in *nec*) and QGA (1.0–1.6 times greater in *und* and 1.5–2.3 times greater in *nec*). The RU and QGA were also further enhanced by maturation. Scab infection (Slatnar et al. 2010) and bitter pit also increased the synthesis of RU and quercetins in apples (Zupan et al. 2013). The highest level of KR and IR was measured in *nec*. Ripening had no influence on KR but increased IR between *t*0 and *t*9. Higher levels of kaempferol glycosides were also found in strawberry fruit infected with *Cn* (Mikulic Petkovsek et al. 2013a).

The relative ranges of individual flavonols during ripening were as follows: 79–81 (*h*), 75–80 (*und*), and 74–80% (*nec*) for RU, 3.8–4.2 (*h*), 3.0–4.1 (*und*), and 3.0–3.9% (*nec*) for QGA, 3.9–4.0 (*h*), 5.1–13.1 (*und*), and 6.6–13.2% (*nec*) for QGL, 3.5–4.3 (*h*), 2.8–3.5 (*und*) and 2.7–3.5% (*nec*) for KR, 8.1–8.8 (*h*), 6.3-6.9 (*und*), and 5.9–6.5% (*nec*) for IR (Fig. 1d).

The composition of flavonols was analysed through four *ilr* coordinates representing the log-ratio between phenolic compounds: ilr_1 = $const_1$ln(QGA / RU), ilr_2 = $const_2$ln(KR/QGL), ilr_3 =

Table 4. Average contents with standard errors (SE) (g/kg FW) of individual flavonols for healthy (*h*), undeformed (*und*) and necrotic (*nec*) plum tissue (tissue) during ripening (time); n = 5

Time	Tissue	Rutin	SE	Isorhamnetin-3-rutinoside	SE	Quercetin-3-galactoside	SE	Quercetin-3-glucoside	SE	Kaempferol-3-rutinoside	SE
	h	0.03^{cB}	0.01	0.003^{bB}	0.001	0.002^{cB}	0.000	0.002^{d}	0.001	0.002^{bA}	0.000
*t*0	*und*	0.03^{bB}	0.01	0.003^{bB}	0.001	0.002^{bB}	0.000	0.002^{cd}	0.001	0.002^{bA}	0.001
	nec	0.05^{aB}	0.02	0.004^{aB}	0.001	0.003^{aB}	0.001	0.004^{bc}	0.001	0.002^{aA}	0.001
	h	0.04^{cA}	0.02	0.004^{bA}	0.002	0.002^{cAB}	0.001	0.002^{d}	0.001	0.002^{bA}	0.001
*t*9	*und*	0.07^{bA}	0.02	0.006^{bA}	0.002	0.003^{bAB}	0.001	0.009^{ab}	0.002	0.003^{bA}	0.001
	nec	0.09^{aA}	0.02	0.007^{aA}	0.001	0.003^{aAB}	0.001	0.012^{a}	0.002	0.003^{aA}	0.001
	h	0.04^{cA}	0.01	0.004^{bA}	0.001	0.002^{cA}	0.000	0.002^{d}	0.001	0.002^{bA}	0.001
*t*22	*und*	0.06^{bA}	0.01	0.005^{bA}	0.002	0.003^{bA}	0.000	0.011^{a}	0.004	0.002^{bA}	0.001
	nec	0.07^{aA}	0.01	0.006^{aA}	0.001	0.004^{aA}	0.001	0.013^{a}	0.004	0.003^{aA}	0.001

for explanation see Table 2

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$const_3\ln(g(\text{QGL´KR})/\text{IR})$, and $ilr_4 = const_4\ln(g(\text{QGL´KR´IR}) / g(\text{RU´QGA}))$. There was no significant effect of tissue type or time on the ilr_1 coordinate. The interaction between tissue and time was statistically significant for the ilr_2, ilr_3 and ilr_4 coordinates. This means that the composition of symptomatic fruit differed significantly from *h* and between harvesting dates. The ilr_3 and ilr_4 coordinates of *und* and *nec* during ripening increased because of increased levels of QGL (ilr_3) and decreased levels of RU (ilr_4). Ripening had no effect on the ilr_3 and ilr_4 coordinates of *h*.

The composition of phenolics in plum fruit was significantly modified by PPV infection. The alteration in the composition of phenolics is only one of the symptoms and the consequence of a defense response against the pathogen. The infection causes stress, against which the plant responds with synthesis of flavonoids. The other studies have linked flavonoids with the control of the polar transport of auxins (Falcone Ferreyra et al. 2012). Manipulating the plant by its own growth-promoting signalling molecules might be a widespread strategy of pathogens (Ludwig-Műller 2015). Apart from the elevated level of flavonoids the content *p*-coumaroylquinic acid was higher in both undeformed and necrotic tissues when compared to healthy tissue. Since similar changes were observed in tissues of other fruits stressed due to biotic and abiotic causal agents, *p*-coumaroylquinic acid can be considered as a good indicator of stress. It could therefore be used in different studies e.g. of fruit physiology or as an indicator of stress caused by unknown factor even before the appearance of visual symptoms. As such, it could be a useful also in plant breeding for detection of latent or hidden infections.

References

Cambra M., Capote N., Myrta A., Llácer G. (2006): *Plum pox virus* and estimated costs associated with sharka disease. Bulletin OEPP, 36: 202–204.

Clemente-Moreno M.J., Hernández J.A., Diaz-Vivancos P. (2015): Sharka: how do plants respond to *Plum pox virus* infection? Journal of Experimental Botany, 66: 25–35.

Diaz-Mula H.M., Zapata P.J., Guillén F., Castillo S., Martinez-Romero D., Valero D. (2008): Changes in physicochemical and nutritive parameters and bioactive compounds during development and on tree ripening of eight plum cultivars: a comparative study. Journal of the Science of Food and Agriculture, 88: 2499–2507.

Falcone Ferreyra M.L., Rius S.P., Casati P. (2012): Flavonoids: biosynthesis, biological functions, and biotechnological applications. Frontiers in Plant Science, 3: 1–15.

Gordon A., Cruz A.P.G., Cabral L.M.C., de Freitas S.C., Taxi C.M.A.D., Donangelo C.M., Mattietto R.A., Friedrich M., de Matta V.M., Marx F. (2012): Chemical characterization and evaluation of antioxidant properties of Açaí fruits (*Euterpe oleraceae* Mart.) during ripening. Food Chemistry, 133: 256–263.

Kamenova I., Milusheva S. (2005): Sharka disease in Bulgaria: past, present and future. Biotechnology & Biotechnological Equipment, 19: 22–40.

Lácer G., Cambra M. (2006): Hosts and symptoms of *Plum pox virus*: fruiting *Prunus* species. Bulletin OEPP, 36: 219–221.

Ludwig-Műller J. (2015): Bacteria and fungi controlling plant growth by manipulating auxin: Balance between development and defence. Journal of Plant Physiology, 172: 4–12.

Mikulic Petkovsek M., Slatnar A., Veberic R., Stampar F., Solar A. (2011): Phenolic response in green walnut husk after the infection with bacteria *Xanthomonas arboricola* pv. *juglandis*. Physiological and Molecular Plant Pathology, 76: 159–165.

Mikulic Petkovsek M., Schmitzer V., Slatnar A., Weber N., Veberic R., Stampar F., Munda A., Koron D. (2013a): Alteration of the content of primary and secondary metabolites in strawberry fruit by *Colletotrichum nymphaeae* infection. Journal of Agricultural and Food Chemistry, 61: 5987–5995.

Mikulic Petkovsek M., Slatnar A., Schmitzer V., Stampar F., Veberic R., Koron D. (2013b): Chemical profile of black currant fruit modified by different degree of infection with black currant leaf spot. Scientia Horticulturae, 150: 399–409.

Milosevic, T.M., Glisic I.P., Milosevic N.T., Glisic I.S. (2010): Plum pox virus as a stress factor in the vegetative growth, fruit growth and yield of plum (*Prunus domestica*) cv. 'Cacanska rodna'. European Journal of Plant Pathology, 126: 73–79.

Naczk M., Shahidi F. (2004): Extraction and analysis of phenolics in food. Journal of Chromatografy A, 1054: 95–111.

R Development Core Team (2013): R, a language and environment for statistical computing. Austria; R foundation for statistical computing, Vienna. Available at: http://www.R-project.org/

Slatnar A., Mikulic Petkovsek M., Halbwirth H., Stampar F., Stich K., Veberic R. (2010). Response of the phenylpropanoid pathway to *Venturia inaequalis* infection in maturing fruit of 'Braeburn' apple. Journal of Horticultural Science and Biotechnology, 85: 465–472.

Sochor J., Babula P., Adam V., Krska B., Kizek R. (2012). Sharka: the past, the present and the future. Viruses, 4: 2853–2901.

doi: 10.17221/196/2015-HORTSCI

Usenik V., Stampar F., Fajt N. (2007): Pomological and phenological characteristics of some autochthonous Slovenian plum cultivars. Acta Horticulturae (ISHS), 734: 53–59.

Usenik V., Kastelec D., Veberic R., Štampar F. (2008): Quality changes during ripening od plums (*Prunus domestica* L.). Food Chemistry, 111: 830–836.

Usenik V., Stampar F., Veberic R. (2009): Anthocyanins and fruit colour in plums (*Prunus domestica* L.) during ripening. Food Chemistry, 114: 529–534.

Usenik V., Stampar F., Kastelec D. (2013): Phytochemicals in fruits of two *Prunus domestica* L. plum cultivars during ripening. Journal of the Science of Food and Agriculture, 93: 681–692.

Valentinuzzi F., Mason M., Scampicchio M., Andreotti C., Cesco S., Mimmo T. (2015): Enhancement of the bioactive compound content in strawberry fruits grown under iron and phosphorus deficiency. Journal of the Science of Food and Agriculture, 95: 2088–2094.

Virscek Marn M., Mavric Plesko I., Zindovic J., Miladinovic Z. (2012): Diversity of *Plum pox virus* isolates in Montenegro. Journal of Plant Pathology, 94: 201–204.

Wang S.Y., Zheng W., Galletta G.J. (2002): Cultural system affects fruit quality and antioxidant capacity in strawberries. Journal of Agricultural and Food Chemistry, 50: 6534–6542.

Zupan A., Mikulic Petkovsek M., Cunja V., Štampar F., Veberič R. (2013): Comparison of phenolic composition of healthy apple tissues and tissues affected by bitter pit. Journal of Agricultural and Food Chemistry, 61: 12066–12071.

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