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# Rootstock effects on polyphenol content in grapes of 'Regent' cultivated under cool climate condition

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## Summary

Rootstocks are well known as the most efficient way to limit phylloxera. However, they can be useful in order to improve grape quality. This study aimed to compare the content of polyphenol compounds in vine fruits of the cultivar 'Regent' grafted on 'Couderc 161-49', 'Sori', 'Kober 125AA', 'Börner' or 'Kober 5BB' rootstocks, or planted on own-roots. Grape samples were collected in three consecutive seasons (2013-2015) at a research station of the West Pomeranian University of Technology Szczecin in Poland. Thirty-three phenolic compounds were determined in the juice of examined samples using ultra-pressure liquid chromatography with photodiode array and mass spectrometry (UPLC-PDA/MS) method. A significant influence of rootstock on the content of polyphenols in grapes has been proven. The highest content of polyphenols was shown in fruits from a scion grafted on 'Sori' and 'Kober 125AA' rootstocks (675 and 643 mg · 100 $g^{-1}$ FW, respectively). 'Börner' and 'Kober 5BB' rootstocks did not have a significant influence on the creation of polyphenol compounds in comparison to own-root plants. In addition, the use of the 'Börner' rootstock resulted in fruits with an especially low content of phenolic acids.

## Introduction

It has been reported that there is an inverse association between the consumption of some fruits and vegetables and the mortality from age-related diseases. This can be partially attributed to a diet rich in antioxidants, especially phenolic compounds (Dudonne et al., 2009). Polyphenols are compounds that occur naturally in large amounts in some food products, including fruits. Until now, 8,000 structures of those compounds have been discovered. Natural polyphenols are safer and more acceptable by the consumers than synthetic antioxidants like butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT), which is why they constitute an important ingredient of our everyday diet (Liu et al., 2015).

Grapes belong to the most frequently eaten fruits in the world, both in their fresh and processed forms. Furthermore, they have one of the highest content of phenolic compounds (Manach et al., 2005) and a high bioactive potential due to their antioxidant, anti-inflammatory, anticancer and antimicrobial properties (Gris et al., 2011). These health benefits have been associated with some groups of polyphenol compounds such as flavonoids, phenolic acids or stilbenes. Among flavonoids, the most important ones are: anthocyanins, flavonols, flavones, flavanones (Espín et al., 2007; Stintzing and Carle, 2004), catechins epicatechins, and procyanidins (Wang et al., 2002; Fuleki and Ricardo-da-Silva, 2003). The health benefits of polyphenols depend on the amounts consumed and their bioavailability (Manach et al., 2004).

The grapevine, as a plant, has been cultivated since ancient times; it was used for consumption and religious purposes (Censi et al., 2014). In the 19th century, the plantation in Europe was almost completely destroyed due to phylloxera (Garnett et al., 2001). A solution to the problem was found in using rootstocks of the *Vitis* sp. kind. A rootstock is a root system of a phylloxera resistant grapevine onto which a scion of a chosen cultivar is grafted (Ozden et al., 2010). Until now, it is still the most efficient way to limit phylloxera (Vršič et al., 2015). On the areas "free from" phylloxera, rootstocks are used in order to improve the quality of the harvest and to limit the influence of adverse soil and climate conditions (Reynolds and Wardle, 2001). However, due to the effects of global warming, the area of pest occurrence has been shifted more to the North. This means that the use of rootstocks may also protect Northern plantations against phylloxera in the future.

Nowadays, the interest in grapevine cultivation in Poland is increasing, and new vineyards have been established where new varieties with resistances to major pests, like downy and powdery mildews dominate. Grapevine cultivar 'Regent', which is the subject of this study, was developed in 1967, and it is characterised by the dark red colour of its fruit skin (Ehrhardt et al., 2014). The studies focused on the evaluation of the influence of rootstock upon the content of polyphenols in grapes of 'Regent'.

## Materials and methods

### Characteristics of the area of research and plant material

The experiment was conducted in three consecutive years (2013-2015) at a Research Station of West Pomeranian University of Technology in Szczecin. The research station is located in subzone 7A (Heinze and Schreiber, 1984) in the North-Western part of Poland in the Szczecin Lowland at a distance of approx. 65 km from the Baltic Sea (53°40' N, 14°46' E). The soil in the orchard was an agricultural soil with a natural profile, developed from silt loam (sand 42.7%, silt 52.9%, clay 4.4%) with considerably lower density of 1.25 Mg · $m^{-3}$, pH 6.8-6.9 and higher water capacity of 46.2 [% $ww^{-1}$]. It also contained much more organic matter – 34.3 g in kg of soil. Regardless of the site, the soil was characterised by similarly low salinity – EC 0.33-0.42 mS $cm^{-1}$. At depth of 20-40 cm it was characterised by a high content of P (72 mg $kg^{-1}$), K (157 mg $kg^{-1}$) and Mg (47 mg $kg^{-1}$). In turn, Ca content was 455 mg $kg^{-1}$ for 20-40 cm of depth, 1336 mg $kg^{-1}$ for 60-80 cm, and 1577 mg $kg^{-1}$ for 120-140 cm. Ground water level was 140-160 cm.

The study involved the dark-skinned grapevine cultivar 'Regent', which is a German cultivar with interspecific hybrids in ancestry. The vines were planted in 2010 with a North-South row orientation at 2.3 × 1 m. 'Regent' vines grafted onto five rootstocks, viz. 'Couderc 161-49', 'Sori', 'Kober 125AA', 'Börner' and 'Kober 5BB' were grown, while own-root 'Regent' vines served as a control. The vines were pruned with a Guyot (one arm) training system and vertically positioned with eight shoots and two clusters each. Other standard vineyard management practices, including pest treatment, were performed during all growing seasons.

The experimental treatments were arranged in a randomised complete block design. Each experimental unit was comprised of 6 vines. Fruits were collected at physiological maturity on the first decade of October in successive seasons.

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**Reagents and standards**

Formic acid and methanol were purchased from Sigma-Aldrich (Steinheim, Germany). Acetonitrile was purchased from Merck (Darmstadt, Germany). quercetin-3-*O*-glucoside, quercetin-3-*O*-rutinoside, kaempferol-3-*O*-glucoside, myricetin-3-*O*-glucoside, isorhamnetin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, peonidin-3-*O*-glucoside, (-)-epicatechin, (+)-catechin, procyanidins, and gallic acid were purchased from Extrasynthese (Lyon, France).

**Extraction procedure**

Three replicates of 25 randomly chosen berries were kept frozen at -27 °C until analysis, and then prepared according to the methodology of Oszmiański et al. (2013). The fruits were extracted with methanol acidified with 2.0% formic acid. The separation was conducted twice by incubation for 20 min under ultrasonic treatment (Sonic 6D, Polsonic, Warsaw, Poland) followed by shaking from time to time (a few times or rarely). Subsequently, the suspension was centrifuged MPW-251 (MPW MED. INSTRUMENTS, Warsaw, Poland) at 19,000 × *g* for 10 min. Prior to analysis, the supernatant was additionally purified with a Hydrophilic PTFE 0.20 μm membrane (Millex Samplicity Filter, Merck). The polyphenol content in each extract was specified by means of the ultra-performance liquid chromatographyphoto-diode array detector-mass spectrometry (LC-PDA-MS, Waters Corporation, Milford, MA USA) method. All extractions were carried out in triplicate.

**Identification of phenolic compounds by the UPLC-PDA/MS method**

Analyses were performed by the methodology Oszmiański et al. (2013). In 'Regent' grapes extracts' polyphenols identification was executed by using an ACQUITY Ultra Performance LC system appointed with a binary solvent manager's, a photodiode array detector (Waters Corporation, Milford, MA) and a G2 Q-TOF micro mass spectrometer (Waters, Manchester, UK) equipped with an electrospray ionisation (ESI) source operating in following modes: negative and positive. Individual polyphenols separations were executed by using a UPLC BEH C18 column (1.7 μm, 2.1 mm × 100 mm, Waters Corporation, Milford, MA) at 30 °C. The elution of injected samples (10 μL) was fulfilled in 15 min followed by a sequence of linear gradients and isocratic flow rates of 0.45 mL $min^{-1}$. The mobile phase consisted of solvent A (0.1% formic acid, v/v) and solvent B (100% acetonitrile). The examination commenced with and initial isocratic elution of 99% solvent A (0-1 min), while applying a linear gradient for 12 min uncovered lowering solvent A to 0%. Next, at 12.5 to 13.5 min, the gradient was returned back to the initial composition (99% A), with the tore-equilibrate column being kept constant. The overall analysis was based on full data-dependant MS scanning with a *m/z* range from 100 to 2500. The reference compound used for the examination was Leucine encephalin, at a concentration of 500 pg/μL and a flow rate of 2 μL/min. The $[M - H]^-$ ion at 554.2615 Da and $[M + H]^+$ ion at 556.2771 were detected. The $[M - H]^-/[M + H]^+$ ions were recognised during a 15 min analysis performed within ESI-MS accurate mass experiments, which were constantly introduced via the LockSpray channel using a Hamilton pump. The mass spectrometer operated in both negative-and positive-ion modes, adjusted to base peak intensity (BPI) chromatograms, scaled to 12,400 counts per second (cps) (100%) within a locked mass correction of ±1.000 for the mass window. The MS conditions were optimised according to the following parameters: a capillary voltage of 2500 V, a cone voltage of 30 V, a source temperature of 100 °C, a desolvation temperature of 300 °C and a desolvation gas (nitrogen) flow rate of 300 L/h. Collision-induced fragmentation experiments were performed using argon as the collision gas, with voltage ramping cycles from 0.3 to 2 V. Characterisation of each and every single component was conducted via retention time and accurate molecular masses while individual compounds were optimised to their estimated molecular mass in both negative and positive modes, prior to and as a result of fragmentation. Afterwards, the data collected from UPLC-MS were uploaded to the MassLynx 4.1 ChromaLynx Application Manager software (MassLynx 4.1 SCN802, Waters Corporation, Milford, MA USA). Quantification was achieved by injection of solutions of known concentrations ranging from 0.05 to 5 mg/mL ($R^2 \leq 0.9998$) of phenolic compounds as standards. The results were expressed as mg per 100 mL for must.

Basing on the delivered data the software itself is developed to scan multiple samples for defined substances. The various data analysis runs were monitored at the following wavelengths: flavan-3-ols at 280 nm, phenolic acids at 320 nm, flavonol glycosides at 360 nm and anthocyanins at 520 nm.

The PDA spectra were measured over the wavelength range of 200-600 nm in steps of 2 nm. Finally the retention times and spectra were compared with authentic standards.

**Statistical analysis**

All statistical analyses were performed with Statistica 12.5 (StatSoft Polska, Cracow, Poland). The data were subjected to one and two-factor variance analysis (ANOVA). Mean comparisons were performed using Tukey's least significant difference (LSD) test; significance was set at $p < 0.05$. To determine the relation between the rootstock and phenolic content the results obtained were subjected to an agglomerative cluster analysis and classified into groups in a hierarchical order by means of the Ward's method.

## Results and discussion

**General**

Identification of polyphenol compounds belonging to anthocyanins, phenolic acids, flavonols and flavan-3-ols was based on a comparison of their retention times, MS and MS/MS data with available standards and published data. The identification results are presented in our previous paper (Mijowska et al., 2016). The results obtained in our study, as well as by other authors (Jogaiah et al., 2015; Koundouras et al., 2009; Suriano et al., 2016), showed an important influence of the rootstock on the content of polyphenols in grapes. However, in the study of Koundouras et al. (2009), rootstocks affected only seed phenolic concentrations.

Basic seasonal weather characteristics are shown in Tab. 1. In 2015, the temperatures were significantly different from what they typically are in Szczecin and its surrounding areas. In May and June of 2015, the temperatures recorded were 0.5 °C and 0.8 °C lower than averages of years 1951-2012, respectively. Then in August of 2015, the air temperature was 3.5 °C higher than the mean value of the multi-year period. During the 2015 growing period, the rainfall was 38% lower than normal, which, together with the high temperature, created highly atypical weather conditions. Regardless of the rootstock used, the content of polyphenols was significantly higher in grapes harvested in 2015 (Fig. 1). The highest content of polyphenols was shown in grapes with the 'Sori' and 'Kober 125AA' rootstocks in all studied years. A cluster analysis conducted using Ward's method (Fig. 2) permitted the isolation of a separate group of these two rootstocks with similar influence on polyphenols in the fruits. Another group was formed out of the single 'Börner' rootstock, whose fruits had the lowest content of polyphenols in three years average. Additionally, 'Börner' and 'Kober 5BB' rootstocks resulted in statistically lower level of polyphenols in grapes or without

**Tab. 1:** Weather conditions during the vegetative season (April-October) in the years 2013-2015 with reference to the average growing season during the multi-year period (1951-2012).

| | Month | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **IV** | **V** | **VI** | **VII** | **VIII** | **IX** | **X** | |
| **Year** | **Average temperature (°C)** | | | | | | | **Mean** |
| 2013 | 8.4 | 14.4 | 16.9 | 19.3 | 18.7 | 13.0 | 10.9 | 14.5 |
| 2014 | 10.8 | 13.4 | 16.3 | 21.3 | 17.5 | 15.4 | 11.8 | 15.2 |
| 2015 | 8.7 | 12.5 | 15.6 | 18.6 | 21.1 | 14.1 | 13.7 | 14.9 |
| 1951-2012 | 8.0 | 13.0 | 16.4 | 18.2 | 17.6 | 13.8 | 9.2 | 13.7 |
| | **Rainfall (mm)** | | | | | | | **Total** |
| 2013 | 20.8 | 88.1 | 112.5 | 50.4 | 35.9 | 43.9 | 45.8 | 397 |
| 2014 | 47.5 | 85.3 | 26.5 | 70.8 | 104.6 | 80.9 | 32.8 | 448 |
| 2015 | 29.0 | 48.0 | 32.8 | 62.0 | 14.7 | 34.4 | 22.1 | 242 |
| 1951-2012 | 39.7 | 62.9 | 48.2 | 69.6 | 74.2 | 58.7 | 37.3 | 391 |

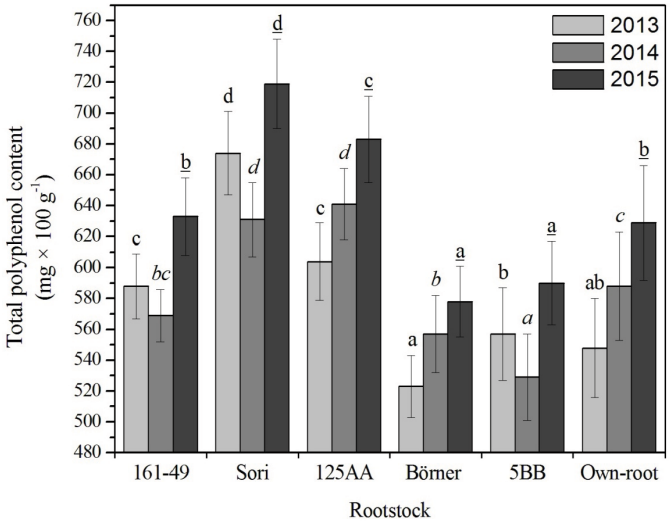


**Fig. 1:** Total polyphenol content in mg · 100 $g^{-1}$ FW of grapes of 'Regent' in three years studied (2013-2015), as related to rootstocks and own-root vines. Means having same letter were not significantly different by Tukey's comparison at $p < 0.05$ level. Lowercase letters (a) indicate the means of 2013, italic letters (*a*) of 2014, and underlined letters (a) of 2015.

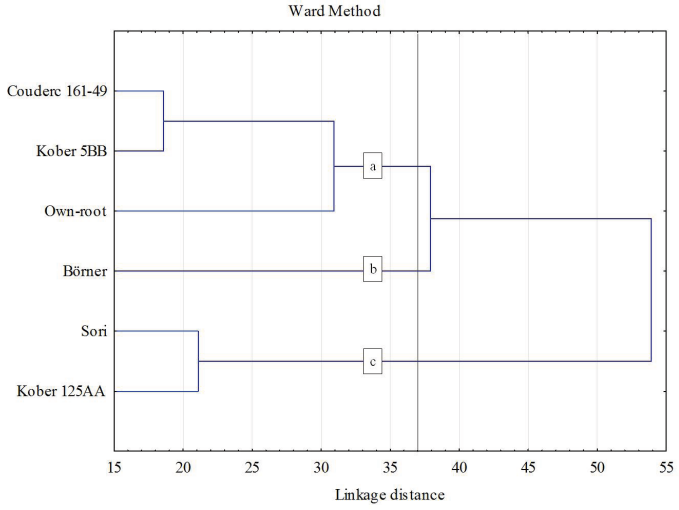


**Fig. 2:** Dendrogram of cluster analysis for rootstocks based on average for phenolic compositions. The vertical line (linkage distance 37) indicate the cut-off used to form the groups.

significant differentiation compared to own-rooted plants in all studied years (Fig. 1).

The average polyphenol compound concentrations of years 2013-2015 are shown in Tab. 2. The sum of polyphenols in grapes of 'Regent' ranged from 554 to 675 mg · 100 $g^{-1}$ FW, for 'Börner' and 'Sori' respectively. Statistically similar content of polyphenols as in the case of 'Sori' was shown in the fruits of 'Kober 125AA' rootstock (643 mg · 100 $g^{-1}$ FW). On the other hand, grapes from 'Kober 5BB' rootstock and own-root plants showed no significant diversity compared with fruits of 'Börner' rootstock. According to our results published previously (Mijowska and Ochmian, 2015), grapes from 'Sori' and 'Kober 125AA' rootstocks were also characterised by higher total soluble solids, while fruits from 'Börner' and 'Kober 5BB' rootstocks were classified as two with lower concentrations. These parameters could depend on the differences of vigour. Refer to other authors findings, 'Sori' is suggested as the low vigour rootstock (Schmidt et al., 2005), while 'Kober 5BB' and 'Börner' are served as a medium/high vigour (Cousins, 2005; Schreiner, 2003). Grapevine fruits are a rich source of polyphenolic compounds; however, they contain half of the total amount of compounds as compared to the blue-berried honeysuckle (Oszmiański et al., 2016).

## Anthocyanins

In the grapes studied, fifteen anthocyanin compounds were identified. Depending on the type of the rootstock, they constituted 60-64% of all polyphenols identified. The highest contents of anthocyanins were found in grapes from plants with the 'Sori' and 'Kober 125AA' rootstocks (respectively: 423, 400 mg · 100 $g^{-1}$ FW), and the lowest, in the fruit from own-root plants, as well as with the 'Börner' and 'Kober 5BB' rootstocks (respectively: 350, 355, 360 mg · 100 $g^{-1}$ FW). In turn, Suriano et al., 2016, observed the highest levels of anthocyanins in berries of vines 'Greco Nero' grafted onto '775 Paulsen' and 'Kober 5BB'. In the study of Ehrhardt et al. (2014), the 'Regent' cultivar fruits cultivated in Germany and Italy had 120-130 mg · 100 $g^{-1}$ FW level of anthocyanins, respectively. The most frequently found anthocyanin compounds in the fruits of 'Regent' studied were the 3-*O*-glucoside forms of petunidin, peonidin, delphinidin, malvidin and cyanidin, in order. Those compounds

**Tab. 2:** Polyphenol compound concentrations in grapes of 'Regent' depending on rootstock [mg · 100 $g^{-1}$ FW] – as the average of years 2013-2015.

| Compounds | Rootstock | | | | | | |
|---|---|---|---|---|---|---|---|
| | Couderc 161-49 | Sori | Kober 125AA | Börner | Kober 5BB | Own-root | **mean** |
| Cyanidin-3-*O*-glucoside | 56.1±4.9ab* | 68.8±3.0c | 66.9±4.7c | 50.0±4.2a | 67.5±3.5c | 64.2±5.3bc | **62.3** |
| Delphinidin-3-*O*-glucoside | 71.1±4.3b | 82.9±5.4c | 64.8±6.1a | 67.1±5.7ab | 69.2±6.3ab | 65.0±7.2a | **70.0** |
| Malvidin-3-*O*-glucoside | 59.7±6.0ab | 69.1±4.4c | 70.7±7.8d | 65.4±5.8bc | 51.7±5.2a | 58.6±5.7ab | **62.5** |
| Peonidin-3-*O*-glucoside | 71.4±7.7b | 79.2±6.8c | 80.9±7.2c | 56.7±5.4a | 70.9±6.5b | 64.7±7.5a | **70.6** |
| Petunidin-3-*O*-glucoside | 77.8±6.8b | 83.8±7.2c | 80.7±6.5bc | 77.1±7.0b | 71.8±5.5a | 67.5±8.3a | **76.5** |
| Cyanidin-3-*O*-acethyl-glucoside | 1.07±0.09ab | 1.32±0.12c | 0.99±0.08ab | 1.16±0.08bc | 0.91±0.10a | 1.67±0.13d | **1.19** |
| Delphinidin-3-*O*-acethyl-glucoside | 1.36±0.11ab | 1.35±0.12ab | 1.20±0.08a | 1.58±0.09bc | 1.08±0.07a | 1.84±0.12c | **1.40** |
| Malvidin-3-*O*-acethyl-glucoside | 6.30±0.44b | 6.37±0.48b | 5.23±0.31a | 6.18±0.25b | 4.76±0.23a | 5.26±0.36a | **5.68** |
| Peonidin-3-*O*-acethyl-glucoside | 0.99±0.06b | 1.15±0.08c | 1.13±0.08c | 0.90±0.07b | 0.55±0.04a | 0.41±0.03a | **0.86** |
| Petunidin-3-*O*-acethyl-glucoside | 1.27±0.08c | 0.87±0.07a | 1.10±0.08b | 1.37±0.06c | 0.80±0.05a | 1.04±0.07b | **1.08** |
| Cyanidin-3-*O*-(6-*p*-coumaroyl)-glucoside | 5.79±0.24bc | 6.17±0.32c | 5.91±0.22bc | 5.44±0.21b | 4.20±0.19a | 3.60±0.21a | **5.19** |
| Delphinidin-3-*O*-(6-*p*-coumaroyl)-glucoside | 1.99±0.09ab | 2.17±0.11b | 2.35±0.10b | 2.92±0.13c | 1.59±0.09a | 2.98±0.15c | **2.33** |
| Malvidin-3-*O*-(6-*p*-coumaroyl)-glucoside | 7.52±0.23c | 7.20±0.27bc | 6.99±0.28b | 7.53±0.30c | 5.68±0.22a | 6.16±0.25a | **6.85** |
| Peonidin-3-*O*-(6-*p*-coumaroyl)-glucoside | 5.51±0.27c | 5.29±0.19c | 5.14±0.21c | 4.64±0.18b | 4.48±0.17b | 3.20±0.15a | **4.71** |
| Petunidin-3-*O*-(6-*p*-coumaroyl)-glucoside | 6.88±0.38de | 7.14±0.41e | 5.79±0.30c | 6.49±0.32cd | 4.90±0.25b | 3.84±0.27a | **5.84** |
| **Anthocyanins** | **375AB** | **423C** | **400BC** | **355A** | **360A** | **350A** | |
| *cis*-Caftaric acid | 1.73±0.13d | 1.33±0.09b | 1.68±0.11cd | 1.08±0.08a | 1.53±0.11c | 1.80±0.12d | **1.53** |
| *trans*-Caftaric acid | 2.55±0.20d | 1.40±0.11b | 1.72±0.15b | 1.02±0.12a | 2.18±0.20c | 2.70±0.22d | **1.93** |
| *cis*-Coutaric acid | 2.73±0.16cd | 2.29±0.15b | 2.52±0.15bc | 1.94±0.14a | 2.93±0.17d | 1.82±0.15a | **2.37** |
| *trans*-Coutaric acid | 0.58±0.03a | 0.72±0.05c | 0.64±0.04b | 0.58±0.02a | 0.66±0.04b | 0.53±0.04a | **0.62** |
| *cis*-Fertaric acid | 0.79±0.05c | 0.40±0.03a | 0.56±0.04b | 0.45±0.03a | 0.76±0.05c | 0.91±0.07d | **0.65** |
| *trans*-Fertaric acid | 0.64±0.05b | 0.72±0.05bc | 0.83±0.04d | 0.53±0.03a | 0.94±0.05e | 0.76±0.05c | **0.74** |
| Gallic acid | 1.36±0.08b | 1.04±0.05a | 1.52±0.08c | 1.03±0.06a | 1.31±0.06b | 1.72±0.08d | **1.33** |
| **Phenolic acid** | **10.38C** | **7.90B** | **9.47C** | **6.63A** | **10.31C** | **10.24C** | |
| Myricetin-3-*O*-glucoside | 4.32±0.27b | 6.21±0.33d | 5.05±0.28c | 3.93±0.19ab | 4.16±0.22b | 3.24±0.15a | **4.49** |
| Quercetin-3-*O*-rutinoside | 2.79±0.11a | 4.07±0.15c | 3.59±0.15bc | 3.04±0.14ab | 3.03±0.12ab | 3.63±0.16bc | **3.36** |
| Quercetin-3-*O*-glucoside | 9.06±0.38b | 12.15±0.53d | 10.54±0.48c | 7.20±0.37a | 9.13±0.33b | 7.91±0.46a | **9.33** |
| Quercetin-3-*O*-glucuronide | 7.75±0.28a | 11.84±0.35d | 11.10±0.31cd | 8.76±0.30ab | 7.52±0.22a | 9.79±0.36bc | **9.46** |
| Kaempferol-3-*O*-glucoside | 0.53±0.03a | 0.73±0.05c | 0.74±0.04c | 0.63±0.05b | 0.45±0.03a | 0.68±0.04bc | **0.63** |
| Isorhamnetin-3-*O*-glucoside | 2.09±0.11bc | 3.04±0.13d | 3.09±0.11d | 2.37±0.09c | 1.55±0.04a | 1.76±0.05ab | **2.32** |
| **Flavonols** | **26.54A** | **38.04B** | **34.11B** | **25.93A** | **25.84A** | **27.01A** | |
| Procyanidin B1 | 17.61±0.55c | 16.94±0.51c | 13.78±0.58b | 8.88±0.36a | 12.35±0.43b | 14.86±0.49b | **14.07** |
| Procyanidin B2 | 27.54±0.94a | 34.09±1.98b | 28.37±1.07a | 23.67±1.23a | 25.45±1.45a | 37.97±3.11b | **29.52** |
| Procyanidin B3 | 10.98±0.34c | 9.31±0.22bc | 8.22±0.30b | 4.45±0.17a | 5.85±0.19a | 7.82±0.24b | **7.77** |
| (+)-Catechin | 81.90±5.43a | 99.54±4.89bc | 105.63±5.11c | 94.43±3.94b | 81.22±3.62a | 77.17±4.40a | **89.98** |
| (-)-Epicatechin | 46.41±2.92c | 45.89±2.30c | 42.84±2.45bc | 34.65±2.03a | 40.22±1.88b | 62.88±3.19d | **45.48** |
| **Flavan-3-ols** | **184B** | **206C** | **199BC** | **166A** | **165A** | **201C** | |
| **TOTAL POLYPHENOLS** | **596B** | **675C** | **643C** | **554A** | **561AB** | **588AB** | |

* Means in the same row followed by the same letter are not significantly different at $p < 0.05$ according to Tukey test; ±SD: standard deviation

belong to the most important anthocyanins in grapes (Ivanova et al., 2010). Their average content ranged from 62.3 to 76.5 mg · 100 $g^{-1}$ FW, and their highest level was found in the fruits from plants with the 'Sori' and 'Kober 125AA' rootstocks. Delphinidin-3-*O*-glucoside was an exception as its content was the lowest in the case of the 'Kober 125AA' rootstock. Own-root plants also had a similarly low content of this compound. The content of other anthocyanin compounds was significantly lower than the 3-*O*-glucoside forms and, on average, it varied from 0.86 (peonidin-3-*O*-acethyl-glucoside) to 6.85 mg · 100 $g^{-1}$ FW (malvidin-3-*O*-(6-*p*-coumaroyl)-glucoside).

According to other authors (Río Segade et al., 2009; Figueiredo-González et al., 2012), the largest group of anthocyanin compounds

of red varieties of grapes were derivatives of malvidin – 40.6-84.9%. Peonidins also constituted a large group of 22-44% anthocyanins (Figueiredo-González et al., 2012). In the case of the grapes of 'Regent', the distribution of anthocyanins was different. Derivatives of petunidin constituted the largest group (83.31 mg · 100 $g^{-1}$ FW), which was equal to 22%. The remaining groups of anthocyanins occurred in similar numbers. The content of cyanidinswas the lowest (68.57 mg · 100 $g^{-1}$ FW); however, it constituted as much as 18% of all anthocyanins identified.

**Phenolic acids**

The profile of phenolic acids in the fruit from the cultivar 'Regent' was diversified and depended on the rootstock used. The lowest amounts of phenolic acids were found in grapes growing with the 'Börner' rootstock (6.63 mg · 100 $g^{-1}$ FW). The remaining plants had a significantly higher content of those compounds in the fruit (7.9-10.38 mg · 100 $g^{-1}$ FW). In the case of grapes of the cultivar 'Regent', seven different compounds belonging to the group of phenolic acids were identified. The ones occurring in the largest amounts were *cis*-coutaric acid (2.37 mg · 100 $g^{-1}$ FW) and *trans*-caftaric acid (1.93 mg · 100 $g^{-1}$ FW). According to Ehrhardt et al. (2014), the level of *trans*-caftaric acid depends on the location of cultivation. Authors noted that in the fruit of the grapevine 'Regent' cultivated in Italy, it was 1.27 mg · 100 $g^{-1}$ FW, while in the cooler climate of Germany it was significantly higher: 3.60 mg · 100 $g^{-1}$ FW. Additionally, our previous study on the evaluation of cluster zone leaf removal on grapes cultivar 'Regent' polyphenol content showed that berries under shaded condition achieved higher level of phenolic acids. The amount of *trans*-caftaric acid was even five times higher compared to grapes from defoliated vines (Mijowska et al., 2016).

**Flavonols**

Six compounds among the flavonols were identified in the fruits. Similarly, as for the case of anthocyanins, the highest level of flavonols was found in fruits harvested from plants with the 'Sori' (38.04 mg · 100 $g^{-1}$ FW) and 'Kober 125AA' rootstocks (34.11 mg · 100 $g^{-1}$ FW). Grapes growing on own-roots or with other type of rootstock were characterised by a low content of flavonols (25.84-27.01 mg · 100 $g^{-1}$ FW). In a study by Satisha et al. (2008), the flavonol content of 'Thompson Seedless' grapes was higher in the case of vines grafted on rootstocks as compared with ungrafted ones. Derivatives of myricetin, quercetin, kaempferol and isorhamnetin were found in the flavonol group of grapes, and the most frequently found ones were quercetin-3-*O*-glucuronide (9.46 mg · 100 $g^{-1}$ FW) and quercetin-3-*O*-glucoside (9.33 mg · 100 $g^{-1}$ FW).

**Flavan-3-ols**

The highest content of flavan-3-ols was found in grapes with the 'Sori' rootstock (206 mg · 100 $g^{-1}$ FW) and own-root plants (201 mg · 100 $g^{-1}$ FW), while the lowest was found in plants with the 'Kober 5BB' (165 mg · 100 $g^{-1}$ FW) and 'Börner' (166 mg · 100 $g^{-1}$ FW) rootstocks. As reported by Suriano et al. (2016), 'Kober 5BB' seemed the rootstock that let the genotype 'Greco Nero' to better express flavans both in grapes and in derived wines. Among flavan-3-ols, B type procyanidins as well as (+)-catechin and (-)-epicatechin were identified. The most frequent compounds occurring in grapes from that group were (+)-catechin (89.98 mg · 100 $g^{-1}$ FW) and (-)-epicatechin (45.48 mg · 100 $g^{-1}$ FW), which is consistent with reports of other authors (Ehrhardt et al., 2014; Antoniolli et al., 2015). The highest content of (+)-catechin was recorded in fruits of plants with the 'Kober 125AA' and 'Sori' rootstocks (105.63 and 99.54 mg · 100 $g^{-1}$ FW respectively). Grapes harvested from own-root plants had the lowest content of (+)-catechin and the highest content of (-)-epicatechin (62.88 mg · 100 $g^{-1}$ FW).

## Conclusions

The study showed an important influence of rootstock on the content of polyphenols in grapes of 'Regent'. The content of polyphenols was significantly higher in grapes harvested in the year 2015, which was characterised by a low amount of rainfall and a high temperature during the growing season.

The use of 'Sori' and 'Kober 125AA' rootstocks in cultivation of 'Regent' on silt loam soil was beneficial for obtaining fruits with the highest content of polyphenol compounds. Such a correlation has been shown in studies from all years. Furthermore, 'Sori' and 'Kober 125AA' rootstocks had the most significant influence on the increase in the anthocyanin and flavonol content in grapes. Additionally, 'Sori' rootstock, next to own-rooted plants, enabled to achieve the highest concentration of flavan-3-ols in berries. 'Börner' and 'Kober 5BB' rootstocks did not have a significant influence on the level of polyphenol in fruits when compared to own-root plants. In addition, it has been shown that using the 'Börner' rootstock reduces the content of phenolic acids in fruits.

Among all polyphenols determined, the majority of them were anthocyanin compounds, which occured most frequently in a form of 3-*O*-glucoside. The contents of derivatives of petunidin, peonidin, delphinidin, malvidin and cyanidin were at a similar level (18-22%).

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