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# Scion-Rootstock Interaction Affects the Phytochemical Composition of *Prunus* Phloem

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**Summary**

The focus of our investigation was the influence of various rootstocks for peach of different genetic origin on phytochemical composition in the phloem above and below the graft union, and the role of phenols in graft compatibility/incompatibility. Assays of phloem with cambium from 6-year-old 'Royal Glory' peach trees, grafted on rootstocks of various genetic origin, peach seedling (*Prunus persica* L.), GF 677 and Monegro (both hybrids of *Prunus persica* L. × *Prunus amygdalus* L.), Cadaman and Barrier (both hybrids of *Prunus persica* L. × *Prunus davidiana* L.), and Penta and Tetra (both belonging to *Prunus domestica* L.) were analysed with HPLC-MS. Twenty-one phenolics were detected in the phloem of *Prunus persica* and their hybrid rootstocks, and 10 phenolics in *Prunus domestica* rootstocks. The most abundant phenolic group in the phloem of *Prunus persica* and *Prunus domestica* rootstocks was the flavanols. 'Royal Glory' is highly compatible with peach seedling, GF 677 and Monegro rootstocks. In Cadaman rootstock, accumulation of all flavonols, and in Barrier, the flavonols quercetin 3-glucoside and isorhamnetin 3-glucoside, and the flavanone prunin were observed above the graft union, which is related to graft incompatibility. Hydoxycinnamic acids, unknown phenols as well as two dimeric procyanidins were present only in plum rootstocks. The level of all analysed flavonols, flavanone naringenin hexoside, and flavanols (except catechin, epicatechin and procyanidin B1) were significantly higher above the graft union in *Prunus domestica* rootstocks, which indicates that flavonols could also be involved in the graft incompatibility process.

**Key words.** graft union – HPLC-MS – incompatibility – individual phenols – peach rootstock

## Introduction

Peach is the third most important deciduous fruit tree worldwide, ranking after apples and pears (FAOSTAT 2012), where the rootstock represents an important consideration for a productive peach orchard, particularly in an orchard replanting situation. Apart from peach seedlings (*Prunus persica* L.), various species of stone fruit can be used as peach rootstocks, for example, plum (*Prunus domestica* L.) and peach × almond hybrids. These are chosen to tolerate undesirable soil properties, such as alkalinity, waterlogging and drought (Hudina et al. 2006; Byrne et al. 2012). The appearance of phenomena related to soil sickness has been known for many years in the practice of replanting (Massai and Loreti 2004).

Special scion/rootstock combinations have been developed for optimized fertility, fruit yield and high fruit quality – all of which benefit from the rootstock's influence on the uptake and transport of water and minerals (Gruppe 1985). The interaction between the two partners also changes the hormonal situation in both the scion and the rootstock (Stevens and Westwood 1984). In addition, rootstocks of different genetic origin have an influence on the vigour, yield (Massai and Loreti 2004; Hudina et al. 2006; Jiménez et al. 2011; Orazem et al. 2011a; Font i Forcada et al. 2012), phenology (Beckman et al. 1992) and fruit quality (Orazem et al. 2011b; Tavarini et al. 2011; Font i Forcada et al. 2013) of peach trees.

It is known that the accumulation of polyphenols is also related to different types of stress and defense responses (Bennett and Wallsgrove 1994). Environmental conditions, site effects and orchard management modify the phenol content (Mikulic Petkovsek et al. 2010) as well as the physiological stage of the tissues (Usenik et al. 2008). On the other hand, phenolic profiles are specific and can be used for cultivar identification, a method which was applied for sweet cherries, sour cherries, and plums (Treutter 2010), for determining genuineness of some fruit jams and juices (Andrade et al. 1998); these profiles are also involved in graft compatibility/incompatiblity.

The compounds potentially most suited as indicators of graft incompatibility are, in general, those found in both scion and stock (Musacchi et al. 2000). Differences in the phenol content between grafting partners are important, when accumulation of a specific phenol occurs above the graft union. Qualitative and quantitative differences in phenol content produced by heterospecific grafts may result in metabolic dysfunctions between the cells of the scion-stock graft union (Errea 1998; Musacchi et al. 2000).

The effect of grafting on the phenolic compounds in the bark was studied for cherries and apricots. Treutter et al. (1986) noticed in the bark just above the union an accumulation of *p*-coumaroylglucose, of flavanones (*i.e.*, naringenin 7-glucoside (prunin)), of the isoflavone genistin and of flavan 3-ols in cherries and Errea et al. (1994) of flavan 3-ols in apricot. Accumulation of those metabolites may indicate a lack of differentiation in the phloem and cambium region of the bark (Treutter et al. 1985), since naringenin 7-glucoside and high concentrations of flavan 3-ols are potent markers of undifferentiated callus-like cells (Feucht et al. 1988).

The aim of this study was to detect major phenolic compounds in the phloem of the 'Royal Glory' peach cultivar, grafted on rootstocks of different genetic origin, below and above the graft union. Individual phenolic compounds were determined using the HPLC-MS. Another objective of this research was to determine whether the differences in phenolic compounds above and below the graft union in the peach cultivar 'Royal Glory' grafted on various rootstocks of different genetic origin could be attributed to graft compatibility/incompatibility.

## Materials and Methods

### *Plant material and sampling*

The investigation was carried out using phenol content in plant tissues of peach (*Prunus persica* L.) trees. The content of phenols in 6-year-old peach trees of the 'Royal Glory' cultivar, which were grafted on rootstocks of different genetic origin: peach seedling (*Prunus persica* L.), GF 677 and Monegro (*Prunus persica* L. × *Prunus amygdalus* L.), Cadaman and Barrier (*Prunus persica* L. × *Prunus davidiana* L.), Penta and Tetra (*Prunus domestica* L.), were analysed. Twelve trees for each scion/rootstock combination were planted in an experimental orchard, the Fruit Growing Center Bilje near Nova Gorica (Slovenia, 45.89° N, 13.62° E). Trunk circumferences were measured 20 cm above the graft union on 12 trees. Three trees per scion/rootstock combination were selected for the analysis of phenolic compounds. Samples of phloem were collected on 6$^{th}$ June 2012. Small sections of bark (1 cm above and below the graft union, 3 cm in length and 1 cm in width) were removed with a knife and samples were instantly frozen in liquid nitrogen.

### *Extraction of phenolic compounds*

The samples for phenolic analysis were extracted according to the method described by Escarpa and González (1998). 100 mg of finely ground phloem was extracted with 2 ml of extraction solution (methanol containing 3 % formic acid and 1 % 2,6-di-*tert*-butyl-4-methylphenol (BHT)) in an ultrasonic ice bath for 1 h before centrifuging at 10,000 rpm for 7 min at 5 °C. The supernatant was filtered through a Chromafil AO-45/25 polyamide filter (Macherey-Nagel, Düren, Germany) prior to injection into the HPLC system.

The analysis of phenolic compounds was carried out using a Thermo Finnigan Surveyor HPLC system with a diode array detector at 280 and 350 nm. The spectra of compounds were recorded between 210 and 400 nm. The flavanols (catechin, epicatechin, procyanidins) were analysed at 280 nm, and flavonols (quercetin 3-galactoside, quercetin 3-glucoside, quercetin hexoside, isorhamnetin 3-galactoside, isorhamnetin 3-glucoside and isorhamnetin 3-hexoside), and flavanones (naringenin 7-glucoside (prunin), naringenin hexoside) were analyzed at 350 nm (Pereira et al. 2013). The compounds were separated on a Phenomenex Gemini C18 (150 × 4.6 mm 3 μm) (Torrance, CA) column, operated at 25 °C with the elution solvent A (1 % aqueous formic acid) and B (100 % acetonitrile). Flow rate was 1 ml min$^{-1}$. The injection volume was 20 μl. The gradient method used was as described by Marks et al. (2007). Quantification of individual phenolic compounds was achieved according to the concentrations of the corresponding external standard.

All phenolic compounds presented in our results were identified by an HPLC-MS detector and a LCQ Deca XP MAX (Thermo Finnigan, San Jose, CA) instrument with an electrospray interface (ESI) operating in negative ion mode. The analyses were performed using full-scan, data dependent MS$^n$ scanning from *m/z* 115 to 2000. Column and chromatographic conditions were identical to those used for the HPLC-DAD analyses. Identification of compounds was achieved by comparing the retention times and spectra as well as by addition of the corresponding external standard.

### *Statistical analysis*

Statistical significance was determined by one-way analysis of variance (ANOVA) using the Statgraphics Plus 4.0 program (Manugistics, Rockville, Maryland, MD, USA). Differences in phenolic content between rootstocks were estimated by the HSD test at $P < 0.05$, and differences between sampling locations (above and below the graft union) were tested with t-test. Two-way analysis of variance was used to determine the effect of rootstocks and sampling locations (above and below the graft union) and their interaction on the phenolic content at a significance level of 0.05.

## Results and Discussion

### *Determination of individual phenols*

HPLC tandem MS was used to determine individual phenols in the phloem of 'Royal Glory', grafted on rootstocks of different genetic origin, below and above the graft union. Fig. 1 shows a typical HPLC chromatogram of *Prunus persica* rootstock at 280 nm, and Fig. 2 at 350 nm. HPLC and MS data of *Prunus persica* and *Prunus domestica* rootstocks are summarised in Table 1. Many phenolic compounds were detected in *Prunus persica* phloem by the

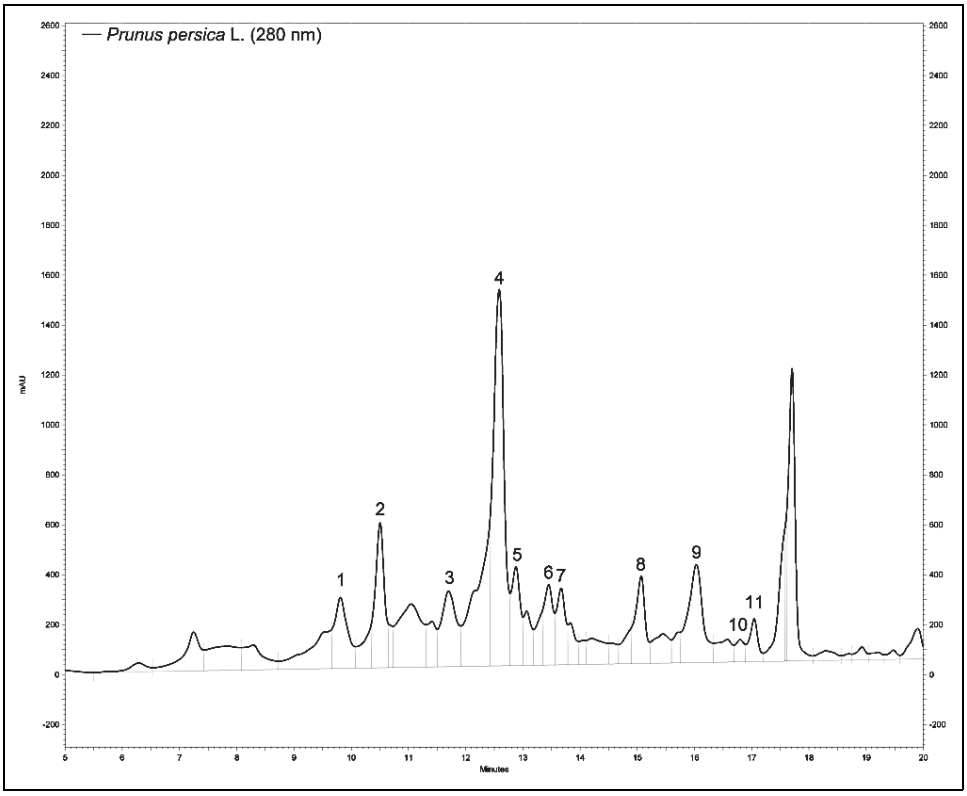



Fig. 1. HPLC chromatogram of phenolic compounds in *Prunus persica* phloem below the graft union at 280 nm: 1 – procyanidin dimer 1 (procyanidin B1); 2 – procyanidin dimer 2; 3 – procyanidin trimer 1; 4 – catechin; 5 – procyanidin trimer 2; 6 – procyanidin tetramer 1; 7 – procyanidin dimer 3; 8 – epicatechin; 9 – procyanidin trimer 3; 10 – procyanidin tetramer 2; 11 – procyanidin dimer 4.

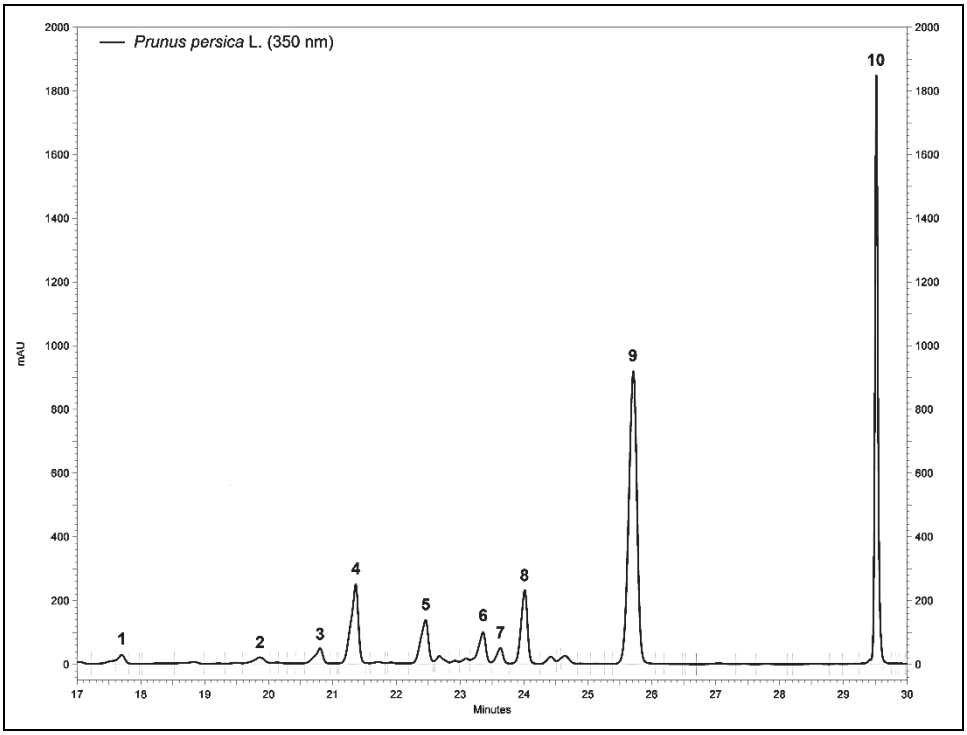



Fig. 2. HPLC chromatogram of phenolic compounds in *Prunus persica* phloem below the graft union at 350 nm: 1 – myricetin hexoside; 2 – naringenin hexoside; 3 – quercetin 3-galactoside; 4 – quercetin 3-glucoside; 5 – prunin (naringenin 7-glucoside), 6 – quercetin hexoside 1; 7 – isorhamnetin 3-galactoside; 8 – isorhamnetin 3-glucoside; 9 – quercetin hexoside 2; 10 – isorhamnetin hexoside.

Table 1. Identification of phenolic compounds in *Prunus persica* L. and *Prunus domestica* L. rootstocks in negative ions with HPLC–MS and $MS^2$.

| Origin | Peak | λ (nm) | $[M-H]^-$ (*m/z*) | $MS^2$ (*m/z*) | Tentative identification |
|---|---|---|---|---|---|
| *Prunus persica* L. | 1 | 280 | 577 | 451, 425, 407, 289 | Procyanidin dimer 1 (procyanidin B1) |
| | 2 | 280 | 577 | 425, 407, 289 | Procyanidin dimer 2 |
| | 3 | 280 | 865 | 577, 425, 407, 289 | Procyanidin trimer 1 |
| | 4 | 280 | 289 | 245 | Catechin |
| | 5 | 280 | 865 | 577, 425, 407, 289 | Procyanidin trimer 2 |
| | 6 | 280 | 1153 | 865, 577, 407 | Procyanidin tetramer 1 |
| | 7 | 280 | 577 | 425, 407, 289 | Procyanidin dimer 3 |
| | 8 | 280 | 289 | 245 | Epicatechin |
| | 9 | 280 | 865 | 577, 425,407, 289 | Procyanidin trimer 3 |
| | 10 | 280 | 1153 | 865, 577, 407 | Procyanidin tetramer 2 |
| | 11 | 280 | 577 | 451, 425, 407, 289 | Procyanidin dimer 4 |
| | 12 | 350 | 479 | 317 | Myricetin hexoside |
| | 13 | 350 | 433 | 271 | Naringenin hexoside |
| | 14 | 350 | 463 | 301 | Quercetin 3-galactoside |
| | 15 | 350 | 463 | 301 | Quercetin 3-glucoside |
| | 16 | 350 | 433 | 271 | Naringenin 7-glucoside (Prunin) |
| | 17 | 350 | 463 | 301 | Quercetin hexoside 1 |
| | 18 | 350 | 477 | 315 | Isorhamnetin 3-galactoside |
| | 19 | 350 | 477 | 315 | Isorhamnetin 3-glucoside |
| | 20 | 350 | 463 | 301 | Quercetin hexoside 2 |
| | 21 | 350 | 477 | 315 | Isorhamnetin hexoside |
| *Prunus domestica* L. | 22 | 280 | 577 | 451, 425, 407, 289 | Procyanidin dimer 1 (procyanidin B1) |
| | 23 | 280 | 577 | 425, 407, 289 | Procyanidin dimer 5 |
| | 24 | 280 | 353 | 191, 179 | Caffeoylquinic acid |
| | 25 | 280 | 289 | 245 | Catechin |
| | 26 | 280 | 577 | 425, 407, 289 | Procyanidin dimer 6 |
| | 27 | 280 | 289 | 245 | Epicatechin |
| | 28 | 280 | 325 | 163, 119 | *p*-coumaric acid hexoside |
| | 29 | 350 | 383 | 221, 206 | Unknown phenol 1 |
| | 30 | 350 | 343 | 181 | Unknown phenol 2 |
| | 31 | 350 | 433 | 271 | Naringenin 7-glucoside (Prunin) |

HPLC system, and we identified 21 of these by MS (Table 1). The HPLC chromatogram of *Prunus domestica* rootstock at 280 nm is shown in Fig. 3, and at 350 nm in Fig. 4. We identified 10 phenolic compounds in *Prunus domestica* rootstock by MS.

The major group of phenols in the phloem of *Prunus persica* rootstock was the flavan 3-ols. Cherry phloem is also rich in flavan-3-ols and their oligomeric compounds (Feucht et al. 1992). Peaks 4 and 8 had a negatively charged molecular ion ($[M-H]^-$) at *m/z* 289 and $MS^2$ fragmentation at *m/z* 245 (Table 1). After comparison of retention time with the standards, peak 4 was identified as (+)-catechin and peak 8 as (−)-epicatechin. Peaks 1 and 11 had a fragmentation pattern of $[M-H]^-$ at *m/z* 577 and $MS^2$ fragmentation at *m/z* 451, 425, 407 and 289. Tomas-Barberan et al. (2001) reported that procyanidin dimers had $[M-H]^-$ at *m/z* 577 and $MS^2$ fragmentation at m/z 425 and 289. Li et al. (2012) found in the $MS^2$ spectrum of *m/z* 577 four fragment ions at *m/z* 451, 425, 407 and 289. The first fragment ion at *m/z* 451 resulted from the elimination of a phloroglucinol molecule from the B-type procyanidin dimer. The fragment ion at *m/z* 425 and at *m/z* 407 came from the RDA-F (retro-Diels-Alder fission), while the latter eliminated a water molecule (Friedrich et al. 2000; Shui and Leong 2004). After comparison of retention times with the standard, peak 1 was found to be procyanidin B1. Peak 2 and 7 also had a fragmentation pattern of $[M-H]^-$ at *m/z* 577 and $MS^2$ fragmentation at *m/z* 425, 407 and 289 and were identified as procyanidin dimers, since Li et al. (2012) identified the

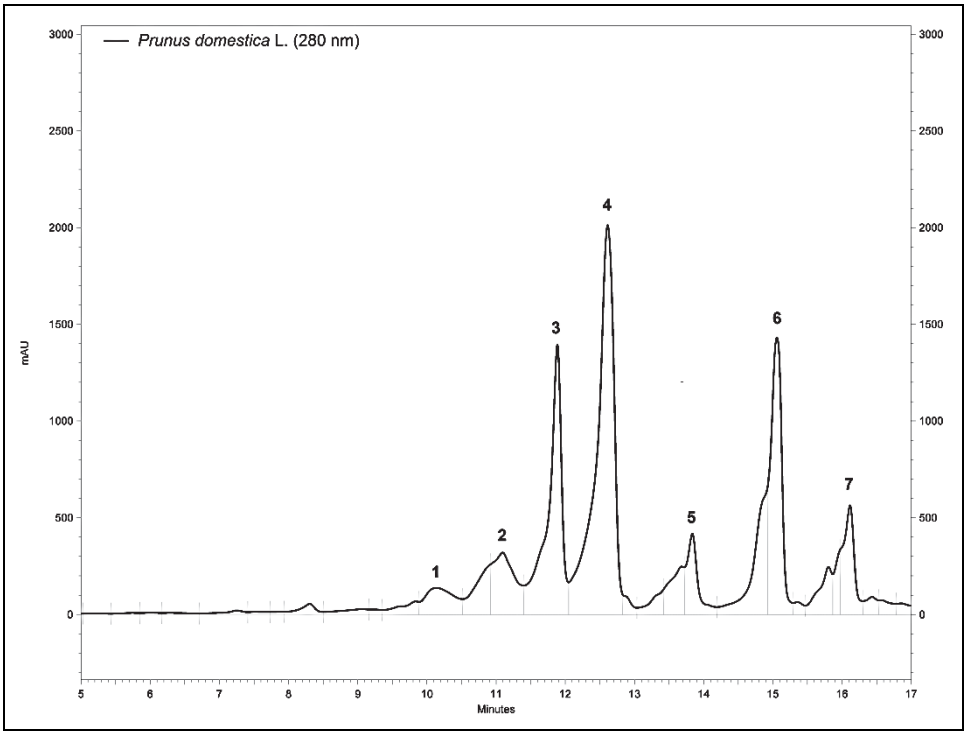



Fig. 3. HPLC chromatogram of phenolic compounds in *Prunus domestica* phloem below the graft union at 280 nm: 1 – procyanidin dimer 1 (procyanidin B1); 2 – procyanidin dimer 5; 3 – caffeoylquinic acid; 4 – catechin; 5 – procyanidin dimer 6; 6 – epicatechin; 7 – *p*-coumaric acid 4-glucoside.

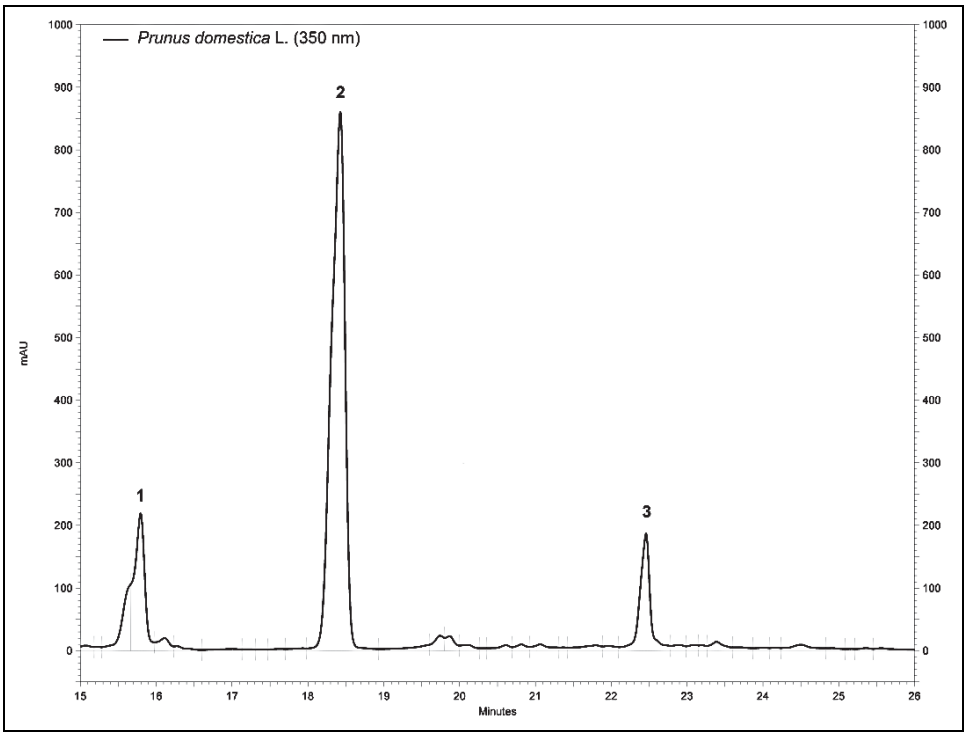



Fig. 4. HPLC chromatogram of phenolic compounds in *Prunus domestica* phloem below the graft union at 350 nm: 1 – unknown phenol 1; 2 – unknown phenol 2; 3 – prunin (naringenin 7-glucoside).

same fragmentation as a B-type procyanidin dimer. Peaks 3, 5, and 9 were all detected at λ = 280 nm, molecular ion $[M–H]^-$ at *m/z* 865, $MS^2$ fragment at *m/z* 577, 425, 407 and 289. Since Tomas-Barberan et al. (2001), Jakopic et al. (2011) and Li et al. (2012) reported that procyanidin trimers have a negatively charged molecular ion at *m/z* 865 and yield $MS^n$ at *m/z* 577 and 289, these peaks were identified as procyanidin trimers. We also found two procyanidin tetramers (peak 6 and 10) which had a negatively charged molecular ion ($[M–H]^-$) at *m/z* 1153 and $MS^2$ fragmentation at *m/z* 865, 577 and 407.

The second major group of phenols in the phloem of *Prunus persica* rootstocks was the flavonols, which were detected at λ = 350 nm. Peaks 14, 15, 17 and 20 had a

fragmentation pattern of $[M-H]^-$ at *m/z* 463, $MS^2$ fragment at *m/z* 301 (Table 1). This fragmentation pattern is indicative of quercetin. The neutral loss associated with *m/z* 301 (from *m/z* 463) indicates the presence of a 162 amu hexose sugar (either glucose or galactose) (Reed 2009). After comparison of retention time with the standards, peak 14 was identified as quercetin 3-galactoside, a compound that had been previously reported in peach fruit (Henning and Herrmann 1980), and peak 15 as quercetin 3-glucoside. The HPLC-MS analysis confirmed and indicated that peak 17 and 20 were quercetin hexoside. Peak 12 had the $[M-H]^-$ at *m/z* 463 and $MS^2$ fragmentation yielded a myricetin ion at *m/z* 317 and was identified as myricetin hexoside. Peak 19 had a fragmentation pattern of $[M-H]^-$ at *m/z* 477, $MS^2$ fragment at *m/z* 315 (Table 1). This fragmentation pattern is indicative of isorhamnetin. The neutral loss associated with *m/z* 315 equals 162 amu, revealing the presence of a single glucose unit. As seen by the fragmentation patterns of the established standards, Reed (2009) stated that this dissociation scheme closely follows the ion spectra for the flavonoid isorhamnetin. Therefore, it is hypothesized that this compound is most likely isorhamnetin 3-glucoside. Peak 18 was identified as isorhamnetin 3-galactoside according to comparison of retention time with the standard and peak 21 as isorhamnetin hexoside.

In the phloem of *Prunus persica* rootstocks, two flavanones (naringenin hexoside and naringenin 7-glucoside (prunin)) were also identified. Both had the $[M-H]^-$ at *m/z* 433, and $MS^2$ fragmentation yielded a naringenin ion at *m/z* 271, what is in accordance with Pereira et al. (2013). Peak 13 was identified as naringenin hexoside and peak 16 as prunin.

The major group of phenols in the phloem of *Prunus domestica* rootstocks was the flavan 3-ols. The HPLC chromatograms of *Prunus domestica* rootstock were quite different from those of *Prunus persica* and their hybrid rootstocks, although they contained the same groups of compounds. Peaks 25 and 27 in Table 1 had a negatively charged molecular ion ($[M-H]^-$) at *m/z* 289 and $MS^2$ fragmentation at *m/z* 245. Peak 25 was identified as (+)-catechin and peak 27 as (−)-epicatechin. Peaks 22, 23 and 26 were identified as procyanidin dimers. Peaks 24 and 28 were also detected at $\lambda = 280$ nm. Peak 24 had a negatively charged molecular ion $[M-H]^-$ at m/z 353, an $MS^2$ fragment at *m/z* 191 and 179; it was identified as caffeoylquinic acid, a finding also reported by Clifford et al. (2003). Peak 28, with the molecular ion $[M-H]^-$ at *m/z* 325, and an $MS^2$ fragment at *m/z* 163 and 119, was identified as *p*-coumaric acid hexoside, since Reed (2009) reported that *p*-coumaric acid has a negatively charged molecular ion at *m/z* 325 and yields $MS^n$ at *m/z* 163 and 119.

At $\lambda = 350$ nm, three phenolic compounds were identified. Peak 29 (Table 1) had the $[M-H]^-$ at *m/z* 383, $MS^2$ fragmentation at *m/z* 221 and 206, while peak 30 had a negatively charged molecular ion $[M-H]^-$ at *m/z* 343 and $MS^2$ fragmentation at *m/z* 181. On the basis of their $MS^n$ spectra, we could identify none of these. Peak 31, with a molecular ion $[M-H]^-$ at *m/z* 433 and an $MS^2$ fragment at *m/z* 271, was identified as prunin.

### *Comparison of phenol content above and below graft union among rootstock of different genetic origin*

Content of all phenolic compounds was determined in 7 rootstocks for peach of different genetic origin: two rootstocks of *Prunus persica* × *Prunus amygdalus* (GF 677 and Monegro), peach seedling (*Prunus persica*), two rootstocks of *Prunus persica* × *Prunus davidiana* (Cadaman and Barrier) and two rootstocks of *Prunus domestica* (Penta and Tetra) (Table 2 and 3). All samples of *Prunus persica* and their hybrid rootstocks exhibited similar phenolic profiles, which consisted of catechin, epicatechin, procyanidin dimers (1 to 4), procyanidin trimers, procyanidin tetramers, quercetin 3-glucoside, quercetin 3-galactoside, quercetin hexoside 1 and 2, myricetin hexoside, naringenin hexoside, prunin, isorhamnetin 3-galactoside, isorhamnetin 3-glucoside, isorhamnetin hexoside. Besides catechin, epicatechin, procyanidin B1 and prunin in the phloem above and below the graft union of *Prunus persica* and their hybrids, *Prunus domestica* rootstocks Penta and Tetra also contained procyanidin dimer 5 and procyanidin dimer 6, caffeoylquinic acid, *p*-coumaric acid 4-glucoside and two unknown phenols, which were not found in the phloem of *Prunus persica* and their hybrid rootstocks.

Rootstock had a significant influence on all phenolic compounds analysed, except on quercetin 3-glucoside and quercetin hexoside 2 (Table 4). The sampling location (above and below the graft union) significantly influenced the levels of all analysed flavonols, hydoxycinnamic acids, unknown phenols, flavanone naringenin hexoside, and most of the flavanols (except procyanidin dimer 3, procyanidin trimers 2 and 3, procyanidin tetramer 1).

In our experiment, we noted no significant differences in peach seedling between individual phenolic compounds above and below the graft union, except for procyanidin tetramer 1, where the content level was significantly higher below the graft union (Table 3). Prunin and high concentrations of flavan 3-ols are potent markers of undifferentiated callus-like cells (Feucht et al. 1988). In addition, catechin and other phenolic compounds which accumulate above the graft union could be the first biochemical response to incompatibility (Cooman et al. 1996), and relatively small quantities of phenols could be enough to produce locally limited dysfunctions in the interphase between two cells (Errea 1998). Since there was no accumulation of phenolic compounds above the graft union, it can be concluded that 'Royal Glory' is highly compatible with peach seedling rootstock.

The same results were noted in *Prunus persica* × *Prunus amygdalus* rootstocks GF 677 and Monegro, where the procyanidin dimer 3 level was significantly higher below the graft union. In Monegro rootstock, epicatechin

Table 2. The content of individual phenolic compounds (mg $kg^{-1}$ of phloem) ± standard error in the phloem above and below the graft union of 'Royal Glory' grafted on *Prunus persica* × *Prunus amygdalus* and *Prunus persica* × *Prunus davidiana* rootstocks. Values represent means of 3 replications ± standard error.

| Genetic origin | *Prunus persica* × *Prunus amygdalus* | | | | *Prunus persica* × *Prunus davidiana* | | | |
|---|---|---|---|---|---|---|---|---|
| Rootstock | GF 677 | | Monegro | | Cadaman | | Barrier | |
| Phenolics | Above* | Below | Above | Below | Above | Below | Above | Below |
| Catechin | 8.459 ± 0.332[x] | 8.190 ± 0.0453 | 10.073 ± 0.596 | 8.490 ± 0.297 | 8.775 ± 0.996 | 9.845 ± 1.264 | 10.254 ± 0.526 | 9.157 ± 1.096 |
| Epicatechin | 2.951 ± 0.676 | 3.733 ± 0.161 | **2.512 ± 0.221 b** | **8.668 ± 1.187 a** | **1.865 ± 0.375 b** | **4.489 ± 0.561 a** | 2.645 ± 0.314 | 3.619 ± 0.605 |
| Procyanidin dimer 1 (B1) | 3.218 ± 0.371 | 2.340 ± 0.171 | 2.828 ± 0.860 | 2.477 ± 0.519 | 4.120 ± 0.960 | 2.526 ± 0.417 | 4.003 ± 0.367 | 4.894 ± 0.723 |
| Procyanidin dimer 2[y] | 8.161 ± 0.384 | 7.411 ± 0.439 | 9.115 ± 1.035 | 8.627 ± 0.423 | 10.090 ± 0.585 | 8.849 ± 0.534 | 9.300 ± 0.774 | 7.688 ± 0.427 |
| Procyanidin dimer 3[y] | **2.359 ± 0.812 b** | **6.109 ± 0.428 a** | **1.755 ± 0.174 b** | **3.417 ± 0.383 a** | 1.792 ± 0.194 | 1.948 ± 0.214 | 2.325 ± 0.273 | 1.794 ± 0.087 |
| Procyanidin dimer 4[y] | 2.120 ± 0.054 | 1.845 ± 0.127 | 2.156 ± 0.287 | 2.759 ± 0.436 | 2.080 ± 0.114 | 2.047 ± 0.147 | 2.630 ± 0.336 | 2.117 ± 0.157 |
| Procyanidin trimer 1[y] | 4.207 ± 0.529 | 4.187 ± 0.614 | 5.708 ± 0.670 | 6.136 ± 0.777 | 4.980 ± 0.780 | 4.141 ± 0.669 | 6.760 ± 0.670 | 5.357 ± 0.203 |
| Procyanidin trimer 2[y] | 3.039 ± 0.443 | 2.591 ± 0.127 | 2.726 ± 0.213 | 3.024 ± 0.288 | **2.786 ± 0.157 b** | **4.959 ± 0.526 a** | 2.819 ± 0.316 | 4.643 ± 0.888 |
| Procyanidin trimer 3[y] | 2.889 ± 0.321 | 2.821 ± 0.321 | **2.328 ± 0.261 b** | **4.970 ± 0.577 a** | 2.584 ± 0.218 | 3.754 ± 0.559 | 2.733 ± 0.335 | 4.874 ± 1.118 |
| Procyanidin tetramer 1[y] | 1.650 ± 0.462 | 2.287 ± 0.488 | 1.513 ± 0.029 | 1.440 ± 0.635 | 1.618 ± 0.161 | 2.862 ± 0.487 | 1.847 ± 0.320 | 1.865 ± 0.441 |
| Procyanidin tetramer 2[y] | 1.721 ± 0.357 | 1.462 ± 0.356 | 1.490 ± 0.0198 | 1.195 ± 0.702 | **1.758 ± 0.122 a** | **0.842 ± 0.186 b** | **2.076 ± 0.336 a** | **1.195 ± 0.109 b** |
| Quercetin 3-galactoside | 0.017 ± 0.003 | 0.014 ± 0.002 | 0.022 ± 0.001 | 0.019 ± 0.005 | **0.021 ± 0.001 a** | **0.015 ± 0.001 b** | 0.024 ± 0.005 | 0.014 ± 0.001 |
| Quercetin 3-glucoside[z] | 0.073 ± 0.012 | 0.090 ± 0.037 | 0.112 ± 0.002 | 0.104 ± 0.035 | **0.115 ± 0.014 a** | **0.029 ± 0.003 b** | **0.114 ± 0.021 a** | **0.043 ± 0.014 b** |
| Myricetin hexoside[z] | 0.015 ± 0.001 | 0.013 ± 0.001 | 0.014 ± 0.001 | 0.015 ± 0.002 | **0.014 ± 0.001 a** | **0.011 ± 0.001 b** | 0.015 ± 0.001 | 0.013 ± 0.001 |
| Quercetin hexoside 1[z] | 0.059 ± 0.014 | 0.060 ± 0.015 | 0.036 ± 0.009 | 0.051 ± 0.008 | **0.022 ± 0.003 a** | **0.013 ± 0.002 b** | 0.046 ± 0.013 | 0.016 ± 0.001 |
| Quercetin hexoside 2[z] | 0.249 ± 0.073 | 0.031 ± 0.071 | 0.404 ± 0.027 | 0.346 ± 0.146 | **0.422 ± 0.033 a** | **0.079 ± 0.007 b** | 0.389 ± 0.097 | 0.131 ± 0.059 |
| Isorhamnetin 3-galactoside[w] | 0.016 ± 0.003 | 0.015 ± 0.004 | 0.020 ± 0.001 | 0.020 ± 0.007 | **0.023 ± 0.001 a** | **0.013 ± 0.001 b** | 0.019 ± 0.003 | 0.012 ± 0.001 |
| Isorhamnetin 3-glucoside[w] | 0.056 ± 0.010 | 0.077 ± 0.029 | 0.094 ± 0.008 | 0.084 ± 0.026 | **0.106 ± 0.009 a** | **0.031 ± 0.004 b** | **0.092 ± 0.016 a** | **0.036 ± 0.011 b** |
| Isorhamnetin hexoside[w] | 0.197 ± 0.050 | 0.244 ± 0.082 | 0.309 ± 0.002 | 0.257 ± 0.045 | **0.329 ± 0.012 a** | **0.099 ± 0.009 b** | 0.028 ± 0.061 | 0.146 ± 0.029 |
| Naringenin hexoside[z] | 0.020 ± 0.005 | 0.019 ± 0.004 | 0.013 ± 0.001 | 0.018 ± 0.003 | 0.011 ± 0.002 | 0.014 ± 0.001 | 0.018 ± 0.004 | 0.012 ± 0.001 |
| Prunin[z] | 0.129 ± 0.055 | 0.139 ± 0.055 | 0.049 ± 0.008 | 0.112 ± 0.034 | 0.029 ± 0.002 | 0.025 ± 0.002 | **0.075 ± 0.014 a** | **0.026 ± 0.001 b** |

* Sampling location: above and below the graft union; [x] Different letters in rows of individual rootstock indicate statistically significant differences in the content of a specific phenolic compound above and below the graft union at $P \leq 0.05$ (in bold); [y] Present as equivalents of procyanidin B1; [z] Present as equivalents of quercetin 3-galactoside; [w] Present as equivalents of isorhamnetin.

Table 3. The content of individual phenolic compounds (mg $kg^{-1}$ of phloem) ± standard error in the phloem above and below the graft union of 'Royal Glory' grafted on *Prunus persica* and *Prunus domestica* rootstocks. Values represent means of 3 replications ± standard error.

| Genetic origin | *Prunus persica* | | *Prunus domestica* | | | |
|---|---|---|---|---|---|---|
| Rootstock | Peach seedling | | Penta | | Tetra | |
| Phenolics | Above | Below | Above | Below | Above | Below |
| Catechin | 11.522 ± 1.771 | 12.598 ± 0.919 | 10.471 ± 0.678 | 14.992 ± 2.235 | **10.182 ± 1.078 b** | **15.048 ± 0.969 a** |
| Epicatechin | 2.481 ± 0.115 | 2.633 ± 0.178 | **2.678 ± 0.211 b** | **6.091 ± 0.436 a** | **2.268 ± 0.138 b** | **5.670 ± 0.444 a** |
| Procyanidin dimer 1 (B1) | 4.888 ± 1.105 | 3.338 ± 0.378 | 5.060 ± 0.641 | 3.581 ± 0.074 | 4.522 ± 0.707 | 3.314 ± 0.262 |
| Procyanidin dimer 2[y] | 9.423 ± 0.534 | 9.883 ± 0.660 | **9.623 ± 0.625 a** | **0.000 ± 0.000 b** | **9.052 ± 0.504 a** | **0.000 ± 0.000 b** |
| Procyanidin dimer 3[y] | 2.200 ± 0.272 | 2.078 ± 0.099 | **2.834 ± 0.508 a** | **0.000 ± 0.000 b** | **1.729 ± 0.061 a** | **0.000 ± 0.000 b** |
| Procyanidin dimer 4[y] | 2.500 ± 0.203 | 2.527 ± 0.292 | **2.630 ± 0.101 a** | **0.000 ± 0.000 b** | **2.383 ± 0.204 a** | **0.000 ± 0.000 b** |
| Procyanidin dimer 5[y] | | | **0.000 ± 0.000 b** | **4.928 ± 0.383 a** | **0.000 ± 0.000 b** | **4.903 ± 0.181 a** |
| Procyanidin dimer 6[y] | | | **0.000 ± 0.000 b** | **2.787 ± 0.773 a** | **0.000 ± 0.000 b** | **2.454 ± 0.232 a** |
| Procyanidin trimer 1[y] | 6.214 ± 1.091 | 6.039 ± 0.987 | **5.850 ± 0.636 a** | **0.000 ± 0.000 b** | **5.471 ± 0.834 a** | **0.000 ± 0.000 b** |
| Procyanidin trimer 2[y] | 2.935 ± 0.174 | 2.431 ± 0.183 | **2.905 ± 0.260 a** | **0.000 ± 0.000 b** | **2.504 ± 0.169 a** | **0.000 ± 0.000 b** |
| Procyanidin trimer 3[y] | 2.761 ± 0.144 | 2.879 ± 0.339 | **2.844 ± 0.345 a** | **0.000 ± 0.000 b** | **2.433 ± 0.227 a** | **0.000 ± 0.000 b** |
| Procyanidin tetramer 1[y] | **1.691 ± 0.099b** | **2.611 ± 0.173a** | **1.449 ± 0.106 a** | **0.000 ± 0.000 b** | **1.159 ± 0.134 a** | **0.000 ± 0.000 b** |
| Procyanidin tetramer 2[y] | 1.973 ± 0.063 | 2.157 ± 0.394 | **1.886 ± 0.024 a** | **0.000 ± 0.000 b** | **1.701 ± 0.197 a** | **0.000 ± 0.000 b** |
| Quercetin 3-galactoside | 0.022 ± 0.03 | 0.023 ± 0.004 | **0.024 ± 0.003 a** | **0.000 ± 0.000 b** | **0.017 ± 0.003 a** | **0.000 ± 0.000 b** |
| Quercetin 3-glucoside[z] | 0.113 ± 0.030 | 0.104 ± 0.023 | **0.144 ± 0.008 a** | **0.000 ± 0.000 b** | **0.098 ± 0.023 a** | **0.000 ± 0.000 b** |
| Myricetin hexoside[z] | 0.014 ± 0.001 | 0.014 ± 0.002 | **0.014 ± 0.001 a** | **0.000 ± 0.000 b** | **0.012 ± 0.001 a** | **0.000 ± 0.000 b** |
| Quercetin hexoside 1[z] | 0.024 ± 0.002 | 0.034 ± 0.005 | **0.039 ± 0.008 a** | **0.000 ± 0.000 b** | **0.055 ± 0.023 a** | **0.000 ± 0.000 b** |
| Quercetin hexoside 2[z] | 0.383 ± 0.107 | 0.378 ± 0.098 | **0.546 ± 0.049 a** | **0.000 ± 0.000 b** | **0.340 ± 0.087 a** | **0.000 ± 0.000 b** |
| Isorhamnetin 3-galactoside[w] | 0.023 ± 0.005 | 0.023 ± 0.004 | **0.025 ± 0.003 a** | **0.000 ± 0.000 b** | **0.018 ± 0.003 a** | **0.000 ± 0.000 b** |
| Isorhamnetin 3-glucoside[w] | 0.103 ± 0.027 | 0.097 ± 0.021 | **0.118 ± 0.006 a** | **0.000 ± 0.000 b** | **0.080 ± 0.022 a** | **0.000 ± 0.000 b** |
| Isorhamnetin hexoside[w] | 0.307 ± 0.079 | 0.318 ± 0.074 | **0.398 ± 0.031 a** | **0.000 ± 0.000 b** | **0.258 ± 0.053 a** | **0.000 ± 0.000 b** |
| Naringenin hexoside[z] | 0.012 ± 0.001 | 0.014 ± 0.001 | **0.050 ± 0.002 a** | **0.000 ± 0.000 b** | **0.017 ± 0.006 a** | **0.000 ± 0.000 b** |
| Prunin[z] | 0.041 ± 0.008 | 0.055 ± 0.008 | 0.055 ± 0.010 | 0.134 ± 0.043 | 0.101 ± 0.059 | 0.810 ± 0.002 |
| Caffeoylquinic acid[y] | | | **0.000 ± 0.000 b** | **13.799 ± 0.666 a** | **0.000 ± 0.000 b** | **11.966 ± 0.543 a** |
| *p*-coumaric acid 4-glucoside[s] | | | **0.000 ± 0.000 b** | **4.445 ± 0.605 a** | **0.000 ± 0.000 b** | **4.038 ± 0.569 a** |
| Unknown phenol 1[z] | | | **0.000 ± 0.000 b** | **0.795 ± 0.021 a** | **0.000 ± 0.000 b** | **0.759 ± 0.070 a** |
| Unknown phenol 2[z] | | | **0.000 ± 0.000 b** | **4.645 ± 0.282 a** | **0.000 ± 0.000 b** | **4.065 ± 0.274 a** |

[*] Sampling location: above and below the graft union; [x] Different letters in rows of individual rootstock indicate statistically significant differences in the content of a specific phenolic compound above and below the graft union at $P \leq 0.05$ (in bold); [y] Present as equivalents of procyanidin B1; [z] Present as equivalents of quercetin 3-galactoside; [w] Present as equivalents of isorhamnetin; [s] Present as equivalents of *p*-coumaric acid.

and procyanidin trimer 3 content level were also significantly higher below the graft union. The results indicated highly compatible scion function between 'Royal Glory' and GF 677 and Monegro rootstocks.

In Cadaman rootstock (*Prunus persica* × *Prunus davidiana*), significant accumulations of epicatechin and procyanidin trimer 2 below the graft union were noticed. In Barrier rootstock there were no significant differences in flavanol content levels above and below the graft union, except for procyanidin tetramer 2, where significantly higher content levels were observed above the graft union. Accumulation of all flavonols was observed above the graft union in Cadaman rootstock. In Barrier rootstock, a significantly higher content level of the flavonols quercetin 3-glucoside and isorhamnetin 3-glucoside and flavanone prunin was detected above the graft union. Treutter et al. (1986, 1987) and Feucht and Treutter (1991) found an increase in prunin above the graft union

Table 4. Significance levels for the influence of rootstock, sampling place above and below graft union and their interaction on phenolic compound content in the phloem of 'Royal Glory' (NS – not significant; * – significant at $P \leq 0.05$; ** – significant at $P \leq 0.01$; *** – significant at $P \leq 0.001$).

| Phenolics | Rootstock (R) | Above/below the graft union (AB) | R × AB |
|---|---|---|---|
| Catechin | *** | * | * |
| Epicatechin | *** | *** | *** |
| Procyanidin dimer 1 (B1) | * | * | NS |
| Procyanidin dimer 2 | *** | *** | *** |
| Procyanidin dimer 3 | *** | NS | *** |
| Procyanidin dimer 4 | *** | *** | *** |
| Procyanidin dimer 5 | *** | *** | *** |
| Procyanidin dimer 6 | *** | *** | *** |
| Procyanidin trimer 1 | *** | *** | *** |
| Procyanidin trimer 2 | *** | NS | *** |
| Procyanidin trimer 3 | *** | NS | *** |
| Procyanidin tetramer 1 | *** | NS | *** |
| Procyanidin tetramer 2 | ** | *** | ** |
| Quercetin 3-galactoside | *** | *** | ** |
| Quercetin 3-glucoside | NS | *** | ** |
| Myricetin hexoside | *** | *** | *** |
| Quercetin hexoside 1 | ** | ** | ** |
| Quercetin hexoside 2 | NS | *** | ** |
| Isorhamnetin 3-galactoside | ** | *** | ** |
| Isorhamnetin 3-gluctoside | * | *** | ** |
| Isorhamnetin hexoside | * | *** | ** |
| Naringenin hexoside | ** | * | ** |
| Prunin | * | NS | NS |
| Caffeoylquinic acid | *** | *** | *** |
| *p*-coumaric acid 4-glucoside | *** | *** | *** |
| Unknown phenol 1 | *** | *** | *** |
| Unknown phenol 2 | *** | *** | *** |

in incompatible combinations. An accumulation of prunin inhibits the synthesis of ATP and has an influence on a decrease in the polarity of the IAA transport (Treutter et al. 1985). Prunin also inhibits growth as well as enhancing oxidative decarboxylation of IAA (Treutter and Feucht 1988).

In the phloem of *Prunus domestica* rootstocks, higher content levels of all identified flavonols above the graft union were detected (Table 3), which indicates that flavonols could also be involved in graft incompatibility. Hudina et al. (2014) reported that, alongside arbutin, catechin, procyanidin B1 and chlorogenic acid, flavonols could also forecast emerging problems in pear prior to the visible external symptoms. Moing et al. (1987) found "translocated" incompatibility in peach/plum combinations, which was associated with functional and biochemical alterations at the graft interface, inducing a carbohydrate blockage in the scion above the graft union. Flavanols (except for catechin, epicatechin and procyanidin B1) and flavanone naringenin hexoside also had significantly higher content levels above the graft union in both *Prunus domestica* rootstock. Hydoxycinnamic acids (phenolic acids), unknown phenols and procyanidin dimer 5 and procyanidin dimer 6 were present only in plum rootstock, but not in 'Royal Glory' cultivar. Treutter and Feucht (1988) reported that phenolic acid inhibits plant growth, which is in accordance with our results. According to trunk circumference, trees on Tetra, Penta and peach seedling rootstocks were significantly less vigorous compared to GF 677, Monegro, Cadaman and Barrier rootstocks (Fig. 5). Reighard (2000) also reported that trees on Tetra rootstock were as vigorous as peach seedling. Massai and Loreti (2004) stated that *Prunus domestica* rootstock yielded less vigorous 'Flavorcrest' trees than GF 677 rootstock, and Garcia Brunton et al. (2004) that rootstocks GF 677, Monegro, Barrier and Cadaman yielded

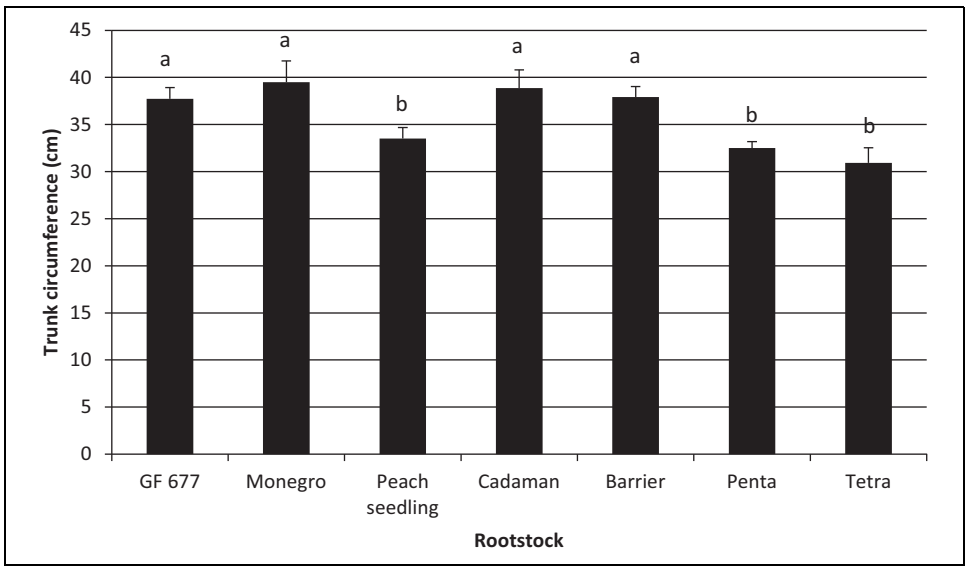



Fig. 5. Average trunk circumference (cm) of 'Royal Glory' grafted on different rootstocks. Vertical bars represent means of 12 replicates ± standard error. The means are not significantly different when followed by the same letter, according to the HSD multiple-range test at $P < 0.05$.

vigorous trees, which is in accordance with our results. Trunk circumference, tree volume, tree vigour and other visible symptoms are not the only symptoms of incompatibility. These visible symptoms should be compared with the response of plants to different types of stress and to the stress metabolites. In heterospecific cultivar/rootstock combinations, low level stress is always present, owing to the metabolism adjustment of two different genotypes. A certain level of stress causes weaker growth of *Prunus* trees; when stress is augmented by the presence of viruses and various environmental factors, this can lead to severe incompatibility. Plants grown under stress conditions often produce and accumulate phenolic stress metabolites, which may be explained by the action of elicitors stimulating signal chains towards biosynthesis and the accumulation of metabolites (Usenik et al. 2008). The abnormal accumulation of phenols at the incompatible graft interface was previously reported in sweet cherry (Usenik and Štampar 2000), pear (Ermel et al. 1999) and apricot (Errea et al. 1994, 2001). Zarrouk et al. (2010) suggest that accumulation of phenolic compounds in the cambium cell interface of incompatible peach/plum combinations is also related to graft incompatibility and may deter the establishment of the union between scion and rootstock by inhibiting the lignification of cells and consequently impairing a good union.

*Conclusion*

Ten phenolics were detected in the phloem of *Prunus domestica* rootstocks, and 21 phenolics in *Prunus persica* and their hybrid rootstocks. The flavan 3-ols was the major group of phenols in the phloem of *Prunus persica* and *Prunus persica* rootstocks. From the results presented in this study, it can be concluded that according to the phenolic compound content, 'Royal Glory' is highly compatible with peach seedling, GF 677 and Monegro rootstocks. The level of all analysed flavonols, hydoxycinnamic acids, unknown phenols, flavanone naringenin hexoside, and flavanols (except procyanidin B1 and catechin at Penta rootstock) differ significantly above and below the graft union in *Prunus domestica* rootstocks. Only catechin, epicatechin, procyanidin B1 and prunin were detected in 'Royal Glory' cultivar and *Prunus domestica* rootstocks. Hydoxycinnamic acids, unknown phenols as well as procyanidin dimer 5 and procyanidin dimer 6 were present only in plum rootstocks and not in 'Royal Glory' cultivar. The proportion of phenolic compounds above and below the graft union is very important, especially their accumulation above the graft union. Accumulation of flavonols above the graft union could be the first biochemical response to incompatibility what indicates that flavonols might also be involved in the graft incompatibility process and can be used to predict the incompatibility at an early stage. Incompatibility is a complex physiological phenomenon occurring in some plants, and is subject to environmental and growing conditions, the presence/absence of viruses, the nutritional status of plants, and defined by adjustment of the metabolisms of the grafted union partners (cultivar/rootstock combinations), as well as other unpredictable stress – inducing factors. Intensive care and optimal growth conditions in the orchard must be provided in order to avoid stress or decrease the potential for stress, which can cause decline of trees because of incompatibility.

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